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REVIEWS: CURRENT TOPICS

Plant sterols combined with exercise for the treatment of hypercholesterolemia: overview of independent and synergistic mechanisms of action

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Abstract

At present, dyslipidemia is most commonly treated with lipid-altering pharmacological therapies. However, safety concerns regarding the use of these agents have prompted the need for safe and efficacious nonpharmacological lipid-altering interventions. One such natural therapy is the combination of plant sterols and endurance training. This combination lifestyle intervention has been shown to decrease total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride concentrations while increasing high-density lipoprotein (HDL) cholesterol concentrations. However, the mechanisms that underlie these positive lipid alterations have yet to be clarified. Thus, the purpose of this review is to evaluate individual effects of plant sterols and exercise training on lipid levels while attempting to elucidate the possible independent and synergistic mechanisms of action responsible for these modulations. Results reveal that plant sterols decrease both total and LDL cholesterol levels by reducing exogenous cholesterol absorption by way of cholesterol displacement in the intestinal lumen. Additionally, the intestinal membrane transport proteins, ABCG5, ABCG8, as well as NPC1L1, have also been implicated in plant sterol-mediated cholesterol lowering. Conversely, exercise decreases triglyceride levels by reducing hepatic very low-density lipoprotein secretion and increasing skeletal lipoprotein lipase activity. In addition, endurance training was shown to increase HDL cholesterol levels by way of HDL subfraction alterations, in conjunction with changing reverse cholesterol transport enzyme activities. Moreover, plant sterols and exercise may work synergistically to alter lipid levels by modulating lipoprotein transport, composition, release and metabolism. In sum, the present review lends further insight as to the metabolic benefits of adopting a healthy lifestyle, including plant sterols and endurance training, in the treatment of dyslipidemia.

Keywords: Plant sterols; Exercise; Mechanism of action; Cholesterol; Hypercholesterolemia; Coronary heart disease

1. Introduction

Dyslipidemia has been identified as one of the most important modifiable risk factors for coronary heart disease (CHD) [1,2]. At present, dyslipidemia is most commonly treated with certain pharmacological therapies, including 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, also known as statins, ezetimibe, bile acid sequestrants, nicotinic acid and fibric acids [3]. Although these drug therapies have been shown to favorably modulate lipid and lipoprotein levels, safety concerns regarding the long-term use of these agents have recently emerged [4–8]. Thus, in view of these safety issues, the need for an efficacious nonpharmacological lipid-lowering therapy, which poses no risk of adverse events, has become apparent [9].

One such natural therapy that has recently been shown to be both effective and safe is the combination of plant sterols and endurance training [10]. In a recent trial [10], it was demonstrated that this combination lifestyle therapy decreased total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride concentrations by 7.7%, 8.3% and 11.8%, respectively, while increasing high-density lipoprotein (HDL) cholesterol concentrations by 7.5%. From the data presented, the authors concluded that the combination of plant sterols and exercise was an effective means of reducing the risk of future CHD, as the therapy resulted in favorable alterations in all four of the above mentioned lipid parameters [10]. However, in reviewing the data, certain questions arise: What are the individual effects of plant sterols and exercise on

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each of the four lipid parameters measured? Furthermore, what are the independent and synergistic mechanisms that underlie these positive alterations? Thus, in an attempt to answer these questions, the objective of this review is to summarize the effects of plant sterols and exercise on individual lipid parameters, and to investigate the underlying independent and synergistic mechanisms of action by which these interventions exert their effects.

2. Plant sterols for the treatment of hypercholesterolemia

2.1. Plant sterols and LDL cholesterol lowering: mechanisms of action

Plant sterols are naturally occurring constituents of plants and are structurally similar to cholesterol. Consumption of 1.8 to 2.0 g/day of plant sterols has been shown to lower both total and LDL cholesterol concentrations by 10% to 15% in a variety of different population groups [11–14]. Although beneficial effects have been repeatedly demonstrated for total and LDL cholesterol levels, plant sterol supplementation generally produces little or no change in HDL cholesterol or triglyceride concentrations [12,15,16]. As such, the decrease noted in total cholesterol levels is almost completely accounted for by the reduction in LDL cholesterol [12]. As described below, the mechanisms by which plant sterols lower LDL cholesterol concentrations have been shown to involve plant sterol-induced alterations in cholesterol absorption (Fig. 1) and synthesis pathways.

2.1.1. Effect of plant sterol supplementation on cholesterol absorption

Previous findings indicate that plant sterols reduce cholesterol absorption by 30% to 50% [17,18]. This decrease in cholesterol absorption is thought to result from the direct competition of plant sterols with cholesterol for incorporation into the mixed micelle [19–23]. Competition is thought to occur due to the limited capacity of the micelle to solubilize lipophilic molecules [24]. Therefore, when plant sterols and cholesterol are administered simultaneously in the diet, competition for solubilization between these two sterol compounds occurs [19,21,22]. Plant sterols are thought to be preferentially absorbed into the micelle in place of cholesterol because they are slightly more hydrophobic [23]. This physical-chemical property increases plant sterol affinity for the micelle, so that plant sterol uptake is energetically favored [23]. In turn, as a result of the micellar displacement of cholesterol by plant sterols, less cholesterol passes across the brush border membrane into the enterocyte.

Although a significant amount of diet-derived plant sterols pass into the intestinal cell, only 5% of these plant sterols pass into the circulation [25,26]. Thus, a transport mechanism that shuffles plant sterols back into the lumen must exist to prevent plant sterols from accumulating within the intestinal cell. The intestinal transporters responsible for this action are termed adenosine triphosphate-binding cassette (ABC) proteins and include specific proteins such as ABCG5 and ABCG8 [27]. Evidence suggests that sitosterolemia, an autosomal recessive disorder where plasma and tissue levels of plant sterols are increased 30- to 200-fold, may be caused by mutations in ABCG5 and ABCG8 proteins [27]. The role of these transporters as plant sterol efflux mechanisms has also been elucidated in experiments involving ABCG5 and ABCG8 knockout mice [28]. In a study by Yu et al. [28], it was shown that mice lacking these transporters experienced a more than 30-fold increase in mean plant sterol plasma concentrations. Taken together, these results suggest that ABCG5 and ABCG8 transport proteins prevent the absorbed plant sterols from accumulation inside the enterocyte. Nevertheless, the direct effect of plant sterols on the

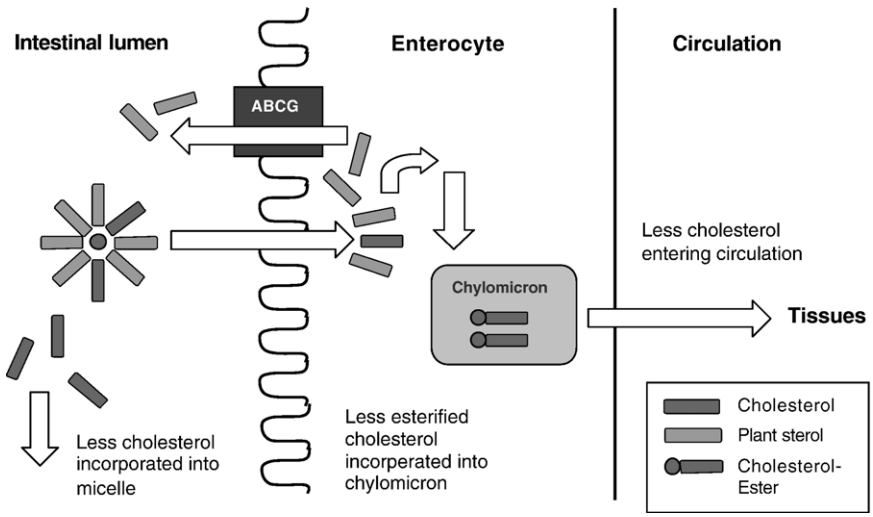

Fig. 1. Mechanism of action by which plant sterols decrease cholesterol absorption. (1) Plant sterols reduce the amount of cholesterol incorporated into the mixed micelle. (2) Excess plant sterols are pumped out of the enterocyte via the ABC proteins, ABCG5 and ABCG8.

up-regulation of these membrane transport proteins has yet to be fully clarified. The expression of ABCG5 and ABCG8 transporters are controlled by liver X receptors [27]. Dietary cholesterol, as well as other endogenous sterol compounds, directly activates these receptors [27]. Kaneko et al. [29] demonstrated that plant sterol derivatives, but not plant sterols themselves, were able to activate these receptors, in turn, up-regulating ABC transporters in the gut. The reason why plant sterol derivatives, and not plant sterols themselves, resulted in these changes remains unclear. These findings highlight the need for more research to be performed in the area of plant sterol-induced up-regulation of the ABC transporters.

Very recently, a specific transporter termed the Niemann-Pick C1 Like 1 (NPC1L1) protein was identified as being critical for the absorption of both cholesterol as well as plant sterols [30–33]. The transporter is localized in the jejunum of the small intestine and has been shown to assist with the trafficking of sterol compounds across the basal membrane into the enterocyte [31]. In a study by Davis et al. [33], it was noted that NPC1L1 null mice had substantially reduced intestinal uptake of both cholesterol and β-sitosterol, with subsequent reductions in plasma plant sterol levels. Furthermore, it was shown that the NPC1L1 null mice were completely resistant to diet-induced hypercholesterolemia [33]. From these results, the authors concluded that the NPC1L1 protein is required for intestinal uptake of both cholesterol and plant sterols, and thus plays a major role in regulating cholesterol homeostasis [33]. Undoubtedly, further research needs to be conducted to determine the potential interactions between plant sterols and cholesterol when absorbed via this transporter. In addition, the implications of these interactions on resulting cholesterol levels within the plasma should also be examined.

2.1.2. Effect of plant sterol supplementation on cholesterol synthesis

As a result of decreased cholesterol absorption by the above-mentioned mechanisms, cholesterol synthesis has been shown to increase in order to maintain whole-body cholesterol homeostasis [34–36]. Changes in cholesterol biosynthesis are measured experimentally by several methods including the rate of incorporation of deuterium from body water into free cholesterol [34,35]. In a study where cholesterol absorption was shown to decrease by 56% as a result of plant sterol administration, cholesterol synthesis, as determined by the deuterated water method, was shown to increase by 25% [34]. Alternatively, cholesterol synthesis may also be assessed by the quantification of certain cholesterol precursors, such as lathosterol levels in plasma [36]. For instance, in a study by De Graaf et al. [36], lathosterol concentrations were shown to increase by 20.7% in response to plant sterol-induced decreases in cholesterol absorption. It must, however, be noted that the increase in cholesterol synthesis resulting from plant sterol administration does not fully compensate for the decrease in cholesterol absorbed; thus, a net reduction in circulating cholesterol levels still occurs [34,35].

Mechanistically, the increase in cholesterol biosynthesis noted when cholesterol absorption decreases is thought to occur by way of HMG CoA reductase gene modulation. 3-Hydroxy-3-methylglutaryl coenzyme A reductase is an enzyme that plays a key role in the cholesterol synthesis pathway and is thought to be a limiting factor that controls the amount of cholesterol synthesized by individual cells [37]. The expression of this enzyme is regulated by certain transcription factors termed sterol regulatory element-binding proteins (SREBPs) [37]. When intracellular cholesterol levels decrease in response to a reduction in cholesterol absorption, SREBPs levels increase [38]. The increase in these elements results in a corresponding increase in the HMG CoA reductase protein, and thus, cholesterol biosynthesis is raised [38].

3. Endurance exercise for the treatment of hypercholesterolemia

3.1. Endurance exercise and triglyceride lowering: mechanisms of action

Accumulating evidence suggests that participating in moderate intensity endurance exercise for 1 h or more can significantly reduce fasted and postprandial blood triglyceride levels (Fig. 2) [39–43]. However, controversy exists with respect to the mechanisms by which this reduction occurs. Although there is no consensus, it has been proposed that reductions in plasma triglyceride concentrations are primarily a result of the up-regulation of skeletal muscle lipoprotein lipase (LPL) [44–49] and reduced hepatic very low-density lipoprotein triglyceride (VLDL-TG) secretion [39,40,50,51].

Most studies investigating mechanisms by which exercise decreases circulating levels of triglycerides have attributed the observed changes to increases in both the amount and activity of skeletal muscle-derived LPL. This mechanism was proposed when it was observed that the effects of endurance exercise on LPL regulation were also related to concurrent reductions in serum triglycerides of up to 20% [44,45,52]. Lipoprotein lipase is synthesized in many tissues, one of which includes skeletal muscle. Once synthesized, LPL is secreted to local capillary beds where triglycerides, transported via chylomicrons and VLDL, are cleaved. This cleavage results in the influx of free fatty acids (FFA) into the adjacent muscle cells [53]. Moreover, studies have shown that endurance exercise can produce significant increases in both LPL protein mass and enzyme activity at the sight of the working muscle [44–46,49]. This is important with respect to endurance exercise bioenergetics because it has also been suggested that during moderate intensity endurance exercise, intramuscular triglycerides are the primary lipid energy source [54,55]. Furthermore, a positive correlation has been observed between levels of LPL mRNA and intramuscular lipid repletion for up to 30 h postexercise [56]. This would

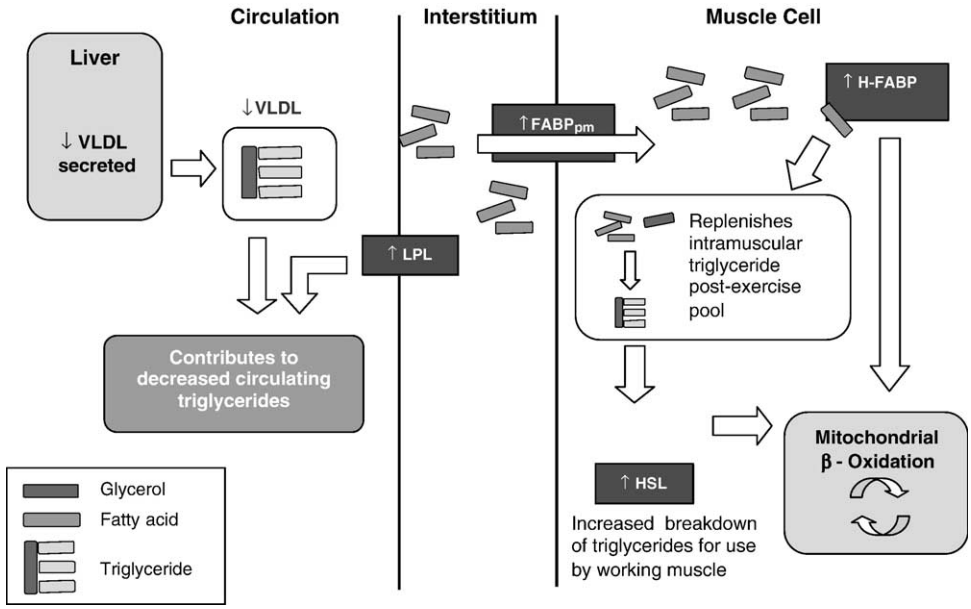

Fig. 2. Mechanism of action by which exercise training decreases circulating concentrations of triglycerides. (1) Exercise results in less VLDL secreted from the liver. (2) Exercise up-regulates LPL activity, thus, resulting in a greater release of fatty acids, which enter the interstitium. (3) Plasma membrane fatty acid binding protein ($FABP_{PM}$) also increases, permitting more fatty acids to enter the muscle cell from the interstitium. (5) Increased intramuscular fatty acid binding protein (H-FABP) allows for increased FFA shuttling capacity within the muscle cell to replenish intramuscular triglycerides and the mitochondria for β-oxidation. (6) Hormone-sensitive lipase (HSL) is up-regulated during exercise, resulting in the increased breakdown of intramuscular triglycerides for use by the working muscle.

imply that LPL up-regulation is persistent, serving as an energy regenerator, by helping replace depleted intramuscular triglyceride stores [49]. Thus, participating in endurance exercise on a regular basis would provide a sustained reduction in circulating triglycerides for a considerable amount of time after training.

Very low-density lipoprotein secretion may also be implicated in the regulation of circulating triglyceride levels in response to endurance exercise. Very low-density lipoprotein is produced by the liver and contains high amounts of triglycerides. If hepatic production of triglycerides, or triglyceride incorporation into the VLDL particle, was blunted, blood triglyceride levels would in turn decrease. A study examining the effects of brisk walking on lipid metabolism observed a 20% and 31% reduction in both fasting triglycerides and VLDL-TG, respectively, independent of any change in triglyceride clearance [39]. Furthermore, 6 months of moderate endurance training has been shown to reduce VLDL secretion by 51% [50].

3.2. Endurance exercise and HDL cholesterol raising: mechanisms of action

High-density lipoprotein cholesterol is an important lipoprotein due to its ability to shuttle excess cholesterol away from peripheral tissues by way of reverse cholesterol transport (RCT). The RCT process begins with the release of lipid-poor pre-β-HDL from the liver or intestine. The pre-β-HDL particle travels to the periphery, where it receives an efflux of cholesterol from tissues forming HDL_3. The free cholesterol within the HDL_3 particle is esterified by lecithin-cholesterol acyltransferase (LCAT) producing HDL_2. Both HDL_2 and HDL_3 are known as mature HDL particles. The enzyme cholesteryl ester transfer protein (CEPT) catalyzes the exchange of HDL_2 cholesterol esters for triglycerides with lipoproteins such as VLDL. HDL_2 then travels back to the liver where it is reformed into either pre-β-HDL and/or HDL_3 [57,58].

Recent reports indicate that endurance exercise can increase HDL cholesterol concentrations from 6% to 21% [59–61]. Furthermore, this increase is often accompanied by a reduction in serum VLDL-triglycerides [62,63]. This concomitant observation is probably important mechanistically for deciphering the observed increase in HDL levels because triglycerides play an valuable role in RCT [57,58]. However, pinpointing an exact mechanism as to how endurance exercise increases HDL concentrations is complicated because inconsistencies exist between studies with respect to the RCT enzyme activities and the subsequent HDL subfractions that are effected by physical activity [60,62–64].

With regards to HDL_3, one study reported that a 14% increase in total HDL was primarily due to an 11% increase in the HDL_3 subfraction, with no changes in LCAT and CETP activities [63]. Although not measured, this observation could be attributed to an increase in pre-β-HDL from synthesis or HDL_2 catabolism and the observed stability in LCAT activity. Other investigations implicate significant increases to the HDL_2 subfraction, coinciding to a 63% decrease in VLDL associated triglyceride, along with a

29% and 14% reduction in CEPT mass and enzyme activity [62]. The authors speculated that both the reductions in VLDL levels and CETP activity could account for the observations pertaining to HDL_2. This could have resulted from an accumulation of HDL_2 before the CETP-mediated transfer and/or possibly the decrease in the catabolism of triglyceride-enriched HDL_2, post-CETP, thus, disabling their conversion to HDL_3 [62]. Furthermore, it has also been shown that lipolysis of VLDL alone can result in higher levels of HDL_2 by way of HDL_3 particles scavenging excess cholesterol and phospholipids, and becoming stable particles that resemble HDL_2 [65]. This is feasible due the previously described effects that exercise has on VLDL metabolism due to higher energy demands. Finally, some studies report that exercise-induced increases in HDL levels are specific to changes to pre-β-HDL, coinciding to higher levels of LCAT activity and cholesterol efflux, and no detectable changes to CETP activity [59,64]. However, in these investigations, results could be attributed to an increase in the availability of the HDL_2 subfraction to move through the CETP pathway, resulting in a faster turnover of HDL_2 and production of pre-β-HDL. Despite these observed differences with respect to how endurance training can induce a pronounced increase in HDL cholesterol specific to particular subfractions, it is more likely that these changes result from the interaction of several variables in the HDL metabolic pathway. It has been shown that submaximal exercise can simultaneously increase both HDL_2 and HDL_3 subfractions over a 24-h period while decreasing circulating triglyceride and VLDL levels [61]. Additionally, RCT has also been shown not to change despite significant increases in LPL activity [66]. In summary, the specific mechanism pertaining to how endurance training increases HDL has not been completely resolved. Further research examining the effects of exercise on all HDL subfractions and related RCT enzymes are needed in order formulate more assertive conclusions.

4. Plant sterols and exercise for the treatment of hypercholesterolemia: possible synergistic mechanisms of action

Although plant sterols and endurance exercise have independently been shown to improve specific lipid parameters, combining these two therapies may give rise to synergistic effects resulting in a wider spectrum of circulating lipid improvements. The following section overviews how these two therapies may work together if both are adopted as part of a lipid-lowering regimen.

As previously discussed, plant sterols lower LDL cholesterol concentrations by reducing exogenous cholesterol absorption through micellar displacement in the small intestine, thus, resulting in less cholesterol being transported to the liver. Endurance exercise may enhance this action due to its effects on both splanchnic and mesenteric blood flow. To be specific, endurance exercise has been shown to significantly reduce blood flow to the liver as well as to the gastrointestinal system [67–69]. These effects have been observed in both fasting and postprandial states [69–71]. If an individual consumes a meal containing plant sterols and then partakes in endurance exercise, the overall capacity for the lipids to make their way to the liver will synergistically be blunted. Initially, overall cholesterol absorption will be lessened due to the ability of plant sterols to reduce cholesterol absorption. If training were to be administered after the ingestion of the plant sterols, blood flow to the gastrointestinal tract and liver would be reduced, resulting in less chylomicron shuttling from the enterocyte into the circulation. These events would subsequently decrease the amount of lipid transported to the liver and hence decrease the amount of VLDL particles released. Therefore, combining plant sterols with exercise would lead to greater reductions in circulating lipids when compared to their effects as individual therapies.

As described above, reducing the amount to of lipid transported to the liver ultimately decreases the amount of lipid released into the circulation via VLDL. The ability of endurance exercise to significantly lower VLDL secretion could result in lower levels of both circulating triglycerides and cholesterol. Although training could result in higher levels of LDL production due to its ability to increase VLDL processing at the site of the working muscle, the resultant LDL particles could contain lower levels of cholesterol due to the initial actions of plant sterols. The induced change in LDL composition would then reduce the likelihood of cholesterol deposition in tissues and arteries. Hence, the combined effects of endurance training and dietary incorporation of plant sterols to reduce levels of circulating lipids could potentially enhance the individual protective properties of each therapy.

Plant sterols and exercise may also work synergistically to favorably alter certain enzymatic pathways involved in cholesterol metabolism. As previously described, endurance exercise has been shown to significantly reduce CETP activity. Moreover, decreased levels of circulating LDL have been shown to result in reductions in CETP gene expression [72,73]. This reduction in CETP activity may therefore be enhanced when plant sterols are combined with exercise, as their combined effects lead to less VLDL and LDL production, which in turn may decrease CETP levels. This decline in CETP activity could result in an accumulation of HDL_2, preventing a fast transfer of cholesterol esters from HDL to VLDL and possibly reducing the efficiency at which cholesterol is transported back to the liver. Then again, chronic plant sterol ingestion may aid such a situation in two ways. First, less scavenging may be necessary due to less cholesterol incorporation into lipoproteins and hence less cholesterol deposition in tissues and arteries, resulting in a reduced need for cholesterol to be shuttled back to the liver for reprocessing. Second, because plant sterols reduce the cholesterol that is initially incorporated into VLDL particles, VLDL may more efficiently receive cholesterol from HDL, preventing reduced CEPT activity from halting tissue

cholesterol scavenging and subsequent transport to the liver. In addition, a lower CETP activity may allow for HDL_2 to act as a cholesterol reservoir, allowing the transfer of cholesterol to VLDL particles to occur at a slower rate and/or increase the amount of cholesterol transferred directly to the liver via HDL_2. These events would therefore minimize the circulating cholesterol pool and hence the amount of cholesterol available to be deposited in arterial walls.

5. Conclusion

The purpose of this review was to evaluate the individual effects of plant sterols and exercise training on lipid levels while attempting to elucidate the possible independent and synergistic mechanisms underlying these alterations. In sum, plant sterols have been shown to decrease both total and LDL cholesterol levels by reducing exogenous cholesterol absorption by way of cholesterol displacement in the intestinal lumen. Additionally, it has been suggested that the intestinal membrane transport proteins, ABCG5, ABCG8, as well as NPC1L1, may play a role in plant sterol-mediated cholesterol lowering. Conversely, exercise has been shown to decrease triglyceride levels through reduced hepatic VLDL secretion and increased skeletal LPL activity. In addition, endurance training was shown to increase HDL cholesterol levels by way of HDL subfraction alterations, in conjunction with changing RCT enzyme activities. Furthermore, plant sterols may work synergistically with exercise to produce more favorable lipid level alterations than what is seen with each intervention alone. The synergistic mechanisms that underlie these enhanced lipid alterations include modulations to lipoprotein transport, composition, release and metabolism. In sum, this paper lends further insight as to the metabolic benefits of adopting a healthy lifestyle, including plant sterols and physical activity, and suggests that this combination lifestyle therapy may significantly reduce one's risk of CHD by favorably altering each of the four key lipid parameters.

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