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Rapid communication

# Evaluation of the effect of plant sterols on the intestinal processing of cholesterol using an in vitro lipolysis model

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ABSTRACT

An in vitro lipolysis model was utilized to study the effect of stigmastanol (lipophilic phytosterol) and disodium ascorbyl phytostanol phosphate (DAPP) (modified hydrophilic phytostanol) on intestinal processing of cholesterol to gain further understanding of their cholesterol lowering mechanism. Lipolysis results showed that stigmastanol, if given in powder alone, had no effect on cholesterol processing probably due to its poor solubility. Stigmastanol suspension formulation re-distributed cholesterol from aqueous phase to oil and sediment phases. The water soluble DAPP has changed cholesterol distribution even more significantly by transferring cholesterol from aqueous phase to sediment phase. Moreover, the results provided evidence that DAPP inhibited triglyceride digestion in vitro. Considering DAPP as a surfactant with the same lipophilic sterol ring as bile salt, its ability to inhibit triglyceride lipolysis may be due to its competition with bile salt for the substrate surface, thereby hindering the lipolysis of triglyceride and inhibiting cholesterol solubilization with the lipolysis products. It can be speculated that the cholesterol lowering mechanism of DAPP during intestinal digestion is related to its ability to act as a surfactant closely resembling bile salt.



Coronary heart disease is the leading cause of death in North America (Gaziano et al., 2010). It is a condition in which plaque builds up inside the coronary arteries (atherosclerosis) and eventually weakens the heart muscle leading to heart failure and arrhythmias. People with hypercholesterolemia have a high risk of developing heart disease (Kannel et al., 1971).

Numerous studies have demonstrated that plant sterols and their analogues lower blood cholesterol levels and reduce the risk of coronary heart disease (Mekhtiev and Misharin, 2007). However, the mechanism of action of the plant sterols is still poorly understood (Mel'nikov et al., 2004).

In recent years, in vitro lipolysis model has been introduced as a useful tool to simulate the intra-luminal processing of dietary lipids in vitro (Dahan and Hoffman, 2007, 2008). This model mimics the digestion process that happens in the physiological environment of the upper gastrointestinal tract. NaOH is used to titrate free acids produced by lipolysis of triglyceride to maintain the pH of the medium. The distribution of the test compound in the oil, aqueous and sediment phases after ultracentrifugation provides insight into its probable fate in the intestine when consumed with lipids. As shown in Fig. 1, the proportion that is recovered in the aqueous phase is most readily available for absorption; the proportion recovered in the oil phase is most likely to have a delayed absorption; while the proportion found in sediment is most likely to be excreted (Gershkovich et al., 2011). So far, various oral lipid-based lipophilic drug formulations have been evaluated using this model and predictions of the relative efficiency of different lipid formulations have been made (Porter and Charman, 2001; Sek et al., 2002; Bonsdorff-Nikander et al., 2005).

However, there have been scarce reports using in vitro lipolysis model to evaluate intestinal processing of cholesterol and the effects of cholesterol lowering agents during the lipid digestion process (Gershkovich et al., 2011; Bonsdorff-Nikander et al., 2005). One of the prerequisites for cholesterol absorption is its solubilization in the intestine during lipid digestion (Ros, 2000). Therefore in vitro lipolysis model, which shows the distribution of cholesterol in different phases during the lipid digestion process, provides valuable information on intra-luminal processing of cholesterol and potentially an insight into the mechanism of action of cholesterol lowering agents (Gershkovich et al., 2011).

In this work, two well known cholesterol lowering plant sterols, stigmastanol and DAPP (Moghadasian and Frohlich, 1999; Wasan et al., 2001a,b), were examined using the in vitro lipolysis system. Stigmastanol is a lipophilic phytostanol, while DAPP is a modified hydrophilic phytostanol (Fig. 2). These two compounds were specifically chosen because of their different lipophilicity in order

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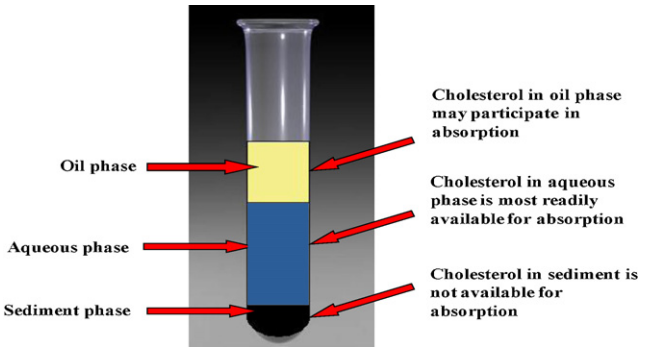


**Fig. 1.** Partition of cholesterol between the various phases indicates cholesterol fate during in vivo absorption processes.

to gain further understanding of their role in cholesterol solubilization during lipid digestion.

The in vitro lipolysis experiments were preformed as previously reported (Gershkovich et al., 2011). Stigmastanol powder and stigmastanol formulation (wetted by Tween 80 and suspended in 2% sodium carboxymethyl cellulose solution) were both tested in the lipolysis model. Stigmastanol suspension was prepared as reported before (Gershkovich et al., 2009). DAPP was added to the lipolysis process as a solution after vigorous vortexing.

In vitro lipolysis results of the relative distribution of cholesterol across oil, aqueous and sediment phases in the presence of stigmastanol alone are shown in Fig. 3 (simulated fasted state) and Fig. 4 (simulate fed state). In both fasted and fed states, there was no significant difference in cholesterol distribution between stigmastanol and the water control groups in all the three phases, which suggests that stigmastanol in the form of powder had no effect on intra-luminal processing of cholesterol during the lipolysis process. In literature, conflicting results have been reported about the effectiveness of this compound when given in different forms, such as in capsules, tablets, or as food additives (AbuMweis et al., 2008; Demonty et al., 2009; McPherson et al., 2005). The lipolysis result observed suggests that formulation plays an important role on the effectiveness of stigmastanol because of its lipophilic nature.

As shown in Figs. 3 and 4, suspension of stigmastanol changed the cholesterol distribution between phases significantly in both fasted and fed state. The suspension reduced cholesterol concentration in aqueous phase by redistributing cholesterol into oil phase and sediment phase, which provided evidence that properly formulated stigmastanol can affect intestinal processing of

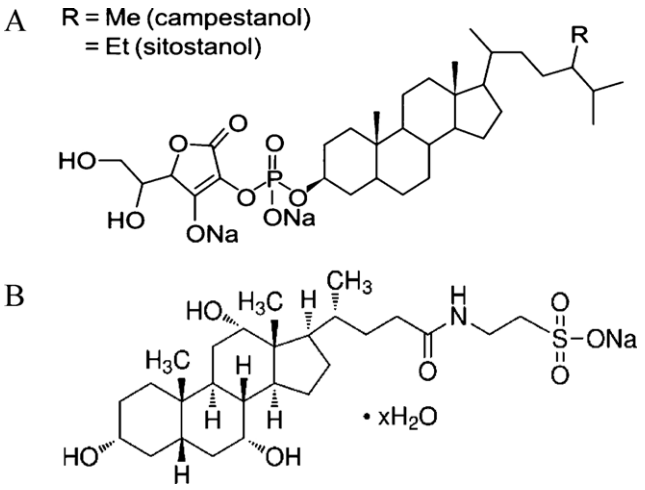


**Fig. 2.** Structure of DAPP (A) and sodium taurocholate (B).

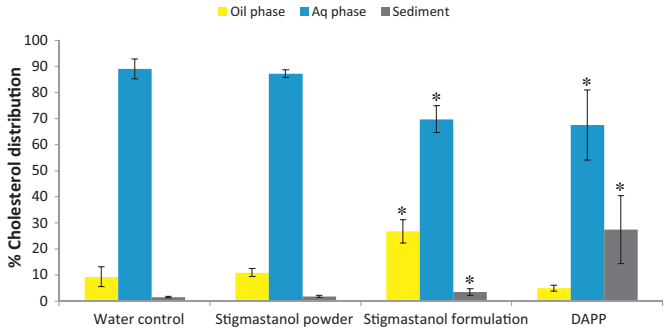


**Fig. 3.** Distribution of cholesterol across the lipid, aqueous and sediment phases following lipolysis in the presence of water control ($n=6$), stigmastanol powder (262.5 mg, $n=4$), stigmastanol formulation (262.5 mg, $n=8$) and DAPP (262.5 mg, $n=5$) under simulated fasted state conditions. $^*P<0.05$ multiple comparisons versus control group.

cholesterol and lead to decreased cholesterol absorption. The result is consistent with previous report by Gershkovich et al. which showed a reduction of plasma cholesterol levels in rats that were given the same formulation (Gershkovich et al., 2009). However, the magnitude of cholesterol reduction obtained in the lipolysis model is relatively low compared to the reduction of plasma cholesterol level (30%) observed in rats (Gershkovich et al., 2009) suggesting that other mechanisms that involve cholesterol absorption and/or metabolism may play a role in stigmastanol's cholesterol lowering property (Mel'nikov et al., 2004; Calpe-Berdiel et al., 2009; Brufau et al., 2011). Disodium ascorbyl phytostanol phosphate (DAPP) significantly reduced cholesterol distribution to the aqueous phase in both the fasted and fed state as seen in Figs. 3 and 4. Significant amount of cholesterol was re-distributed from the aqueous phase to the sediment phase, while no significant change was observed in the oil phase. Compared to suspension of stigmastanol which transferred cholesterol from aqueous phase to oil phase (possible delayed absorption) and sediment phase, DAPP transferred similar amount of cholesterol from aqueous phase to sediment phase only (possible excretion). This observation suggests that DAPP could be a more efficient cholesterol lowering agent than stigmastanol. Vissers et al. reported similar findings in human clinical trials that DAPP reached equal LDL-lowering effect but at a 2–3 times lower dose compared to plant sterol and stanol esters (Vissers et al., 2008). The authors attributed the higher efficiency of DAPP to its higher water solubility and more efficacious competition with cholesterol for mixed micelle positions. However, Ramaswamy et al. reported that no differences were observed in cholesterol accumulation by Caco-2 cells when DAPP was

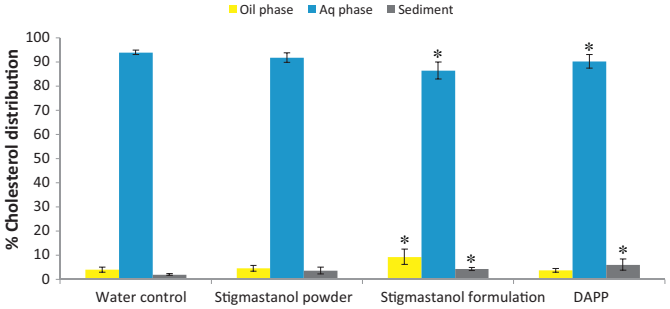


**Fig. 4.** Distribution of cholesterol across the lipid, aqueous and sediment phases following lipolysis in the presence of water control ($n=6$), stigmastanol powder (262.5 mg, $n=6$), stigmastanol formulation (262.5 mg, $n=5$) and DAPP (262.5 mg, $n=7$) under simulated fed state conditions. $^*P<0.05$ multiple comparisons versus control group.