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Mini-review: The effects of apples on plasma cholesterol levels and cardiovascular risk – a review of the evidence

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SUMMARY
Evidence suggests that a high intake of fruits is associated with a reduced risk of cardiovascular disease (CVD) and lowered plasma cholesterol, but the specific effects of individual types of fruit, fruit fractions, and processed fruit are less well-studied. Apples are among the most frequently consumed fruits, and human and animal studies on apple may help to clarify the effect of this fruit on CVD risk markers. The aim of this mini-review is to summarise current evidence for a lowering effect of apple on the risk of CVD and plasma cholesterol levels, and to investigate whether such an effect is influenced by fruit processing or the form of intake. Possible mechanisms behind the cholesterol-lowering effect of apples are also considered. All relevant published experimental studies in humans and animals were identified within the open literature. Nine human studies were identified, of which four concerned the effects of whole apples, two the effects of dried apples, and three the effects of filtered apple juice. Additional studies considered specific apple components. In general, there was a cholesterol-lowering effect, in the range of 5 – 8%, after the intake of approx. three whole apples, whereas the consumption of apple juice (375 – 720 ml) had no effect on plasma cholesterol levels and may result in adverse effects on plasma triglyceride levels. Limitations in the study designs did not allow us to draw conclusions on the effect of the intake of whole, dried apples (15 – 52 g). We also identified a total of nine experimental studies in animal models. Feeding with apple products resulted in decreased levels of plasma (11 – 43%) and liver (23 – 67%) cholesterol in the majority of studies. There was an increased excretion of bile acids (3 – 56%) and cholesterol (5 – 41%) in rats fed with apple products. Based on the current evidence from human observational and intervention studies, it seems likely that a reduction in plasma total and LDL cholesterol occurs after a dietary intake of apples, which could lead to a decreased risk of CVD. On average, a daily intake of approx. three apples resulted in a decrease in total cholesterol of 5 – 8% (approx. 0.5 mmol l^{-1}). The consumption of filtered apple juice may result in adverse effects on plasma triglyceride levels. Evidence from animal studies suggests that the major mechanism behind the cholesterol-lowering effect of apples involves an increased clearance of plasma cholesterol due to enhanced faecal excretion of bile acids and cholesterol.

A high intake of fruit has been associated with a 10 – 40% reduction in the risk of cardiovascular disease (CVD) in prospective cohort studies (Bazzano *et al*., 2002; Hung *et al*., 2004; Johnsen *et al*., 2003; Joshipura *et al*., 2001; Singh *et al*., 1993). These effects have largely been ascribed to the high fibre and anti-oxidant contents of fruit. The effects of whole and processed fruit on early CVD risk factors have been tested in randomised controlled interventions, which demonstrated a significant lowering of plasma total and LDL cholesterol and blood pressure (Djousse *et al*., 2004; Dragsted *et al*., 2006; Jenkins *et al*., 1997; Obarzanek *et al*., 2001). However, different fruits may differ considerably in composition with respect to their content and types of phytochemicals and fibre, and the specific effects of a single type of fruit, fruit fraction, and/or processed fruit are less well studied. An overview of the possible preventive effects of apple consumption on cancer, CVD, diabetes, and other health conditions has been presented by others (Boyer and Liu, 2004); but a focussed review of the effects of apple and apple components on cholesterol-lowering and CVD is lacking. Apples are among the most frequently consumed fruits, especially in northern Europe and North America, and one of the best studied fruits. Human and animal studies on apple consumption may help to clarify the effects of fruit on CVD and CVD risk markers.

This mini-review aims to provide answers, based on all currently available evidence, on whether i) there is *a priori* evidence that apples affect the risk of CVD and CVD risk markers; ii) a difference may exist in the effect of whole apples *vs*. processed apple fractions; and iii) there is evidence for specific mechanisms behind the effects observed, with a focus on plasma cholesterol levels.

Relevant experimental and observational studies on apples and apple products were identified: i) by literature searches in PubMed, Biosis, and Chemical Abstracts using the subject headings - apples, apple juice, apple products, pectin, polyphenols, cardiovascular disease, and plasma cholesterol, alone or in combination; and ii) by checking the reference lists of other publications to identify studies published in Journals that are not indexed in literature databases. The quality of the design of each study, in terms of adequate controls and its power, was evaluated during the reviewing process. Papers with severe limitations were excluded from the final Discussion section.

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TABLE I
Average contents of energy, nutrients, fibres, and phytochemicals in apple

Component	Unit	Content (100 g^{-1} apple)	Reference
Energy	kJ	245	(National Food Institute, 2007)
Protein	g	0.3	"
Total fat	g	0.3	"
Saturated fat	g	0.066	"
Mono-unsaturated fat	g	0.009	"
Poly-unsaturated fat	g	0.17	"
Total carbohydrates	g	12.9	"
Fructose	g	5.86	"
Glucose	g	1.68	"
Sucrose	g	0.57	"
Total dietary fibre	g	2.7	(Schakel *et al*., 2001)
Insoluble fibre	g	2.0	"
Soluble fibre	g	0.70	"
β-carotene	mg	25	(National Food Institute, 2007)
Thiamin	mg	0.016	"
Riboflavin	mg	0.011	"
Vitamin B_6	mg	0.051	"
Folate	mg	9	"
Vitamin C	mg	10.0	"
Calcium	mg	4	"
Iron	mg	0	"
Zinc	mg	0.03	"
Iodine	mg	0.20	"
Sodium	mg	3	"
Potassium	mg	120	"
Magnesium	mg	4	"
Total polyphenols	mg	111.45	(Vrhovsek *et al*., 2004)
Flavanols	mg	96.33	"
Flavonols	mg	5.66	"
Dihydrochalcones	mg	4.18	"
Anthocyanins	mg	1.62*	"
Hydroxycinnamic acids	mg	14.21	"
Total phytosterols	mg	13**	(Normen *et al*., 1999)

*Only in red apples.
**Plant sterol content includes the sum of campesterol, β-sitosterol, stigmasterol, campestanol, and β-sitostanol.

APPLE COMPONENTS

Apples are low in energy and fat, and are a good source of fibre, micronutrients, and phytochemicals (Table I). Several of the components in apple may affect plasma cholesterol levels. The mechanism(s) behind these actions are discussed in more depth later in this mini-review.

Simple carbohydrates: Ninety-percent of the energy from apples is derived from carbohydrates, mainly sugars, of which fructose is the dominant form (Table I). Sucrose and glucose are also abundant. Fructose is lipogenic, and high doses may therefore affect plasma triglyceride and cholesterol levels. Apple fructose and sucrose contents are lower than in most other fruit.

Fibre: The fibre content of apple is approx. 3 g 100 g^{-1} fresh weight (FW) and consists of soluble fibres, mainly pectin, as well as insoluble fibres, predominantly cellulose and hemicellulose (Table I). Pectin is a complex polysaccharide, and apple pectin exhibits a high degree of esterification and a particularly high content of branched side chains (Thakur *et al.*, 1997). These characteristics are responsible for the excellent gelling properties of apple pectin (Thakur *et al.*, 1997). Pectin is resistant to hydrolysis by human digestive enzymes, but, unlike most of the cellulose, it is fermented by the microflora in the large intestine resulting in the formation of short chain fatty acids (SCFA) which are absorbed and metabolised in the colonic mucosa, liver, or peripheral tissues (Kay, 1982). Gel-forming soluble fibres may decrease the re-absorption of cholesterol and bile acids in the lower gut, and SCFA may decrease hepatic cholesterol biosynthesis.

Micronutrients: Apples are rich in vitamins C and E, some pro-vitamin A carotenes, lutein, folic acid, potassium and magnesium (Table I). While these micronutrients are nutritionally essential, in order to avoid negative health effects (Nordic Council of Ministers, 2004), there is no evidence that any of them influence levels of plasma cholesterol. The content of micronutrients was therefore not considered further in the present context.

Phytochemicals: Apples contain phytosterols and polyphenols (Table I). These are secondary plant metabolites which are not regarded as human nutrients. The chemical structures of plant sterols resemble that of cholesterol, but with an additional methyl or ethyl group in the side chain. High doses of phytosterols are known to inhibit the uptake of cholesterol. Apples have a relatively low content of sterols (Table I). Important classes of apple polyphenols include pro-anthocyanidins, flavanols (catechins), flavonols, dihydrochalcones, anthocyanins (in red apples), and hydroxycinnamic acids (Table I). The total polyphenol content of apples may differ by a factor greater than threefold, between varieties, and specific classes of phenols may vary even more (Escarpa and Gonzalez, 1998). The majority of polyphenols in apples, are present as polymers, glycosides, and/or esters, which can only be hydrolysed to a limited extent by human gut enzymes. They therefore require hydrolysis by the colonic microflora before absorption (Manach *et al*., 2004). These characteristics result in most polyphenols having a low degree of absorption and urinary excretion, with catechins being the main exception (Young *et al*., 2002). Flavonols, when present as glucosides, have a higher absorption and their metabolites may reach plasma concentrations in the low µM-range, similar to the catechins (Manach *et al.*, 2005). Apple polyphenols have been implicated in lowering cholesterol in combination with pectins. The processing of apples results in changes in the contents of bio-available polyphenols; for example, lower contents of most polyphenols exist in apple juice.

OBSERVATIONAL STUDIES ON THE EFFECTS OF APPLE INTAKE ON CVD RISK OR CHOLESTEROL-LOWERING

A diet rich in fruits and vegetables has been associated with lowered plasma cholesterol levels in observational studies, and in interventions with mixed fruits and vegetables (Djousse *et al.*, 2004; Dragsted *et al.*, 2006; Jenkins *et al.*, 1997; Obarzanek *et al.*, 2001).

Apples alone have not been the subject of observational studies on cholesterol lowering, but apple consumption has been associated with a lower occurrence of CVD in large prospective cohort studies examining dietary intakes of flavonoids. In the Dutch Zutphen Elderly Study of 805 men aged 65 – 84 years, a high apple consumption (> 110 g d^{-1}) was inversely associated with 5-year mortality from coronary heart disease [adjusted risk ratio (RR) = 0.51; 95% confidence interval (CI) = 0.23 – 1.16], but the association was not statistically significant (*P* for trend = 0.12; Hertog *et al.*, 1993). This study caused a considerable focus on flavonoids as the possible preventive dietary factor against CVD, and several larger studies followed, some of which also reported separately on the effects of the main sources of flavonoids, such as apples. In a Finnish prospective study of 9,208 men and women, followed for 28 years, high apple consumption (> 47 g d^{-1}) was inversely associated with 5-year mortality from ischemic heart disease (IHD), and the incidence of thrombotic stroke, when compared to low apple intakes (0 g d^{-1}; Knekt *et al.*, 2000). The adjusted RR of mortality from IHD were 0.75 (95% CI = 0.60 – 0.94; P for trend = 0.007) and 0.75 for thrombotic stroke (95% CI = 0.57 – 0.99; *P* for the trend = 0.009) between the highest and lowest quartiles of apple intake. In the Women's Health study, a prospective cohort study among 38,445 women, there was a non-significant decrease in CVD risk with increased apple intake at the 6.9-year follow-up, and there was no significant association with dietary flavonoid levels (Sesso *et al.*, 2003). Also, in another large prospective study of 34,492 post-menopausal US women followed for 10 years, the authors were unable to demonstrate any association between apple consumption (1.1 – 4.7 servings per week) and CVD (Yochum *et al.*, 1999). The differences in the results between the European and American studies may be largely explained by the much lower intakes of apple in studies from the USA. Study population groups, and consequently the adjustment variables applied, were not the same in these three studies, and this may also have affected the outcomes. Specifically, the adjustment for cholesterol used in several studies may partially remove the apparent effects of fruits such as apple, which may decrease cholesterol levels. In the Finnish study, gender-specific effects were reported, with a stronger effect in women (Knekt *et al.*, 2000)

INTERVENTION STUDIES ON APPLES AND CHOLESTEROL-LOWERING

Currently, nine published human studies have been undertaken to investigate the effects of apples or apple products on cholesterol-lowering (Table II).

Whole apples: The most recent and well-conducted study was performed by Hyson *et al.* (2000), who studied the effect on plasma total cholesterol in 25 healthy men and women who were each assigned at random to a period with 340 g d^{-1} of whole apples, or a period with clear apple juice supplementation (375 ml), followed by a subsequent period with the other supplementation in a cross-over design. There was a small reduction in LDL cholesterol (5%) and a small rise in triglycerides (10%) compared to baseline values after the consumption of clear apple juice, but none of these differences were significant ($P > 0.05$). This study was un-blinded, but randomised and well controlled with regard to inclusion criteria and dietary intake, and it should be sufficiently statistically powered to detect differences in total and LDL cholesterol.

Four much earlier studies, with limitations in their design, were undertaken to examine the cholesterol-lowering effects of dietary interventions with fresh apples. Gormley *et al.* (1977) conducted a study in which 76 mildly hypercholesterolemic men participated in a time-course, parallel design (i.e., each participant received only one treatment and the two treatment arms were conducted simultaneously). The intervention group was assigned to eat an average of three apples a day, and the control group was allowed to eat a maximum of three apples per week, both over 4 months. The groups were pair-matched according to baseline serum cholesterol levels and fruit fibre intakes. There was no real control of smoking habit, but the authors attempted to control the subjects' diets for fibre intake. The serum cholesterol levels of the intervention group were consistently lower than those of the control group throughout the 4-month study period, but were only considered significantly lower (no statistics presented) at the last two measurements. HDL cholesterol was measured on the last day of intervention, and a higher level was demonstrated in the "apple" group compared to the control.

Canella *et al.* (1963) examined a daily intake of six fresh apples consumed as a blended, graded, or thinly-sliced product using 100 hypercholesterolemic patients at a hospital. Twenty of the patients served as controls. The duration was not stated and, in general, the description of the study is very sparse. All meals were prepared by the hospital, but there were no other restrictions. The patients, who were consuming blended apple pulp experienced the greatest reduction in total cholesterol (by 1.2 mmol l^{-1}), the group consuming graded pulp had a slightly smaller decrease (0.8 mmol l^{-1}), and the last group consuming apple slices had the smallest decrease (0.1 mmol l^{-1}). The level of significance was not given. The decreases in cholesterol were numerically larger than those observed in other studies, which may be caused by the higher dose (six apples d l^{-1}), or by the processing. The authors suggested that the blended form had the greatest effect because of its smaller particle size, which increased the surface area and hence the capacity to bind cholesterol in the gut.

TABLE II
Effect of apple products on total plasma cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels in humans[1]

Reference	Product and/or daily dose	Effects	Notes
(Gormley *et al*., 1977)	3 apples	TC: ↓max 8.1% LDL: – HDL: ↑12.5% TG: –	76 mildly hypercholesterolemic men, 4 months, parallel, matched according to cholesterol and fruit fibre intake
(Hyson *et al*., 2000)	340 g apple	TC: → LDL: NS↓ (5%) HDL: → TG: NS ↑ (10%)	25 healthy men and women, 6 weeks, unblinded randomised cross-over
(Sable-Amplis *et al*., 1983b)	2–3 apples (approx. 350 – 400 g)	Men: TC ↓ 8.6% Women: TC ↓ 11.7% LDL: NS↓ HDL: NS↑	30 hypercholesterolemic men and women, 1 month, intervention without control
(Girault *et al*., 1988)	3 apples	TC: ↓ 5.1%** LDL: ↓ 5.5%** HDL: ↓ 5.8%**	235 men and women (109 overweight or hyperlipidemic), 2 months, intervention without control
(Canella *et al*., 1963)	2 apples (approx. 150 – 180 g) after each meal as blended pulp, graded pulp or thin apple slices	TC: ↓ (blended pulp < graded pulp < apple slices) LDL: – HDL: – TG: –	100 hypercholesterolemic patients, 20 days, parallel
(Mahalko *et al*., 1984)	26 or 52 g of dried whole apple (equiv. to approx. 130 or 260 g fresh apple	TC: 26 g: NS ↓ (4.7%) 52 g: ↑ 6% LDL: 26 g: NS ↓ (8.3%) 52 g: ↑ 11% HDL/TG: → (both)	18 men and women with type 2 diabetes, 1 month, randomised cross-over
(Mayne *et al*., 1982)	15 g of dried whole apple (equiv. to approx. 75 g fresh apple	TC: → LDL: – HDL: → TG: →	12 men and women with type 2 diabetes, 7 weeks, intervention without control
(Hyson *et al*., 2000)	375 ml of filtered juice	TC: → LDL: → HDL: → TG: NS ↑ (13%)	25 healthy men and women, 6 weeks, unblinded randomised cross-over
(Davidson *et al*., 1998)	720 ml of filtered or fibre supplemented juice	TC: → LDL: → HDL: → TG: NS ↑ (15–34%)	85 hypercholesterolemic men and women, 18 weeks, randomised parallel
(Mee and Gee, 1997)	568 ml of filtered or fibre supplemented juice	TC: filtered: →, fiber supplemented: ↓ 8.3% LDL: filtered: →, fiber supplemented: ↓ 14% HDL: filtered: ↓ 9%, fiber supplemented: → TG: →	25 mildly hypercholesterolemic men, 6 weeks, randomised cross-over

[1]↓ or ↑ = Significant difference compared to control or baseline. → = No difference.
NS = non-significant. – = not measured. D, day; Wk, week; mo, month; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.
**only significant decrease in 63 subjects, who were hyperlipidemic. LDL- and HDL- cholesterol only measured in these hyperlipidemic subjects.

Two additional studies used a design without comparisons to controls, and consisted of a single intervention with supplementation of two-to-three apples a day (Girault *et al*., 1988; Sable-Amplis *et al*., 1983b). In the study by Sable-Amplis *et al*. (1983b), a total of 30 men and women were instructed to maintain their habitual diets, while adding apples as prescribed (Sable-Amplis *et al*., 1983b). Their cholesterol levels were measured before and after 1 month of intervention. The authors classified the subjects according to gender and, for both these groups, there was a significant reduction in total cholesterol (by 9 – 12%). The subjects were hypercholesterolemic, but heavy smokers or drinkers were excluded.

Cholesterol-lowering was also observed in the study by Girault *et al*. (1988). A total of 235 men and women participated in this study, and 109 of these subjects were overweight or hypercholesterolemic. Significant results were found only among the hypercholesterolemic subjects. In this group, the levels of total cholesterol and LDL, and also HDL cholesterol, decreased by 5 – 6 %. The intervention lasted 2 months, and there were no inclusion or exclusion criteria.

Whole dried apples: The effects of whole dried apples on lipid metabolism was investigated in two studies with subjects having type-2 diabetes (Mahalko *et al*., 1984; Mayne *et al*., 1982). The study by Mahalko *et al*. (1984) examined the effects of 26 g and 52 g of apple powder (equivalent to 130 or 260 g fresh apples d l^{-1}), respectively, on glucose metabolism and on fasting plasma lipids, in a randomised cross-over study. Eighteen subjects participated, and the subjects were considerably heterogeneous with regard to weight, initial cholesterol level, and chronic diseases. Both intervention periods lasted 4 weeks and, at biweekly intervals, the subjects filled in a 3-day food record and had their body weight measured. The lower dose of apple powder resulted in a non-significantly lower level of total cholesterol (by 5%) and LDL cholesterol (by 8%) compared to the control period. In contrast, the higher dose resulted in adverse effects. Total cholesterol and LDL cholesterol were significantly higher in the intervention period compared to the control period. The adverse effect of the higher dose of apple powder was probably related to the lack of any dietary control, which was supported by recorded weight increases in four of the subjects. Mayne *et al.* (1982) observed no effect of dried apple on plasma lipids. This study was conducted as one intervention period, without a control period, and the intervention consisted of daily additions of 15 g of freeze-dried apple powder (equivalent to approx. 75 g fresh apples) in the form of a slurry added to the subjects' habitual diet over 7 weeks. The 12 subjects had non-insulin-dependent diabetes and were mildly hypercholesterolemic. Individuals with other diagnoses were excluded. There was no substantial control of the diet. The lack of any effect on plasma lipids may be partially ascribed to the low intervention dose.

Apple juice: The effect of filtered apple juice on plasma lipid levels has been examined in three studies (Davidson *et al*., 1998; Hyson *et al*., 2000; Mee and Gee, 1997). Generally, these studies were well-designed and controlled with regard to dietary intakes, but none included a control group, or a period without apple products. No significant effects on plasma cholesterol or on triglycerides were observed after the consumption of filtered apple juice (375 – 720 ml d^{-1}) in any of these studies. A small, but significant decrease in HDL cholesterol from the baseline was observed in a 6-week cross-over study among 25 hypercholesterolemic men (Mee and Gee, 1997). This adverse effect on HDL cholesterol was not observed during a period in which the filtered apple juice was fortified with 10 g soluble fibre [1:1 (w/w) apple fibre and gum Arabic]. In contrast, fibre-fortification resulted in significant (by 10 – 14%) reductions in total and LDL cholesterol compared to the baseline values. These results suggest that the soluble fibre fraction of apples has beneficial effects on plasma cholesterol. Non-significant increases in triglycerides (< 34%) were demonstrated in two of the studies (Davidson *et al*., 1998; Hyson *et al*., 2000), and these increases occurred after the consumption of filtered, as well as fibre-supplemented apple juice (375 or 720 ml d^{-1}). Overall these studies indicate that the effects of whole apples on plasma cholesterol levels may be observable mainly in hypercholesterolemic subjects.

APPLE COMPOSITION AND CHOLESTEROL-LOWERING

Food analyses show that apples contain a wide palette of compounds (Table I), some of which have bio-activity in cell cultures, in animal feeding studies, and in humans. Pectin, phytosterols, and polyphenols are all known to interfere with lipid metabolism and, in apple, these components have the ability alone or in combination to affect plasma cholesterol levels.

Pectins: Pectin has good gelling properties and may decrease the enterohepatic recirculation of bile acids through complex formation. The increased excretion is then compensated-for by faster synthesis of bile acids at the expense of hepatic and plasma cholesterol (Kay, 1982). The cholesterol-lowering effect of pectin from various sources of fruit has been well-documented in human studies (Groudeva, 1997; Hillman *et al*,. 1985; Jenkins *et al*,. 1975; Judd and Truswell, 1982; Kay and Truswell, 1977; Keys *et al*., 1961; Palmer and Dixon, 1966; Stasse-Wolthuis *et al*., 1980), resulting in decreases in total cholesterol of between 6 – 35 %. No effects have been reported on HDL cholesterol or triglycerides, indicating that LDL or very-low-density lipoprotein (VLDL) are the main lipoprotein fractions affected . A minimum dose of 6 g pectin was necessary to demonstrate a significant effect on total cholesterol. This amount would correspond to an intake of seven-to-eight medium-sized apples, indicating that other factors in apples might also affect plasma cholesterol. No cholesterol-lowering effect of cellulose and hemicellulose has been documented. There is, however, limited evidence that the fermentation of apple fibres (soluble and insoluble) may cause a cholesterol-lowering effect through a reduction in hepatic cholesterol synthesis by SCFA. Hara *et al*. (1999) observed that rats fed on a SCFA mixture, in addition to a fibre-free diet, had lowered hepatic cholesterol synthesis. Aprikian *et al*., (2003) observed a significant increase in plasma concentrations of the SCFAs acetate, propionate, and butyrate when rats were fed on different apple products. It is not known whether these results can be extrapolated to humans, since the digestive tract differs between humans and rodents. For example, rodents have a larger caecum.

Sugars: Sucrose is hydrolysed rapidly to glucose and fructose in the stomach. Glucose is then absorbed into the small intestine by the sodium-dependent glucose transporter, SGLT1, but the food matrix can affect the rate of absorption to some extent. Whole fruit has a lower glycemic index (GI) than filtered juice and soft drinks, while the GI of cloudy juice lies between. Glucose metabolism leads to the formation of acetyl coenzyme A (CoA). Surplus acetyl CoA increases the formation of acetoacetyl CoA, thereby forming the building blocks for fatty acid biosynthesis as well as for mevalonate, the first compound in cholesterol biosynthesis. However, since the entry of glucose into the glycolytic pathway is limited at the phosphofructokinase step by feed-back regulation of cellular glucose uptake, glucose is not strongly lipogenic, *per se*. Fructose is absorbed and transported through enterocytes to the portal bloodstream by a facilitative fructose-specific hexose transporter, GLUT5 (Havel, 2005), which is much slower than SGLT1. Fructose is metabolised by an insulin-independent mechanism and cellular uptake of fructose is not controlled by a feed-back mechanism, since fructose bypasses the rate limiting step in glycolysis catalysed by phosphofructokinase (Havel, 2005). The breakdown products of fructose can then enter the citric acid cycle, or can serve as substrates for the formation of mevalonate and triglycerides. This means that the consumption of large and sustained amounts of fructose can facilitate hepatic cholesterol biosynthesis and the production of VLDL. This has been demonstrated in human dietary studies in which the consumption of high-fructose diets (especially drinks) has led to increased plasma triglyceride levels for several hours, post-prandially (Havel, 2005).

Phytosterols: Apple phytosterols can interfere with the intestinal absorption of cholesterol by their incorporation into micelles, leading to enhanced excretion of dietary and endogenous cholesterol (Plat and Mensink, 2005). It is well known that phytosterols, at a dose of 2 – 3 g d^{-1}, have a significant cholesterol-lowering effect in humans (Plat and Mensink, 2005). Apples have a relatively low content of phytosterols compared to the daily dose necessary for any effect. It can be calculated that it would require the consumption of at least 120 apples a day to achieve this dose of phytosterols; so, unless another factor is enhancing their effect, phytosterols may affect plasma cholesterol levels only marginally during ordinary levels of apple consumption.

Polyphenols: The low absorption of most apple polyphenols makes them available to interact with other indigestible apple components, or the intestinal contents. The effects of apple polyphenols on plasma lipids have been investigated in two randomised human placebo-controlled intervention studies, both conducted by the same research group (Nagasako-Akazome *et al*., 2005; 2007). In one of these studies, the highest dose of polyphenols (1,500 mg d^{-1}) resulted in a slight, but significant lowering of total cholesterol (by 5%) and LDL cholesterol (by 8%), but this did not happen after a lower intervention dose (300 or 600 mg d^{-1}; Nagasako-Akazome *et al*., 2005). In the more recent study, intervention with 600 mg d-1 resulted in a small but significant lowering of total cholesterol after 4 weeks (by approx. 0.2 mmol l^{-1}). These doses correspond to five-to-12 apples, a high daily level of consumption, indicating that polyphenols must interact with other factors in the fruit in order to be active at common levels of apple consumption.

EFFECTS OF APPLES ON CHOLESTEROL LEVELS IN ANIMAL STUDIES

The effects of apple products on cholesterol metabolism have been investigated in a number of studies using a hamster model (Sable-Amplis *et al*., 1983a; 1986; 1987). Hypercholesterolemic hamsters fed fresh apples showed decreased accumulation of hepatic cholesterol (by 23 – 67%), especially esterified cholesterol, in combination with decreased plasma levels of cholesterol (by 19 – 43%). This implies increased synthesis and removal (from the plasma) of cholesterol that might be due to an increased production of bile acids. Free cholesterol is the preferred substrate for 7 α-hydroxylase, which is the rate-limiting enzyme in bile acid synthesis. The activity of this enzyme was measured in one of these studies, and was enhanced after the intervention with fresh apples (Sable-Amplis *et al*., 1987). These effects on hepatic metabolism presumably result from an increased elimination of bile acids in the faeces. Bobek *et al*. (1990) also observed an increase in the amount of HDL cholesterol, with a simultaneous decrease in VLDL cholesterol This observation pointed to the enhanced removal of peripheral and plasma cholesterol. In the study by Sable-Amplis *et al*. (1983a), the hamsters showed decreased levels of cholesterol esters in their livers, as well as in their intestinal walls, when apple products were added to their diet. It is possible that this decrease in cholesterol esters was caused by a reduced activity of enzymes such as acyl-CoA:cholesterol acyltransferase 2 (ACAT2) in enterocytes, but the mechanisms are currently unclear.

Five studies have been conducted using rat models to examine the effects of different apple products on lipid metabolism (Aprikian *et al*., 2002; 2003; Leontowicz *et al*., 2001, 2002; Sembries *et al*., 2004). The apple products consisted mostly of dried apple (Aprikian *et al*., 2002; 2003; Leontowicz *et al*., 2002), but pomace (Leontowicz *et al*., 2001), colloids from apple extraction juice (Sembries *et al*., 2004), and apple fibres (Sable-Amplis *et al*., 1983a) were also used. In two studies, faecal excretion of cholesterol was measured and, in both cases, there was a significant increase (by 5 – 41%; Aprikian *et al*., 2003; Sembries *et al*., 2004). The effect of apple-feeding on faecal excretion of bile acids is ambiguous. Sembries *et al*. (2004) and Aprikian *et al*. (2003) observed a significant increase (by 3 – 56%), whereas another study by Aprikian *et al*. (2003) reported a decrease in bile acid excretion (by 50 – 97%). In the majority of these studies, there was also a simultaneous decrease in plasma (11 – 22%) and hepatic (26 – 27%) cholesterol after supplementation with apple products. Thus, in these rat models, the mechanism seems comparable to the one operating in hamsters, but there is limited evidence for an additional effect on cholesterol absorption.

The results from animal studies therefore indicate that the mechanism(s) behind the cholesterol-lowering effect of apple may relate to an effect on the enterohepatic recirculation of bile acids, or to an inhibition of cholesterol absorption. However, the effects observed cannot exclude decreased *de novo* biosynthesis of hepatic cholesterol, which could also be a potential mechanism. The individual contributions of apple components to the overall cholesterol-lowering effect is difficult to clarify, but soluble apple fibre may play a major role, as indicated by the dominant effect on the LDL fraction and the lack of an effect when filtered apple juice was consumed. At a physiologically realistic dietary intake of apples, the doses of bio-active apple components are relatively low, and their cholesterol-lowering effects are therefore most likely the result of synergies between apple components. Aprikian *et al*. (2003) carried out a study in mildly hypercholesterolemic rats that consumed a diet supplemented with either 5% apple pectin, 10% lyophilised apple rich in polyphenols, or a diet supplemented with both apple components (5% pectin and 10% lyophilised apple). The latter resulted in a significant reduction (24%) in total plasma cholesterol, whereas diets supplemented with either pectin or polyphenol-rich apple alone resulted in only small non-significant reductions. Besides the synergistic effect suggested between apple pectin and apple polyphenols, one can imagine synergistic and additive effects among the other phytochemicals present in apple and present in either of these two fractions.

Filtered apple juice had no cholesterol-lowering effect, and its adverse effects on plasma triglyceride levels (Davidson *et al*., 1998) may relate to a stimulation of hepatic lipogenesis by fructose (Havel 2005).

DISCUSSION

Based on current evidence from human observational and intervention studies, it seems likely that there is a reduction in total plasma and LDL cholesterol, and a considerable decrease in the risk of CVD, after a daily intake of two-to-six apples; however the response may be more evident in hypercholesterolemic subjects (Canella *et al*., 1963; Girault *et al*., 1988; Gormley *et al*., 1977; Hyson *et al*., 2000; Knekt *et al*., 2000; Sable-Amplis *et al*., 1983b; Sesso *et al*., 2003). A daily intake of approx. three apples resulted in a decrease in total cholesterol of 5 – 8% (approx. 0.5 mmol l^{-1}; Table II). Apples are therefore clearly a beneficial supplement to a heart-friendly diet. This dose is manageable, and a comparable effect may be achieved by a mixed dietary intake of other whole fruits, although the evidence-base for most single fruits is limited. The effect is possibly dependent on the form of intake (Canella *et al*., 1963). Processed apple products may not have an equal potential for cholesterol-lowering. The literature indicates that filtered apple juice causes an adverse effect on plasma triglyceride levels in experimental animals and in humans (Davidson *et al*., 1998; Hyson *et al*., 2000). Unfortunately, it was not possible to draw any conclusions about the effects of dried apple products, since these studies had an insufficient design. The beneficial effects of whole apples on plasma lipid levels are probably related to synergistic interactions between apple components (Aprikian *et al*., 2003). Evidence from animal studies suggests that the major mechanism behind the cholesterol-lowering effect of apple may involve an increased clearance of plasma cholesterol due to enhanced faecal excretion of bile acids and cholesterol. The adverse effects of filtered apple juice are most likely related to the lack of apple fibre and the high fructose content, in a highly bio-available form, causing hepatic lipogenesis (Havel, 2005).

In conclusion, there is a need for well-designed studies examining dietary apple intake. There is also a need for further studies investigating the different fractions of apple, including different juices, apple puree and pomace, as well as dried apple. In particular, it would be important to examine whether cloudy apple juice had a less adverse effect on plasma lipid levels compared to clear juice. Moreover, to be able to establish the mechanism behind the cholesterol-lowering effect of apples, human studies investigating faecal excretion and the enzymes involved in lipid metabolism should be performed. However, since several animal models seem to show cholesterol-lowering, the mechanisms might be worked out first in these models, then verified in human trials.

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