REVIEW

Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms

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Proanthocyanidins are the most abundant polyphenols in human diets. Epidemiological studies strongly suggest that proanthocyanidins protect against cardiovascular diseases. Despite the antioxidant and anti-inflammatory properties of these flavonoids, one of the mechanisms by which proanthocyanidins exert their cardiovascular protection is improving lipid homeostasis. Animal studies demonstrate that proanthocyanidins reduce the plasma levels of atherogenic apolipoprotein B-triglyceride-rich lipoproteins and LDL-cholesterol but increase antiatherogenic HDL-cholesterol. The results in humans, however, are less clear. This review summarizes the results that have been published on plasma triglyceride, apolipoprotein B, HDL-cholesterol and LDL-cholesterol levels in humans and animal models in response to proanthocyanidin extracts and proanthocyanidin-rich foods. The physiological processes and biochemical pathways that are related to lipid homeostasis and affected by proanthocyanidin consumption are also discussed. Intestinal lipid absorption, chylomicron secretion by the intestine and VLDL secretion by the liver are the processes that are most repressed by proanthocyanidins, which, therefore, induce hypolipidemic effects.

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1 Introduction

Proanthocyanidins are a class of polyphenolic compounds that are one of the most ubiquitously distributed groups of plant secondary metabolites, which makes them important in human diet. Proanthocyanidins are considered to be bioactive compounds because they influence physiological and cellular processes and, therefore, can have an effect on health. Proanthocyanidins have been described as antimicrobial compounds, antioxidants, anti-cancer agents and anti-inflammatory agents with cardioprotective properties [1–10].

This review focuses not only on the hypolipidemic effects of proanthocyanidins, but also on their basic biochemical and molecular mechanisms, one of the less well-known aspects of these compounds that may be an important factor in the protective effect of proanthocyanidins against cardiovascular diseases (CVD).

2 General characteristics, sources and bioavailability of proanthocyanidins

Proanthocyanidins, which are also known as condensed tannins, are the oligomeric or polymeric forms of flavan-3-ols or flavanols. They are the most structurally complex subclass of flavonoids, are one of the main constituents of the phenolic intake in the diet and are mainly provided by fruits, beans, nuts, cocoa, tea and wine. Proanthocyanidins are known as such because, when heated in acidic alcohols, they produce red anthocyanidin pigments. Unlike other

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Abbreviations: CPT1, carnitine palmitoyltransferase 1; **CVD,** cardiovascular diseases; **DP,** degree of polymerization; **FXR,** farnesoid X receptor; **HMG-CoA,** 3-hydroxy-3-methyl-glutaryl-CoA; **LPL,** lipoprotein lipase; **MTP,** microsomal triglyceride transfer protein; **SHP,** small heterodimer partner; **SREBP,** steroid response element binding protein; **TG,** triglyceride

classes of flavonoids, which exist in plants primarily in glucoside forms, flavanols are usually present in the aglycone form as monomers and oligomers or esterified with gallic acid [11]. The basic structural skeleton of flavonoids, and therefore of proanthocyanidins, comprises 15 carbons, with two aromatic rings (A and B) connected by a pyrone ring (C). The benzenoid B ring of the flavan-3-ols is in the 2-position as occurs with flavanones and flavonols. However, the C ring of the flavan-3-ols does not have a carbonyl group in the 4-position or a double bond in the 2-3-position, unlike flavonols and isoflavones. The most studied proanthocyanidins are based on the flavan-3-ols (+)-catechin and (−)-epicatechin. Other important flavan-3-ols are (+)-gallocatechin, (−)-epigallocatechin, and (−)-epigallocatechin gallate. Proanthocyanidins can have a variety of structures due to: (i) different chain lengths (this is known as degree of polymerization (DP)); (ii) different hydroxylation patterns; (iii) different stereochemistries at the three chiral centers and (iv) different locations and types of interflavan linkage [12]. Proanthocyanidins are classified according to their hydroxylation pattern into several subgroups, including procyanidins (3,5,7,3′,4′-OH), prodelphinidins (3,5,7,3′,4′,5′-OH), propelargonidins (3,5,7,4′-OH), profisetinidins (3,7,3′,4′-OH), prorobinetinidins (3,7,3′,4′,5′-OH), proguibourtinidins (3,7,4′-OH), proteracacinidins (3,7,8,4′-OH) and promelacacinidins (3,7,8, 3′,4′-OH) [13]. Procyanidins are the most common group of naturally occurring proanthocyanidins. Flavan-3-ols are nonplanar by virtue of their saturated C3 element. The two chiral centers at C2 and C3 of the flavan-3-ols produce four isomers for each level of B-ring hydroxylation, two of which, (+)-catechin and (−)-epicatechin are widespread in nature whereas (−)-catechin and (+)-epicatechin are comparatively rare [14]. Oligomeric and polymeric proanthocyanidins have one additional chiral centre at C4 of each additional flavan-3-ol unit. These differences in chirality have a significant effect on the 3-D structure of the molecules, and they can be expected to have an effect on the binding properties to proteins [15]. B-type (dimeric) and C-type (trimeric) procyanidins are characterized by single linked flavanyl units, usually between C-4 of the flavan-3-ol upper unit and C-6 or C-8 of the lower unit [13].

Quantitative information on the content of proanthocyanidins in plant products is available in several articles and databases (for example, of the United States Department of Agriculture (USDA)) [16]. The phenolic content of fruit, vegetables and other plant foods varies considerably, not only between different types but also between cultivars of the same type and can even depend on growing conditions and the time of harvest [17]. In addition, food processing can significantly influence the total content of proanthocyanidins and the profile of monomers and oligomers, both favorably and unfavorably [11]. In an excellent review, Crozier *et al.* (2009) reported that proanthocyanidins can occur as polymers of up to 50 units [15]. However, the proanthocyanidin DP of a particular plant or food depends on the moment at which it is consumed. The mean DP in grapes varies between 9.8 and 31.5, and in red wines between 4.8 and 22.1, indicating that there are substantial changes in flavan-3-ol composition during the fermentation and aging of wines [15]. In fresh cocoa beans, oligomers range from dimers to decamers, but during fermentation and processing many of the phenolic components precipitate and, as a result, the proanthocyanidin content of commercial cocoas and chocolate varies considerably. In tea, although monomers are the dominant form, at least 27 proanthocyanidins also occur [15]. Apples and pears are among the main sources of proanthocyanidins in the diet. In apples the average DP is between 3.1 and 8.5 [17] and in some varieties of pears as high as 44 [18]. As far as is known, proanthocyanidins do not occur frequently in vegetables, except in beans, which can contain in excess of 5 g/kg of proanthocyanidins [19]. Although nuts are consumed in small quantities, they often contain quite high concentrations of proanthocyanidins: for example, hazelnuts and pecans are particularly rich with *ca.* 5 g/kg, whereas almonds and pistachios contain 1.8–2.4 mg/kg, walnuts *ca.* 0.67 g/kg and roasted peanuts *ca.* 0.16 g/kg [15]. However, the content of proanthocyanidins in foods could be largely underestimated because it is usually determined by analyses of aqueous-organic extracts [20], and a large amount of proanthocyanidins could remain undissolved during the extraction procedure. Some authors have quantified non-extractable proanthocyanidins in plant foods [21, 22]. This information is of importance because even though they will probably not be absorbed in the small intestine when they are ingested, they will be substrates of the metabolism of the bacterial flora in the colon and their metabolites may be able to explain some of the effects that proanthocyanidins have been reported to have on health.

Proanthocyanidins are believed to exert their biological effects in different ways: as unabsorbable, complex structures with binding properties that can have local effects in the gastrointestinal tract, as absorbable proanthocyanidins (probably low molecular-weight) and as absorbable metabolites from the colonic fermentation of proanthocyanidins that may have systemic effects in various organs [9]. Although long proanthocyanidins are absorbed less efficiently than short proanthocyanidins in the small intestine, they may have important local functions in the gut [23], neutralizing oxidants and carcinogenic compounds [24], exhibiting immunomodulatory, and anti-inflammatory properties [25, 26] and showing antibacterial activity toward pathogens [27]. Intestinal bacteria that are resistant to the antibacterial properties of proanthocyanidins have also been reported [9]. Also, during small intestine digestion, higher proanthocyanidins can form complexes with starch and proteins, resulting in the formation of less digestible complexes [9], and proanthocyanidins can inhibit gastrointestinal lipase activity. Similarly, polymeric proanthocyanidins influence the absorption of proanthocyanidin oligomers [28]. In experimental animals, various combinations of methylated, glucuronidated and sulfated

derivatives of flavan-3-ols, as well as native monomers, dimers and trimers, have been detected in body fluids and tissues after the ingestion of proanthocyanidins [29–33]. Recently, an improved liquid chromatography-tandem mass spectrometry method [34] has been used to analyze rat plasma obtained 2 h after the ingestion of 1 g of grape seed procyanidin extract *per* kilogram of body weight. Conjugated forms were identified and quantified, and their concentrations were respectively: catechin and epicatechin glucuronide, 23.90 and 20.57 μM; catechin and epicatechin methyl glucuronide, 13.75 and 9.06 μM; and catechin and epicatechin methyl-sulfate, 1.05 and 1.30 μM. Moreover, monomers (catechin and epicatechin), dimers and trimers in their native form were detected and quantified in plasma samples, and their concentrations were, respectively, 0.85, 1.28, 2.40 and 8.55 μM. Thus, flavan-3-ols exist in plasma predominantly in their modified forms, as has already been described, although intact molecules are also found at micromolar level. As regards oligomer metabolization, two *in vivo* studies have reported the presence of highly methylated dimers in plasma and tissues [35] and in urine [32] after proanthocyanidin uptake. In humans, the detection of dimers B1 and B2 in plasma has been reported in two studies in which the volunteers consumed approximately 2 g of procyanidins [36, 37]. In another study, procyanidin B2 has been detected in human plasma and urine after cocoa consumption [33]. As mentioned above, proanthocyanidins are thought to be poorly absorbable and highly metabolized by gut microflora before absorption [9]. Only a few species of microorganisms involved in the colonic fermentation of polyphenols have been identified. The *Clostridium* and *Eubacterium* genera, which are phylogenetically associated, are common elements in the metabolism of several phenolic compounds, although the transformation of native phenols into their metabolites depends on the particular microflora of each individual [38]. To date, only a few studies have been performed on crude extracts that are rich in proanthocyanidins but also contain a multitude of other phenolic compounds to reveal human microbial proanthocyanidin metabolites. Therefore, the origin of the metabolites remains uncertain. A recent study by Appeldoorn *et al.* [39] included *in vitro* fermentation of purified procyanidin dimers with human microbiota. Monomeric flavan-3-ols were not found as microbial metabolites of procyanidins, and the most abundant metabolite was 2-(3,4-dihydroxyphenyl)acetic acid. As 2-(3,4-dihydroxyphenyl)acetic acid was not found as a common human metabolite in studies performed with pure monomeric flavan-3-ols [40, 41], Appeldoorn and co-workers proposed that dimers degraded directly instead of being cleaved into flavan-3-ols first.

3 Effects of proanthocyanidins on lipid homeostasis

Proanthocyanidins affects lipid metabolism and has important positive consequences on CVD. Some reviews on flavonoids and proanthocyanidins and their biological action corroborate this hypothesis [4, 7, 8, 42–46]. These reviews conclude that consumption of flavan-3-ol rich foods such as grapes, wine, berries, apples, chocolate and teas has an antiatherogenic activity, thus not only reducing plasma levels of apolipoprotein B (ApoB)-containing triglyceride (TG)-rich proatherogenic lipoproteins (*i.e.* intestinal chylomicrons and hepatic VLDL and LDL) but also improving the serum cholesterol profile.

Since 1994 several studies have shown the beneficial effects of proanthocyanidins in atherosclerosis prevention: both plasma ApoB and TG levels are reduced, suggesting that the liver produces considerably less VLDL.

Studies with animal models demonstrate that proanthocyanidins have a positive effect on the TG metabolism since they significantly reduce plasma TGs especially if the concentration of TGs is unusually elevated. Results obtained by different authors indicate that proanthocyanidins inhibit VLDL secretion by the liver. Table 1 summarizes the state of the art of plasma TGs and VLDL production, and it seems that oligomeric forms of proanthocyanidins specifically control the endogenous liver lipid production.

Nevertheless, the effect on the TG metabolism is less clear in the case of human subjects. Several authors (see Table 2) conclude that the intake of proanthocyanidins may be positive, neutral or negative. These contradictory conclusions may be explained by different doses and, principally, the different composition of extracts administered. More studies must be done to determine the dose and type of proanthocyanidins that are most effective at improving hypertriglyceridemia.

As far as plasma total cholesterol and its distribution in lipoproteins is concerned, several studies in animals and human subjects have reported a significant reduction in plasma total cholesterol, a clear reduction in LDL-cholesterol and an increase in HDL-cholesterol after supplementation with foods containing polyphenols, basically with proanthocyanidins. The results of these studies are summarized in Tables 3 and 4. Nevertheless, not all studies carried out in animals or in humans have reported the same effect, especially if the dose administered is low or not rich in proanthocyanidins.

4 Effects of proanthocyanidins on physiological processes and biochemical pathways related to lipid and lipoprotein metabolism

Lipid and lipoprotein levels in blood are the consequence of many biochemical and physiological processes, each one of which is finely regulated. Particular mention should be made of lipid digestion, absorption and chylomicron synthesis in the intestine, VLDL production in the liver, TG uptake by extra-hepatic tissues, uptake of LDL and remnant-like lipoprotein by the liver and HDL metabolism and cholesterol elimination from the body. The main problem in

Table 1. Effects of proanthocyanidins on TG metabolism in animal models

Animal	Treatment	Extract composition	Dose	Effect	Authors
Rats	Chronic administration of grape seed tannins and hypercholesterolemic diet	Grape seed tannins in: dimeric, trimeric and tetrameric proanthocyanidin forms Monomeric proanthocyanidin forms	2% proanthocyanidins in food pellets for 9 wk	Decrease in plasma TG to control values Decrease in VLDL concentration Decrease in TG in aorta With monomers, slow decrease in TG and VLDL	[59]
Rats	Chronic administration of wine rich in flavonols	Red wine flavonols Dealcoholized red wine flavonols	80 mL/day/kg w/w for 10 wk	Increase in TG associated to red wine consumption.	[104]
Hamster	Chronic administration of red wine to animals on a high-cholesterol diet	Red wine flavonols Dealcoholized red wine flavonols	50–189 mg of catechin equivalents/kg of body weight for 10 wk	Decrease in plasma TG	[105]
Hamsters	Chronic administration of red wine phenolic extract and normal diet	Red wine phenolic extract, containing proantho-cyanidins momoners and dimers, anthocyanins, and phenolic acids	30.4 mg PE/kg of body wt *per* day for 8 wk	Decrease in plasma TG Decrease in ApoB	[106]
Rats	Chronic administration of grape seed proanthocyanidin extract in normolipidemic and hyperlipdemic rats	Grape seed proanthocyanidin extract	Between 0.05 and 1 g/kg of body wt *per* day for 4 wk	Decrease in plasma TG	[107]
Hamster	Chronic administration of a grape seed extract with a hypercho-lesterolemic diet	Grape seed proantho-cyanidin extract	50 and 100 mg/kg for 10 wk	Important reduction in TG at higher doses	[108]
Hamster	Chronic administration of grape seed proanthocyanidin extracts and atherogenic diet	Grape seed proantho-cyanidin extracts rich in monomeric /dimeric forms	18.4 mg of GSE/kg of body wt *per* day	Plasma ApoB concentrations did not differ	[109]
Rats	Acute administration of grape seed proanthocyanidin extract in normolipidemic rats	Grape seed tannins in monomeric, dimeric, trimeric and oligomeric proanthocyanidin forms	5 h after administration of 250 mg/kg body wt	Decrease in plasma TG Decrease in ApoB	[72]
Mouse		Grape seed tannins in monomeric,	5 h after administration of 250 mg/kg body wt	Decrease in plasma TG	[66]

Table 1. Continued

Animal	Treatment	Extract composition	Dose	Effect	Authors
	Acute administration of grape seed proanthocyanidin extract in normolipidemic mouse	dimeric, trimeric and oligomeric proanthocyanidin forms		Decrease in ApoB	
Guinea pigs	Chronic administration of grape powder in ovariectomized guinea pigs	Grape polyphenols	10% polyphenols in solid food for 12 wk	Decrease in plasma TG Decrease in VLDL cholesterol	[110]
Mouse	Chronic administration of proanthocyanidins from persimmon peel extract in genetically diabetic C57BL/Ksj-*db*/*db* mice	Oligomers and polymers of proanthocyanidins from persimmon peel	10 mg/kg of body wt *per* day for 6 wk	Decrease in plasma TG to control values Reduction in liver TG	[97]
Rats	Chronic administration of red wine polyphenol extract in genetically obese Zucker fa/fa rats	Wine polyphenols	20 mg/kg of body wt *per* day for 8 wk	Decrease in plasma TG to control values	[111]
Rats	Chronic administration of grape seed proanthocyanidin extract in obese rats fed with a cafeteria diet for 13 wk	Grape seed tannins in monomeric, dimeric, trimeric and oligomeric proanthocyanidin forms	25 mg/kg of body wt *per* day for 10 days	Decrease in plasma TG to control values Repression of the expression of hepatic regulators of VLDL assembling such as SREBP1, MTP and DGAT2	[69]
Rats	Chronic administration of grape seed proanthocyanidin extract in hyperlipemic rats	Proanthocyanidins	100 mg/kg body weight for 6 wk	Reduction in levels of TGs	[112]

Table 2. Effects of proanthocyanidins on TG metabolism in human intervention studies

Treatment	Ingredient composition	Dose *per* day	Days	Subjects	Effect	Authors
Milk chocolate	Cocoa polyphenols	46 g	27	Forty-two normocholesterolemic men	Reduction in plasma TG	[113]
Wine	Red and white wine polyphenols	550 mL/day	28	Twenty-four healthy men	Total TG concentrations in plasma did not change	[114]
Purple grape juice	Wine polyphenols	7.7 mL/kg	14	Fifteen adults with angiographically documented coronary artery disease	Increase in plasma TG	[115]
Red wine	Red and white wine polyphenols	375 mL/day	14	Eighteen male smokers	Total TG levels were unaffected	[116]
Purple grape juice	Wine polyphenols	4.6–8 mL/kg	56	Eleven adults	Total TG levels were unaffected	[117]
Cocoa powder/dark chocolate	Proanthocyanidins, catechins	466 mg proanthocyanidins	28	Twenty-three healthy subjects	No changes in plasma TGs	[118]
Grape seed extract	Rich in proanthocyanidins	2 × 300 mg/day	Long term	Seventeen healthy and hypercholesterolemic	TG decrease to the normal range	[105]
Purple grape Juice	Wine polyphenols	4.6–8 mL/kg	56	Eleven adults	No modification in plasma TG	[117]
Lyophilized grape powder	Flavans, anthocyanins, quercetin, myricetin, kaempferol and resveratrol	36 g	28	Forty-four menopausal women	Reduction in plasma TG and ApoB	[119]
Concentrated red grape juice	Total polyphenols, 0.64 g/100 mL	50 mL twice a day	14	Twenty-six hemodialysis patients with slight hypertriacylglycerolemia	No modification in plasma TG	[120]
Dark chocolate	Cocoa polyphenols	30 mg *per* day.	18 wk	Forty-four with untreated upper-range prehypertension	No modification in plasma TG	[121]
Grape antioxidant dietary fiber	1400 mg of polyphenols	7.5 g	16 wk	Thirty-four hypercholesterolemic subjects	Reduction in plasma TG	[122]
Concentrated red grape juice plus vitamin E	Total polyphenols, 0.64 g/100 mL	50 mL twice a day	14	Thirty-two hemodialysis patients with slight hypertriacylglycerolemia	No modification of plasma TG	[123]
Modified Mediterranean-style diet	Low glycemic load diet with soy protein, phytosterols, *rho* iso-α acids and proanthocyanidins	60 mg/day proanthocyanidins	12 wk	Forty-nine subjects with metabolic syndrome and hypercholesterolemia	Reduction in plasma TG	[124]
Grape seed extract	Rich in proanthocyanidins	600 mg	28	32 type 2 diabetes mellitus patients	No modification of plasma TG	[125]

Table 3. Effects of proanthocyanidins on cholesterol metabolism in animal models

Animal	Treatment	Extract composition	Dose	Effect	Authors
Rats	Chronic administration of grape seed tannins and hypercholesterolemic diet	Grape seed tannins in dimeric, trimeric and tetrameric proanthocyanidin forms Monomeric proantho-cyanidin forms	2% proanthocyanidins in food pellets for 9 wk	Dietary grape seed tannins have a pronounced anti-hypercholesterolemic effect by enhancing reverse cholesterol transport and also by reducing intestinal cholesterol absorption and increasing bile acid excretion	[59]
Rats	Chronic administration of grape seed tannins and hypercholesterolemic diet	Grape seed tannins in dimeric, trimeric and tetrameric proanthocyanidin forms Monomeric proantho-cyanidin forms	2% proanthocyanidins in food pellets for 9 wk	Polymeric grape seed tannins exert a hypocholestero-laemic effect in high-cho-lesterol-fed rats	[126]
Rats	Chronic administration of wine and grape seed polyphenols	Red wine 0.025% grape seed extract 03% wine phenolic extract	*Ad libitum* in beverage for 2 wk	No changes in cholesterol or HDL	[127]
Mouse	Chronic administration of wine or proanthocyanidin monomers in ApoE-deficient mice	Red wine Catechin or quercetin	Catechin or quercetin (50 μg/day *per* mouse) for 6 wk Red wine (0.5 mL/day *per* mouse) for 6 wk	No changes in LDL or HDL-cholesterol	[128]
Rats	Chronic administration of grape and apple pomace in rats fed a cholesterol diet (0.3%)	Polyphenols from grape and apple pomace	5% in diet	No changes in serum cholesterol levels Apple pomace reduced cholesterol level in liver and in heart Grape pomace reduced cholesterol level in heart Cholesterol absorption was not affected Reduction in the activity of HMG-CoA reductase in liver	[129]
Rats	Chronic administration of dietary fibers rich in polyphenols in hypercholesterolemic rats	Rich in condensed tannins	10 g/100 g for 4 wk	Decrease in serum total cholesterol and LDL-cholesterol Increase in HDL-cholesterol	[130]
Rabbits	Chronic administration of grape seed extracts in cholesterol-fed rabbits	Proanthocyanidin-rich extracts	0.1% and 1% in diets [w/w] for 8 wk	No changes in serum lipid profile	[131]

Table 3. Continued

Animal	Treatment	Extract composition	Dose	Effect	Authors
Rats	Chronic administration of wine and grape polyphenols in hypercholesterolemic rats	Red grape peels, white Grape peels and white grape seeds	100 g/Kg diet for 42 days	Decrease in serum total cholesterol and LDL-cholesterol Increase in HDL-cholesterol	[132]
Rats	Chronic administration of wine rich in flavonols	Red wine flavonols Dealcoholized red wine flavonols	80 mL/day/kg w/w for 10 wk	No changes in total cholesterol Increase in HDL-cholesterol	[104]
Rats	Chronic administration of red wine	Red wine polyphenols	*Ad libitum* for 6 months	Consumption of wine did not influence plasma cholesterol or HDL-cholesterol At 45 days the consumption of wine reduced LDL-cholesterol and VLDL cholesterol At 6 months the rats that consumed wine had reduced LDL-cholesterol	[133]
Hamster	Chronic administration of red wine fed with a high cholesterol diet	Red wine flavonols Dealcoholized red wine flavonols	50–189 mg of catechin equivalents/kg of body weight for 10 wk	Decrease in plasma total cholesterol, LDL and HDL-cholesterol	[134]
Hamster	Chronic administration of a grape seed extract with a hypercholesterolemic diet	Grape seed proanthocyanidins	50 mg/kg and 100 mg/kg for 10 wk	Slow reduction in total cholesterol	[108]
Hamsters	Chronic administration of red wine phenolic extract and normal diet	Red wine phenolic extract, containing proanthocyanidins momoners and dimers, anthocyanins and phenolic acids	30.4 mg PE/kg of body wt *per* day for 8 wk	Reduction in total plasma cholesterol Apo A-1 was not affected	[106]
Rats	Chronic administration of grape seed proanthocyanidins extract in normolipidemic and hyperlipdemic rats	Grape seed proanthocyanidins	Between 0.05 and 1 g/kg of body wt *per* day for 4–5 wk	No significant alterations in cholesterol metabolism were found	[107]
Guinea pigs	Chronic administration of grape powder in ovariectomized guinea pigs	Grape polyphenols	10% polyphenols in solid food for 12 wk	No changes in LDL-cholesterol Less accumulation of cholesterol in the aorta Reduced secretion of LDL particles by kidney	[110]
Hamster	Chronic administration of grape seed proanthocyanidin extracts and atherogenic diet	Grape seed proantho-cyanidins extracts rich in monomeric/dimeric forms	18.4 mg of GSE/kg of body wt *per* day for 12 wk	Reduction in total plasma cholesterol	[109]

Table 3. Continued

Animal	Treatment	Extract composition	Dose	Effect	Authors
Hamster	Chronic administration of red wine and atherogenic diet	White wine enriched with polyphenols Red wine polyphenols	7.14 mL/kg of body wt *per* day for 12 wk	Reduction in total plasma cholesterol No changes in HDL-cholesterol Increase in the ApoA-1/ApoB ratio	[135]
Rats	Acute administration of grape seed proanthocyanidin extract in normolipidemic rats	Grape seed tannins in monomeric, dimeric, trimeric and oligomeric proantho-cyanidin forms	5 h after administration of 250 mg/kg body wt	Proanthocyanidins decreased free fatty acids, ApoB, LDL-cholesterol and non-HDL:non-LDL-cholesterol levels and slightly increased HDL-cholesterol	[72]
Rats	Chronic administration of apple proanthocyanidin extracts and hypercholesterolemic diet	85% catechin oligomers	0.2–1% proantho-cyanidins in solid diet for 30 days	Decrease in serum cholesterol level and increase in HDL-cholesterol Increase in the activity of hepatic cholesterol 7a-hydroxylase Dose-dependent increase in the fecal excretion of neutral steroids	[60]
Rabbits	Chronic administration of grape juice and hypercholesterolemic diet	Grape juice rich in polyphenolics	225 mL/day for 48 and 96 days	Decrease in total serum cholesterol at 96 days	[136]
Mouse	Acute administration of grape seed proanthocyanidin extract in normolipidemic mice	Grape seed tannins in monomeric, dimeric, trimeric and oligomeric proanthocyanidin forms	5 h after administration of 250 mg/kg body wt	No changes in total cholesterol plasma levels	[66]
Mouse	Chronic administration of proanthocyanidins from persimmon peel extract in genetically diabetic C57BL/Ksj-*db*/*db* mice	Oligomers and polymers of proanthocyanidins from persimmon peel	10 mg/kg of body wt *per* day for 6 wk	Decrease in total cholesterol, and nonesterified fatty acids to control values	[97]
Rats	Chronic administration of red wine polyphenol extract in genetically obese Zucker fa/fa rats	Wine polyphenols	20 mg/kg of body wt *per* day for 8 wk	Reduction in total cholesterol levels, as well as the ratio between LDL- and HDL-cholesterol to control values	[111]
Rats	Chronic administration of grape seed proanthocyanidin extract in obese rats fed with cafeteria diet for 13 wk	Grape seed tannins in monomeric, dimeric, trimeric and oligomeric proanthocyanidin forms	25 mg/kg of body wt *per* day for 10 days	Proanthocyanidins normalized plasma LDL-cholesterol	[69]
Rats	Chronic administration of grape seed proanthocyanidin extract in hyperlipemic rats	Proanthocyanidins	100 mg/kg body weight for 6 wk	Reduction in levels of total cholesterol	[112]

Table 4. Effects of proanthocyanidins on cholesterol metabolism in human intervention studies

Treatment	Ingredient composition	Dose *per* day	Days	Subjects	Effect	Authors
Milk chocolate	Cocoa polyphenols	46 g	27	Forty-two normocholesterolemic men	Increased HDL-cholesterol; LDL not affected	[113]
Wine	Red and white wine polyphenols	550 mL/day	28	Twenty-four healthy men	Total cholesterol, LDL-cholesterol and HDL-cholesterol concentrations in plasma did not change	[114]
Red wine	Proanthocyanidins, anthocyanins, quercetin	375 mL	14	Nine healthy men	No changes in LDL- or HDL-cholesterol	[137]
Purple grape juice	Wine polyphenols	7.7 mL/kg	14	Fifteen adults with coronary artery disease	LDL lag time increased	[115]
Pomegranate juice	Polyphenol mixture	1.5 mmol total polyphenols	14	Ten hypertensive patients	No changes in total cholesterol, LDL-cholesterol	[138]
Red wine	Red wine polyphenols	190 ml red wine 5 days/wk	140	Twenty moderately obese women (BMI 29.8)	Blood lipids remained unchanged	[139]
Grape seed extract	Proanthocyanidins	100 mg	60	Forty hypercholesterolemic subjects	Decrease in circulating auto-antibodies to oxidized LDL No changes in LDL/HDL-cholesterol	[140]
Red wine	Red and white wine polyphenols	375 mL/day	14	Eighteen male smokers	Total cholesterol, LDL-cholesterol and HDL-cholesterol levels were unaffected	[116]
Purple grape juice	Wine polyphenols	4.6–8 mL/kg	56	Eleven adults	Total cholesterol, LDL-cholesterol and HDL-cholesterol levels were unaffected	[117]
Purple grape juice	Wine polyphenols	7 mL/kg/day	14	Twenty healthy subjects	No changes in total plasma cholesterol	[141]
Grape seed extract	Rich in proanthocyanidins	2 × 300 mg/day	Long term	Seventeen healthy and hypercholesterolemic	Decrease in plasma cholesterol, LDL-cholesterol and HDL-cholesterol concentrations in subjects with high cholesterol	[134]
Cocoa powder/dark chocolate	Proanthocyanidins, catechins	466 mg proanthocyanidins	28	Twenty-three healthy subjects	LDL oxidation lag time decreased by 8%; HDL-cholesterol 4% higher	[118]
Extract of *Pinus pinaster*	Procyanidins, some (+)-catechin	150 mg	42	Twenty-five healthy subjects	Decrease in LDL-cholesterol; increase in HDL-cholesterol	[142]

Table 4. Continued

Treatment	Ingredient composition	Dose *per* day	Days	Subjects	Effect	Authors
Cocoa	Proanthocyanidins, catechins	500 mg polyphenols	14	Thirteen healthy subjects	No changes in plasma cholesterol	[143]
Grape polyphenol extract	Proanthocyanidins	300 mg	28	Twenty-four male smokers	Increase in LDL oxidation lag phase No changes in HDL, LDL or total cholesterol	[144]
Chocolate	Polyphenols	7 5g/day	3 wk	Forty-five nonsmoking, healthy subjects	Increase in HDL-cholesterol	[145]
Chocolate	Flavonoids	100 g/day	15	Twenty never-treated, grade I patients with essential hypertension	Reduction in serum total and LDL-cholesterol levels	[146]
Red wine	Polyphenols	300 mL/day	28	Sixty-nine healthy subjects	No changes in HDL-cholesterol	[147]
Red wine	Polyphenols	375 mL/day	14	Twenty healthy subjects	Reduction in LDL and increase HDL-cholesterol	[148]
Lyophilized grape powder	Flavans, anthocyanins, quercetin, myricetin, kaempferol and resveratrol	36 g	28	Forty-four menopausal women	Reduction in LDL-cholesterol	[119]
Red wine	Polyphenols	250 mL/day	28	Forty-eight healthy subjects	Decrease in LDL/HDL ratio Increase in HDL-cholesterol	[149]
Concentrated red grape juice	Total polyphenols, 0.64 g/100mL	50 mL twice a day	14	Twenty-six hemodialysis patients with slight hypertriacylglycerolemia	Decrease in LDL-cholesterol and ApoB-100 concentrations, and increase in the concentrations of HDL-cholesterol and apolipoprotein A-I	[120]
Wine	Polyphenols	300 mL/day	15	Twenty healthy subjects	Reduction in LDL-cholesterol	[150]
Red wine	Polyphenols	400 mL/day	42	Forty-five hypercholesterolemic postmenopausal women	Reduction in fasting LDL-cholesterol concentrations and increase in HDL-cholesterol concentrations	[50]
Cocoa	Flavanol-rich cocoa	Up to 26 g/day	28	One hundred and sixty normo and hypercholesterolemic patients	Decrease in plasma levels of LDL and oxidized LDL and	[151]

Table 4. Continued

Treatment	Ingredient composition	Dose *per* day	Days	Subjects	Effect	Authors
					increase in HDL serum concentrations	
Cocoa	Flavanol-rich cocoa	26 g/day	12 wk	Twenty-five normo and mildly hypercholesterolemic patients	Increase in HDL-cholesterol	[152]
Dark chocolate	Cocoa polyphenols	30 mg *per* day	18 wk	Forty-four with untreated upper-range prehypertension	No changes in plasma lipids profile	[121]
Concentrated red grape juice plus vitamin E	Total polyphenols, 0.64 g/100 mL	50 mL twice a day	14	Thirty-two hemodialysis patients with slight hypertriacylglycerolemia	Reduction in LDL-cholesterol. Increase in HDL-cholesterol	[123]
Grape antioxidant dietary fiber	1400 mg of polyphenols	7.5 g	16 wk	Thirty-four hypercholesterolemic subjects	Reduction in LDL-cholesterol	[122]
Modified Mediterranean-style diet	Low glycemic load diet with soy protein, phytosterols, *rho* iso-α acids and proanthocyanidins	60 mg/day proanthocyanidins	12 wk	Forty-nine subjects with metabolic syndrome and hypercholesterolemia	Reduction in cholesterol, non-HDL-cholesterol, cholesterol/HDL and TG/HDL	[124]
Grape seed extract	Rich in proanthocyanidins	600 mg	28	Thirty-two type 2 diabetes mellitus patients	Decrease in total plasma cholesterol No changes in HDL-cholesterol	[125]

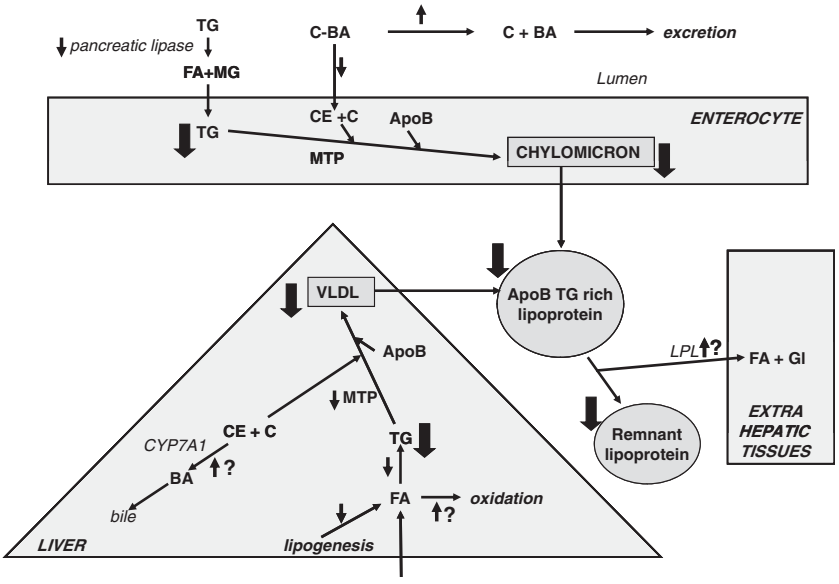

Figure 1. Reported effects of proanthocyanidins on physiological processes and biochemical pathways related to lipid and lipoprotein metabolism. Big arrows indicate reduced lipid or lipoprotein levels. Small arrows indicate changes in process rate. C: cholesterol, CE: cholesteryl esters, BA: bile acids, FA: fatty acids, MG: monoglycerides, GI: glycerol.

Table 5. Effects of proanthocyanidins on TAG metabolism in HepG2 cells

Incubation in the presence of	Extract composition	Dose	Effect	Authors
Green tea catechins	(−)Epicatechin (−)Epigallocatechin gallate	10–50 μM	Dose-dependent inhibitory effect on ApoB secretion	[153]
Dealcoholized red wine	Wine polyphenols	5 mM	Reduction in ApoB100 levels both in cells and in the media	[68]
Red grape juice	High concentrations of anthocyanins, flavonols, and flavan-3-ols, particularly myricetin, quercetin glycosides and procyanidin B2	5 mL/L	Reduction in ApoB and SREBP-1 expression Reduction in the TG content of the cells	[154]
Grape seed proanthocyanidin extract	Grape seed tannins in monomeric, dimeric, trimeric and oligomeric proanthocyanidin forms	50 mg/L	Inhibition of TAG and ApoB secretion into the media	[66]
Pure and purified monomeric and dimeric proanthocyanidin forms	Proanthocyanidin monomers and dimers B1-4 Trimer C1 and oligomeric forms of proanthocyanidins	25 mg/L	No effects on TAG and ApoB secretion into the media with monomers and dimers Trimer C1 inhibited TAG and ApoB secretion into the media	[64]

summarizing the effects of proanthocyanidins upon these pathways is that the composition and content of proanthocyanidins in food and the fractions used are poorly described in the experimental reports published. It is, then, difficult to draw clear conclusions about the effectiveness of proanthocyanidins or the proanthocyanidin forms that are most active. Even so, the papers that compare the effect of proanthocyanidin fractions with the effect of total extracts or monomers (catechin or epicatechin) conclude that proanthocyanidins are the effective molecules in the extracts that improve lipid metabolism. Figure 1 summarizes the most important effects of proanthocyanidins on lipid biochemical processes.

4.1 Lipid digestion, absorption and chylomicron production by the intestine

One of the mechanisms responsible for the hypolipidemic effect of procyanidins could be the delay in fat and cholesterol

absorption, and a reduction in chylomicron secretion. There is clear evidence to suggest that atherosclerosis is a consequence of disordered chylomicron metabolism, and that this is probably the most common etiology [47]. As humans are in postprandial state for much of the day, the reduction of chylomicron production induced by procyanidins seems to be crucial for their protection against CVD.

Foods rich in procyanidins, such as red wine, have been demonstrated to down-regulate chylomicron secretion in humans. Postprandial ApoB48 (a specific marker of chylomicrons and their remnants) was reduced by acute alcoholic and non-alcoholic red wine consumption in dyslipidemic postmenopausal woman 6 h after eating [48]. However, a subsequent study by the same authors with 17 dyslipidemic postmenopausal women [49] shows no effects of acute alcoholized or dealcoholized red wine consumption (400 mL) on ApoB48 measured as the area under the curve for 6 h after eating, although there was a significant reduction in ApoB48 levels at 1 h. In hypercholesterolemic postmenopausal women (45 women), chronic consumption of alcoholized or dealcoholized red wine (400 mL) has no effect on fasting plasma ApoB48 over a 6-week intervention period [50].

In human intestinal CaCo-2 cells, the secretion of ApoB48 is significantly reduced by alcoholic and non-alcoholic red wine [51, 52] and apple polyphenol extract [53], whereas a wine polyphenol extract is not effective [53]. This difference in the effect of foods and extracts upon chylomicron secretion could be dependent on procyanidin content, as each food/extract has a characteristic composition and concentration. It has been demonstrated that only the procyanidin purified/enriched fraction, but not the procyanidin-depleted fraction, of an apple polyphenol extract drastically reduces the secretion of lipids (cholesterol, TG and phospholipids) and reproduces the effects of the total extract [53].

Lipid availability, ApoB and microsomal triglyceride transfer protein (MTP) are known to be central to the efficient assembly and secretion of chylomicrons [54]. Impaired lipid availability in enterocytes seems to be the first cause of the reduction in intestinal chylomicron secretion by procyanidins. Before fat can be absorbed, TGs from foods must be hydrolyzed, and the pancreatic lipase plays a key role in the efficient digestion of TGs [55]. Grape seed extracts [56, 57] and apple procyanidins [58] inhibit the activity of pancreatic lipase, thus suggesting limited dietary TG absorption. The inhibitory effect of procyanidins upon pancreatic lipase is very powerful (IC50 1.4 μg/mL for global procyanidins in apple) and it increases with the DP from dimer to pentamer [58]. As far as cholesterol absorption is concerned, elevated excretion of neutral steroids and bile acids has been described in rats supplemented with grape procyanidins [59], apple procyanidins [60] and cacao procyanidins [61]. On the other hand, monomers (catechin or epicatechin) do not affect cholesterol or bile acid excretion [61]. The micellar solubility of cholesterol *in vitro* is reduced significantly more by procyanidins B2 (dimer), B5 (dimer), C1 (trimer) and A2 (tetramer) than by catechin and epicatechin [61], suggesting that procyanidins inhibit the absorption of cholesterol and bile acids by decreasing micellar cholesterol solubility. Moreover, apple procyanidins reduce the synthesis of cholesteryl esters in Caco-2 cells, without changing cholesterol uptake, most likely by inhibiting cholesterol esterification [53]. No changes were observed in the mRNA levels of enzymes catalyzing cholesterol esterification (ACAT1 and ACAT2) [53], suggesting that, in enterocytes, procyanidins interfere in this process by a mechanism other than gene expression regulation. Red wine (normal or dealcoholized), on the other hand, reduces free cholesterol and total cholesterol in Caco-2, without affecting cholesteryl esters [51].

Little information is available about changes induced by procyanidins on ApoB synthesis and degradation and MTP activity in enterocytes, although the effects seem to be less important than those induced on TG and cholesterol availability. Apple procyanidins do not affect MTP activity, but they do inhibit the synthesis of ApoB without changing its rate of degradation in Caco-2 [53].

4.2 Lipogenesis, cholesterol synthesis and VLDL production in liver

Although procyanidins exert some of their hypolipidemic effect by inhibiting the absorption of dietary lipids and diminishing chylomicron secretion by enterocytes, the repression of VLDL secretion by the liver also has an important role in reducing plasma lipids. Insulin resistance, type 2 diabetes and metabolic syndrome are characterized by dyslipidemia due to an overproduction of VLDL particles [62, 63]. Thus, procyanidin reduction of VLDL secretion by the liver could involve a minor risk of CVD linked to these diseases.

Using human transformed hepatic HepG2, pure procyanidins or extracts – basically from grape seed and wine – inhibit TGs and ApoB (a marker of VLDL) production and secretion into the media. Table 5 summarizes the publications that have helped to draw this conclusion. Working with cell models makes it easier to identify which proanthocyanidins are responsible for this phenomenon. Montagut *et al.* [64] concluded that trimer C1 and other oligomeric forms of proanthocyanidins may be largely responsible for inhibiting TG and ApoB secretion by hepatic cells.

Assembly of VLDL in liver is regulated by lipid availability, ApoB and MTP [65]. Impaired lipid availability in hepatocytes seems to contribute to the reduction of hepatic VLDL secretion by procyanidins. *De novo* synthesis of TGs and cholesterol, as well as their secretion, is inhibited by grape seed procyanidins in HepG2 cells [64, 66]. The use of grape seed procyanidin fractions has demonstrated that trimer-enriched fractions have hypolipidemic activity, whereas monomeric- and dimeric-enriched fractions have no effect [64]. Pure dimer B1, B2, B3 and B4 do not reduce TG and cholesterol secretion, whereas trimer C1 only represses TG secretion [64].

Grape seed procyanidins modulate the hepatic expression of numerous genes related to fatty acid, TG and cholesterol metabolism, which explains the reduction in TG and cholesterol secretion. Acute administration of grape seed procyanidins represses the expression of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (the rate-limiting enzyme in the cholesterol biosynthetic pathway), HMG-CoA synthetase and other enzymes involved in cholesterol biosynthesis in mouse liver [66, 67]. HMG-CoA reductase mRNA levels, on the other hand, increase in rat liver after an acute dose of grape seed procyanidins and in HepG2 cultured with red wine polyphenols [68]. In hyperlipidemic rats fed with a high-fat-diet, chronic administration of grape seed procyanidins (10 days) does not modify HMG-CoA reductase expression in liver [69]. These differences could be related to animal species or the lengths and doses of procyanidin administrations. However, some drugs that repress HMG-CoA reductase activity and cholesterol synthesis do not inhibit transcription of the gene; rather, they inhibit translation of the HMG-CoA reductase mRNA and accelerate the degradation of the enzyme protein [70]. In this regard, procyanidin A-2 inhibits HMG-CoA reductase activity on Vero cells [71].

No changes are detected in fatty acid synthase and acetyl-CoA carboxylase mRNA levels in the livers of rats [72] and mice (results not published) treated with an acute dose of grape seed procyanidins. Nevertheless, mRNA levels of carnitine palmitoyltransferase 1 (CPT1), the enzyme controlling fatty acid oxidation, are increased in mouse liver [66, 67], suggesting that fatty acids are more directed to oxidation than to TG synthesis. On the contrary, grape seed procyanidins do not overexpress CPT1 in the liver of normolipidemic [72] or hyperlipidemic [69] rats. Nevertheless, increased fatty acid oxidation by grape seed procyanidins cannot be ruled out because CPT1 activity is tightly regulated by its physiological inhibitor malonyl-CoA, which physiologically regulates β-oxidation depending on the availability of fatty acids and glucose [73]. The expression of genes related to TG synthesis in liver is strongly repressed by grape seed procyanidins. mRNA levels of phosphatidic acid phosphatase (the enzyme that generates diglycerides from phosphatidic acid for TG synthesis) and diacylglycerol acyl transferase 2 (the enzyme that catalyzes the last reaction of TG synthesis) are reduced in mouse liver [66, 67] and hyperlipidemic rats [69], respectively.

ApoB and MTP are necessary for VLDL assembly [74]. It has been demonstrated that ApoB itself is constitutively synthesized and that ApoB (VLDL) secretion by the liver is regulated mainly by degradation depending on lipid bioavailability and MTP activity [75]. In accordance with these findings, ApoB mRNA levels in liver are not changed by grape seed procyanidins after their acute administration to normolipidemic rats [72] or chronic administration to hyperlipidemic rats [69]. However, MTP mRNA levels are reduced in HepG2 cells (results not published) and normalized (reduced) in hyperlipidemic rats by grape seed procyanidins [69]. Thus, it can be suggested that the reduction of VLDL secretion by the liver is mainly due to a reduction in the bioavailavility of lipids, mainly TGs.

4.3 Lipid uptake by extra hepatic tissues

Several studies have been made on lipid accumulation and lipid metabolism in extrahepatic tissues, mainly adipose, as a result of procyanidin treatment. Pre-treating rats with grape seed procyanidins prevents the isoproterenol-induced accumulation of cholesterol and TGs in the heart [76]. Also, adipose tissue differentiation [77], metabolism [78] and mitochondrial function [79] are modified by procyanidins. However, there are no bibliographic references with direct measures of the effect of procyanidins on extra hepatic lipid uptake. However, studies on lipoprotein lipase (LPL) expression in extrahepatic tissues and studies on apolipoprotein expression in liver suggest that procyanidins have some effect on TG uptake by muscle and adipose tissue. LPL is a multifunctional enzyme produced by many tissues (including adipose tissue, cardiac and skeletal muscle). It is the rate-limiting enzyme for the hydrolysis of TGs from circulating chylomicrons, and VLDL [80]. LPL is regulated at transcriptional, posttranscriptional and posttranslational levels in a tissue-specific manner [80]. LPL mRNA levels are increased in muscle and decreased in adipose tissue by the acute administration of grape seed procyanidins to normal rats [72], suggesting that TGs are directed preferentially to energy production by the muscle instead of to energy storage by the adipose tissue. But this effect is not clear because no changes are observed in LPL mRNA levels of muscle and adipose tissue after the administration of chronic grape seed procyanidins to hyperlipidemic rats [69].

Besides LPL activity, apolipoprotein content in VLDL determines the clearance of TG-rich lipoproteins. Apolipoprotein (Apo)CIII and ApoAV play an important role in TG metabolism [81] ApoCIII delays TG clearance by such mechanisms as inhibition of LPL, and variation in its expression has been documented to have an important role in hypertriglyceridemia [82, 83]. ApoCIII is synthesized mainly by liver, and grape seed procyanidins reduce its expression in rat liver [72]. Also, ApoAV, which is overexpressed by grape seed procyanidins in mouse liver [66, 67], accelerates VLDL catabolism [84]. Thus, it seems that procyanidins probably modify extra hepatic TG uptake.

4.4 LDL metabolism and reverse cholesterol transport

Reverse cholesterol transport is a complex process that ensures the efflux of cholesterol from peripheral cells and its transport back to the liver for its metabolism and biliary excretion [85]. Some elements in this process have been shown to be modulated by procyanidins. The first step in the

process is the transference of free cholesterol to lipid poor ApoAI (HDL) by the ABC-AI transporter [85]. ABC-A1 expression is induced by grape seed procyanidins in macrophages cultured with oxidized LDL [86], thus preventing their transformation into foam cells. On the other hand, it has been reported that ApoA1 levels exert the strongest influence on HDL concentrations and risk of atherosclerosis [87]. Moderate red wine consumption increases plasma ApoA1 levels in healthy men, thus improving cholesterol efflux [88, 89]. Moreover, ApoAI is the human serum protein that most efficiently binds proanthocyanidins from grape seed [90], so reverse cholesterol transport may be modified directly by proanthocyanidins.

Cholesteryl esters in HDL are partially transferred to TG-rich ApoB-containing lipoprotein, so the hepatic uptake of the cholesterol released from peripheral cells may proceed *via* an HDL-receptor, the SR-BI and through the LDL receptor route [85]. Only the effects of procyanidins on LDL receptors have been studied. Red wine polyphenolics increase LDL receptor expression and activity in human HepG2 cells [68], but grape seed procyanidins do not change the expression of the LDL receptor in liver in either normal rats [72] or hyperlipidemic rats [69]. The fact that grape seed procyanidins do not modify the expression of the hepatocyte LDL receptor suggests that the decrease in LDL-cholesterol induced by procyanidins is largely due to the reduced VLDL secretion by liver, which results in lower LDL production.

The last step in reverse cholesterol transport is bile acid synthesis in the liver and excretion. Bile acid synthesis is controlled by the activity of CYP7A1. mRNA levels and CYP7A1 activity are increased by grape seed procyanidins [72] and apple [60], respectively, thus suggesting that procyanidin consumption leads to greater cholesterol elimination. On the other hand, grape seed procyanidins repress the expression of CYP8B1 [72], the enzyme required for the synthesis of cholic acid, thus suggesting a change in the chenodeoxycholate to cholate ratio in the bile acid pool. The hydrophilic–hydrophobic balance of bile acid modifies cholesterol absorption in the intestine, and cholic acid is the most efficient [91]. This effect could be another mechanism by which procyanidins can reduce intestinal cholesterol absorption (see Section 4.1).

5 Molecular mechanisms underlying the hypolipidemic effects of proanthocyanidins

As stated above, one of the causes of the hypotriglyceridemic effect of procyanidins is the blockage of VLDL secretion by the liver as a consequence of changes in the expression of genes related to lipid metabolism. Nuclear receptors are transcription factors that regulate the expression of specific target genes. The farnesoid X receptor (NR1H4/FXR) is of particular importance in lipid metabolism as its activation inhibits hepatic *de novo* lipogenesis [92, 93]. Moreover, FXR-deficient mice display elevated serum levels of TGs whereas the activation of FXR by agonists lowers plasma TGs, thus demonstrating the critical role that FXR plays in plasma TG levels [92]. Using FXR-null mice, it has been demonstrated that FXR is an essential mediator of the hypotriglyceridemic action of procyanidins *in vivo*: the hypotriglyceridemic effect induced by grape seed procyanidins in wild-type mice is annulled in FXR-null mice [67].

The natural agonists of FXR are bile acids, of which chenodeoxycholic acid is the most effective activator [94]. The capacity of procyanidins to activate FXR has been studied *in vitro*. In FXR-driven luciferase expression assays, grape seed procyanidins enhance FXR transcriptional activity in CV-1 and HeLa cells [67]. For activation, procyanidins require the presence of chenodeoxycholic acid, so they act as co-agonists that increase the transcriptional activity induced by chenodeoxycholic acid alone. It has not been demonstrated that procyanidins bind to FXR, so FXR may be activated by the direct interaction of procyanidins or by an indirect mechanism.

Also, the nuclear receptor small heterodimer partner (NR0B2/SHP) mediates the hypotriglyceridemic effect of procyanidins [66]. The hypotriglyceridemic effect induced by grape seed procyanidins in wild-type mice is annulled in SHP-null mice. The involvement of SHP in the hypotriglyceridemic actions of procyanidins has been confirmed using HepG2 cells, in which procyanidins need a transient increase in SHP mRNA and protein to block TG secretion. SHP is a key regulatory factor of the transcription of genes involved in various metabolic pathways, including lipid metabolism [95]. The predominant role of SHP is that of transcriptional co-repressor: SHP protein interacts with several nuclear receptors and transcription factors, and acts as a co-repressor of target genes [95]. The SHP promoter is regulated by many transcription factors and nuclear receptors and SHP expression is induced by FXR [95]. As procyanidins activate the transcriptional activity of FXR, it seems that the molecular mechanism used by procyanidins includes activation of FXR and up-regulation of SHP, which in turn repress the expression of genes related to lipogenesis and TG synthesis.

The study of differential patterns of gene expression in the liver of wild-type, FXR-null and SHP-null mice by procyanidins has made it possible to discriminate the genes that respond to procyanidins through FXR- and SHP-dependent mechanisms. It is interesting to emphasize that one of the genes that grape seed procyanidins down-regulate – in an FXR- and SHP-dependent manner – is the transcription factor steroid response element binding protein 1 (SREBP1) [66, 67]. The role of SREBP1 is to activate the transcription of genes involved in fatty acid synthesis [96]. SREBP (1 and 2) are membrane-bound proteins that must enter the nucleus after their proteolytic cleavage to activate their target genes. But, it seems that SREBP-1c regulates lipogenesis mainly by changing its mRNA level instead of the proteolytic cleavage system [96]. However, the protein level of SREBP1 has also been reported to be reduced in the liver of mice with type 2 diabetes by oligomeric procyanidins in persimmon peel [97]. In accordance with SREBP1 mRNA and protein reduction by

procyanidins, several SREBP1 target genes involved in lipogenesis and genes involved in TG synthesis are repressed by an FXR-dependent mechanism in mice liver.

As a result, procyanidins decrease plasma TGs by activation of FXR, transient up-regulation of SHP expression and subsequent repression of SREBP1. In humans, treatment with chenodeoxycholic acid has been shown to reduce hypertriglyceridemia [98] and the molecular mechanism described for the hypotriglyceridemic action of bile acids is the same of that described for procyanidins [99]. This molecular signaling pathway also works in dislypidemic rats, where the hypotriglyceridemic effect induced by procyanidins correlates with the induction of SHP and the repression of SREBP1 in liver [69].

In HepG2, procyanidins suppress both TG and ApoB secretion. However, only TG secretion is suppressed by SHP-dependent mechanisms, whereas ApoB secretion is inhibited [66] and MTP mRNA reduced (results not published) by SHP-independent mechanisms. Thus, it seems that procyanidins block VLDL secretion by activating FXR together with other molecular signaling pathways. One of the most important regulators of VLDL assembling and secretion is insulin, insulin resistance being a situation in which secretion of VLDL is exacerbated [100, 101]. Insulin binds to its receptors at the cell surface, which activates a cascade of events including insulin receptor substrate, PI3-kinase, and Akt. In turn, this cascade regulates MTP and ApoB production [101]. It has been demonstrated that grape seed procyanidins activate the insulin receptor and key targets of insulin signaling pathways, including phosphorylation of protein kinase B and Akt [102]. Thus, this insulin-like effect of procyanidins, in conjunction with the activation of FXR, could help repress VLDL secretion and the hypotriglyceridemic effect. On the other hand, plasma free fatty acid play an important role in stimulating hepatic VLDL production [103]. As procyanidins strongly reduce plasma free fatty acids levels in normolipidemic [72] and hyperlipidemic [69] rats, the reduction in the availability of free fatty acids to the liver could be another mechanism that helps to repress VLDL secretion by procyanidins.

6 Concluding remarks

Epidemiological studies strongly suggest that food rich in proanthocyanidins protect against CVD. One of the mechanisms by which proanthocyanidins exert their cardiovascular protection is by improving lipid homeostasis. Animal studies clearly demonstrate that proanthocyanidins reduce the plasma levels of atherogenic ApoB-TG-rich lipoproteins and LDL-cholesterol. They also increase antiatherogenic HDL-cholesterol. The strongest effect is the reduction of plasma TGs. Nevertheless, the effect on TG metabolism was less clear in the case of human subjects. Several human intervention studies conclude that the intake of proanthocyanidins may be positive, neutral or negative. The different doses used and, above all, the different compositions of the extracts and food administered may explain these contradictory responses. Thus, more studies will be carried out to determine the dose and the type of proanthocyanidins and food that are most effective at improving hypertriglyceridemia in humans.

In vivo and *in vitro* studies have demonstrated that intestinal lipid absorption, chylomicron secretion by the intestine and VLDL secretion by the liver are the processes that are most repressed by proanthocyanidins, thus inducing hypolipidemia. Because the composition and content of proanthocyanidins in food or the fraction used have been poorly described in published reports, it is difficult to draw clear conclusions about the effectiveness of the proanthocyanidins or the proanthocyanidin forms that are most active. Even so, papers comparing the effect of the proanthocyanidin fraction with the effect of total extracts or monomers (catechin or epicatechin) conclude that proanthocyanidins are the molecules in the extracts that effectively improve lipid metabolism. Moreover, *in vitro* studies, using either animal or human cell lines, show that trimeric proanthocyanidins are the molecules that most effectively repress TG (VLDL) secretion by the liver. Studies on proanthocyanidin bioavailability demonstrate dimer and trimer absorption, but *in vivo* studies using purified trimers or fractions will be required to confirm how effective they are at improving lipid homeostasis.

In normolipidemic mice, it has been shown that procyanidins decrease plasma TGs by activation of FXR, transient up-regulation of SHP expression and subsequent repression of SREBP1 in liver. This molecular signaling pathway also works in dislypidemic rats, in which the hypotriglyceridemic effect induced by procyanidins correlates with the induction of SHP and the repression of SREBP1 in liver. The importance of the proanthocyanidin activation of FXR lies in the functionality of this nuclear receptor. FXR activity plays a key role in controlling not only triglyceridemia but also cholesterol, bile acid and glucose homeostasis. Consequently, modulation of FXR has been proposed as a therapeutic target in the treatment of hyperlipidemia, hyperglycemia and metabolic syndrome. Furthermore, as VLDL assembling and secretion is regulated by insulin, the insulin-like effect of procyanidins in conjunction with the activation of FXR may collaborate in the repression of VLDL secretion and the hypotriglyceridemic effect.

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