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Bergamot (*Citrus bergamia* Risso) Flavonoids and Their Potential Benefits in Human Hyperlipidemia and Atherosclerosis: an Overview

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Abstract: Elevated serum cholesterol, triglycerides and LDL levels are often associated with an increased incidence of atherosclerosis and coronary artery disease. The most effective therapeutic strategy against these diseases is based on statins administration, nevertheless some patients, especially those with metabolic syndrome fail to achieve their recommended LDL targets with statin therapy, moreover, it may induce many serious side effects. Several scientific studies have highlighted a strong correlation between diets rich in flavonoids and cardiovascular risk reduction. In particular, *Citrus bergamia* Risso, also known as bergamot, has shown a significant degree of hypocholesterolemic and antioxidant/radical scavenging activities. In addition, this fruit has attracted considerable attention due to its peculiar flavonoid composition, since it contains some flavanones that can act as natural statins. Hence, the study of bergamot flavonoids as metabolic regulators offers a great opportunity for screening and discovery of new therapeutic agents. Cholesterol metabolism, flavonoid composition and potential therapeutic use of *C. bergamia* Risso will be discussed in the following review.

Keywords: Bergamot fruit, flavonoids, hyperlipidemia, atherosclerosis, 3-hydroxy-3-methylglutaryl-CoA reductase enzyme.

INTRODUCTION

The risk for atherosclerosis and coronary heart disease is increased in patients with elevated serum concentrations of low-density lipoproteins cholesterol (LDL), total cholesterol (TC) and triglicerides (TG) [1-5]. Several meta-analysis studies showed that statin therapy can reduce the 5-year incidence of cardiovascular diseases, by about one fifth per mmol/L reduction in LDL cholesterol [6-8].

It is well-known that statins are able to inhibit 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) activity, the rate-limiting enzyme of cholesterol biosynthesis [9]. Statin administration is one of the most widely used approaches to lower serum LDL level and to reduce cardiovascular event rates [10-12]. However, many patients, especially those with the dyslipidemia associated with metabolic syndrome, are unable to reach their lipid treatment goals on statins alone [2]. Furthermore, patients might be statin-intolerant and experience significant side-effects [3], hence the importance of finding new drugs acting as statins.

Some foods were shown to possess these therapeutic properties; in particular, daily consumption of *Citrus* fruit juice was shown to positively influence serum lipid levels and to decrease coronary heart disease risk [13]. Their hypolipidemic effects can be due to the presence of flavonoids, pectins and ascorbic acid, which have a high antioxidant potential and may interfere with cholesterol metabolism [14-19].

Flavonoids are aromatic secondary plant metabolites, having strong antioxidant and radical scavenging activities [15, 20]. Their intake was associated with a reduced risk for certain chronic diseases such as cardiovascular disorders and cancerous processes [21-23]. Flavonoids exhibited antiviral, antimicrobial and anti-inflammatory activities [23-25], moreover, they were able to inhibit human platelet aggregation [26] and to support a correct immune response [27].

Bergamot, the common name of *Citrus bergamia* Risso, belongs to the family Rutaceae, subfamily Esperidea and it has been widespread in the Mediterranean area for centuries. Over the past few years, thanks to the growing interest in bioactive compounds, bergamot fruit has attracted attention for its remarkable flavonoid composition. The first part of this review will report an overview on cholesterol metabolism, in the second part, literature data regarding flavonoid composition and distribution in bergamot fruit will be analysed. The last part will focus on the scientific evidence concerning the bioactivities of bergamot flavonoids and their potential utility for human health as well as their uses in atherosclerosis and coronary heart disease treatments.

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1. CHOLESTEROL HOMEOSTASIS AND REGULATION

Cholesterol body homeostasis is mainly due to the regulation of its endogenous synthesis, intestinal absorption, excretion and hepatic conversion (Fig. **1**).

Cholesterol *de novo* synthesis occurs mainly in the liver and, in human, it accounts for more than 70% of body cholesterol. Cholesterol intestinal absorption depends on diet composition. Excess liver cholesterol can be directly excreted as biliary sterols or converted into bile acids, both are eliminated via feces.

Cholesterol absorption is controlled by at least two types of transporters, Niemann-Pick C1-Like 1 (NPC1L1) as influx transporter and ATP-Binding Cassette (ABC) proteins as efflux transporters [28]. NPC1L1 transports cholesterol from intestinal lumen into enterocytes and it reabsorbs free cholesterol back into hepatocyte from bile [29]. ABCG5 and ABCG8 reduce cholesterol absorption in the intestinal lumen and exclude cholesterol from liver to the bile duct (Fig. **1**). ABCG1 and ABCA1 are involved in reverse cholesterol transport, the pathway by which peripheral cell cholesterol can be returned to the liver for excretion [30].

Regulation of cholesterol homeostasis is achieved by proteins such as sterol regulatory element-binding proteins (SREBPs) and AMP-Activated Protein Kinase (AMPK); by nuclear receptors such as peroxisome proliferator activated receptors (PPARs) and liver X receptors (LXRs); by microRNAs (miRNAs).

SREBPs are key transcription regulators encoded by two genes, SREBP-1 and SREBP-2. SREBP-1 upregulates the transcription of some hepatic lipogenic genes [31-35]. SREBP-2 modulates the transcription of some sterol

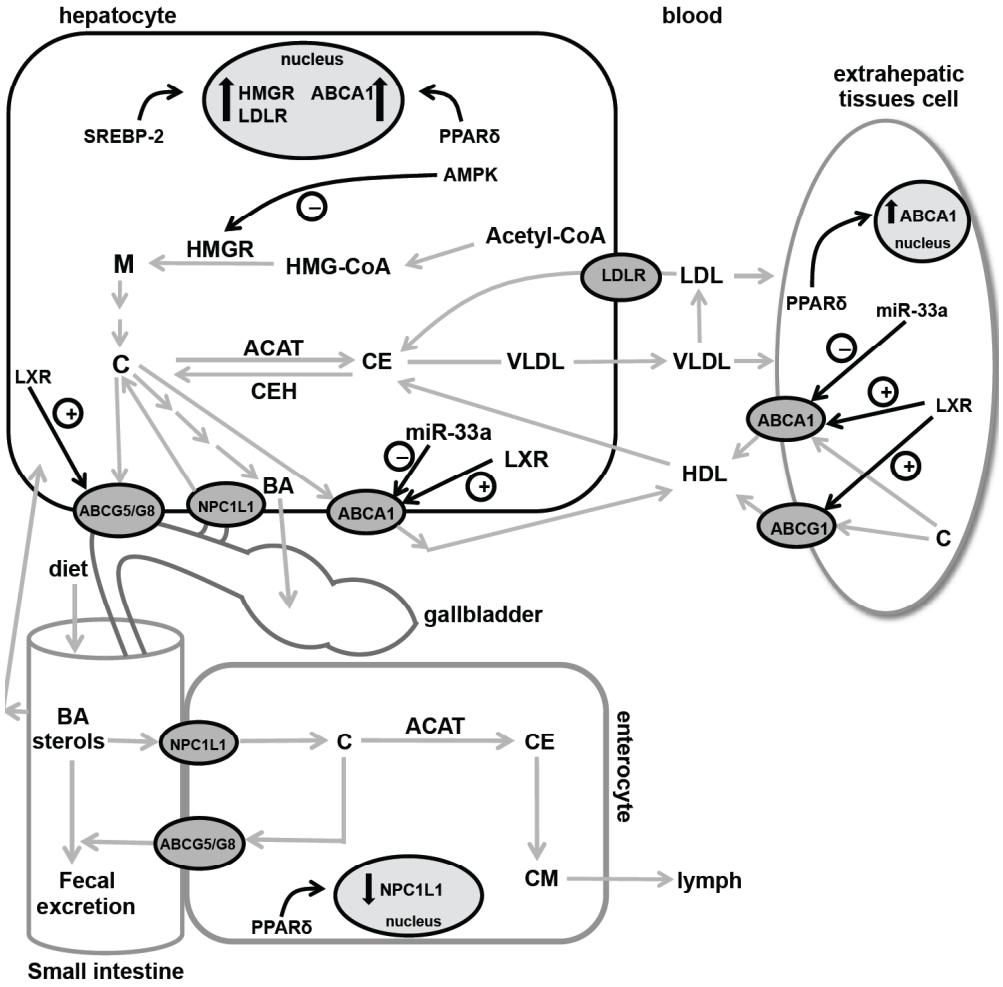


Fig. (1). Overview of cholesterol homeostasis and regulation in liver, small intestine, extraepatic tissues, and plasma. The regulation is indicated with black arrows. ⊕ indicates activation while ⊖ indicates inhibition. ABCA1, ABCG1, and ABCG5/G8: ATP-binding cassette transporters; ACAT: acyl CoA:cholesterol acyltransferase; AMPK: AMP-activated protein kinase; BA: bile acid; C: cholesterol; CE: cholesteryl ester; CEH: cholesteryl ester hydrolase; CM: chylomicrons; HDL: high-density lipoprotein; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; LDL: low-density lipoprotein; LDLR: low-density lipoprotein receptor; LXR: liver X receptor; M: mevalonate; miR-33a: microRNA-33a; MTP: microsomal triglyceride transfer protein; NPC1L1: Niemann-Pick C1-Like 1; PPAR∂: peroxisome proliferator activated receptor delta; SREBP2: sterol regulatory element binding protein-2; VLDL: very low density lipoprotein.

biosynthetic genes [36], for instance, when hepatocyte cholesterol content is low, expressions of HMGR and LDLR are upregulated [36] (Fig. **1**).

AMPK is a critical player in energy homeostasis at both cellular and whole body levels. An increased AMP to ATP ratio leads to AMPK activation through phosphorylation by at least three different upstream kinases [37]; in particular, when cellular cholesterol content is high, AMPK inactivates HMGR by phosphorylation (Fig. **1**) [38].

PPARs are members of nuclear hormone receptors superfamily that act as ligand-dependent transcription factors [39, 40]. PPARα directly upregulates the transcription of genes involved in cholesterol catabolism [41]. PPARγ integrates the control of energy, lipid and glucose homeostasis [42-44] and its activation also redirects effluxed cholesterol from liver toward adipose tissue uptake via scavenger receptor type-BI [45]. PPARδ activation elevates serum HDL levels by increasing the expression of ABCA1 [30], it can reduce cholesterol absorption by decreasing NPC1L1 intestinal expression [29] and it also potentiates fecal neutral sterol secretion by increasing transintestinal cholesterol efflux [46].

LXRs play a primary role in reverse cholesterol transport, modulating the expression of several target genes as ABCA1, ABCG1, ABCG4 ABCG5, ABCG8 and apoE [47-50]. In the liver, when cellular cholesterol content is high, LXRs activation induces cholesterol excretion and/or efflux [50, 51].

MicroRNAs promote the down-regulation of their target genes by binding to specific regions located in the 3' UTR of their target mRNA [52]. MIR-33a is believed to minimize cholesterol export by the post-transcriptional repression of ABCA1 transporter (Fig. **1**) [53, 54].

2. FLAVONOIDS IN BERGAMOT TISSUES

Plant flavonoids are a large group of very different compounds sharing the common feature of phenolic moieties [55]. The presence of a relatively large number of flavonoids is the result of many different possible combinations among polyhydroxylated aglycones and a limited number of mono- and disaccharides. The most commonly found sugars are hexoses, such as glucose, galactose and rhamnose or pentoses such as arabinose and xylose. They are, with a few notable exceptions, plant metabolites deriving from the shikimate pathway and the phenylpropanoid metabolism [56]. In recent years, flavonoids have attracted tremendous attention due to the protection that they provide against some types of cardiovascular diseases [57]. As a consequence, many studies have been directed to the characterization of the flavonoid fractions and to the isolation of the most representative flavonoids present in the most common *Citrus* species, as well as of flavonoids present in many local species such as *C. bergamia* Risso [58, 59]. Bergamot fruit presents an external part, epicarp or flavedo yellow coloured; a middle part, mesocarp or albedo, that is a spongy white inner layer and an inner part, endocarp or pulp. Albedo and flavedo peeled off together are called peel. Bergamot essential oil is obtained from this fraction by cold press; it is composed of a volatile (93–96%) and a non-volatile fraction (4–7%).

The classes of flavonoids present in *C. bergamia* Risso fractions are flavanones and flavones. Flavanones are present as flavanone-O-glycosides, recently, flavanones diglycosides carrying the 3-hydroxy-3-methylglutaric acid (HMG) moiety have also been detected [60-62]. Flavones are present as flavone-O-glycosides, flavone-C-glycosides or polymethoxy-flavones (Table **1**).

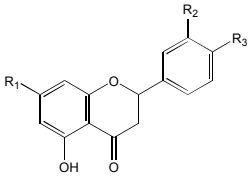
A comparative study on flavonoid composition in fruit tissues of different *Citrus* species has been reported by Nogata *et al.* [59], showing that bergamot fruit has a peculiar flavonoid composition. In particular, it contains neoeriocitrin in exceptionally large amount (288 mg/100 g fresh weight) and it is relatively rich in neohesperidin, naringin, poncirin, rhoifolin, and neodiosmin (590, 438, 1240, 43 and 33 mg/100 g fresh weight, respectively) with respect to the other *Citrus* fruits analyzed. Furthermore, it contains very little amount of hesperidin (2 mg/100 g fresh weight).

Table **1** lists structure and tissue distribution of flavonoids, in *C. bergamia* Risso as described in literature.

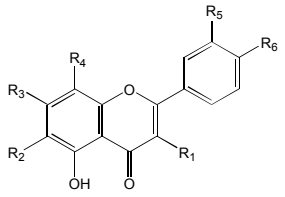
Flavanone-O-glycosides are present in all the analysed parts, in particular the most abundant are naringin, neoeriocitrin, neohesperidin and poncirin, whereas hesperidin and neoponcirin have been detected in a very low amount [59]. They are also present in the peel, but it could be noted that when it is splitted into albedo and flavedo, these compounds are mainly present in albedo [59]. Moreover, poncirin, which is present in huge amount in hand-squeezed juice, is absent in industrial juice, this may be due to the pressing process used to extract industrial juices [58]. In addition, three acylated flavanones, which seem to correspond to di-oxalate derivatives of neoeriocitrin, naringin and neohesperidin, have been identified in bergamot juice [63]. The HMG-conjugated flavanones, brutieridin, melitidin and HMG-neoeriocitrin have also been detected at different concentrations depending on the ripening stage; they may be found in bergamot fruit either in juice or in albedo and flavedo [60-62].

Flavone-O-glycosides present a different tissue distribution. All these compounds are present in the peel, with the exception of chrysoeriol 7-O-neohesperidoside, chrysoeriol 7-O-neohesperidoside-4'-glucoside and rhoifolin 4'- glucoside [59, 64]. Diosmetin mono-glucoside, diosmetin mono-rhamnoside and apigenin mono-glucoside/mono-rhamnoside has been detected in bergamot peel [64] but not in albedo and flavedo. It could be explained because, according to this author, bergamot peel is a mix of seeds, pulp and deoiled flavedo after essential oil and juice extraction. Furthermore, rutin, that is absent in albedo, has been found in large amount in flavedo [64]. All these compounds have been revealed in the juice [58, 59, 63, 65-67], with the exception of diosmetin mono-rhamnoside and diosmetin mono-glucoside; this latter has been detected in industrial juice, probably because fruit industrial processing leads to juices contaminated with peel constituents [58].

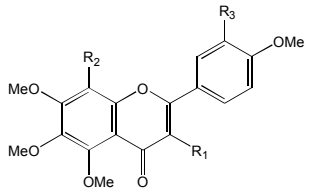
Table 1. Flavonoids identified in bergamot fruit.

Flavanone-O-glycosides	Peel	Albedo	Flavedo	Juice	Industrial juice
	[59, 84, 92]	[59, 60, 79]	[59, 60, 79]	[58-60, 64-67, 79]	[58]
Eriocitrin (eriodictyol 7-O-rutinoside) R_1=O-rutinose, R_2=OH, R_3=OH	X	X	X	X	X
Eriodictyol mono-rhamnoside Most likely 7-O-substituted	X				
Hesperidin (hesperetin 7-O-rutinoside) R_1=O-rutinose, R_2=OH, R_3=OCH_3	X	X		X	
Hesperetin mono-rhamnoside Most likely 7-O-substituted	X				
Naringin (naringenin 7-O-neohesperidoside) R_1=O-neohesperidose, R_2=H, R_3=OH	X	X	X	X	X
Naringenin mono-rhamnoside Most likely 7-O-substituted	X	X	X	X	X
Narirutin (naringenin 7-O-rutinoside) R_1=O-rutinose, R_2=H, R_3=OH	X	X	X	X	
Neoeriocitrin (eriodictyol 7-O-neohesperidoside) R_1=O-neohesperidose, R_2=OH, R_3=OH	X	X	X	X	X
Neohesperidin (hesperetin 7-O-neohesperidoside) R_1=O-neohesperidose, R_2=OH, R_3=OCH_3	X	X	X	X	X
Neoponcirin (isosakuranetin 7-O-rutinoside) R_1=O-rutinose, R_2=H, R_3=OCH_3	X	X	X	X	
Poncirin (isosakuranetin 7-O-neohesperidoside) R_1=O-neohesperidose, R_2=H, R_3=OCH_3	X	X	X	X	
Neoeriocitrin di-oxalate R_1=O-neohesperidose -di-oxalate, R_2=OH, R_3=OH				X	
Naringin di-oxalate R_1=O-neohesperidose-di-oxalate R_2=OH, R_3=OH				X	
Neohesperidin di-oxalate R_1=O-neohesperidose -di-oxalate R_2=OCH_3, R_3=OH				X	
Brutieridin (hesperetin 7-2''-α-rhamnosyl-6''-3''''-hydroxy-3''''-methylglutaryl)-β-glucoside) R_1=O-rhamnosyl-HMG, R_2=OH, R_3=OCH_3		X	X	X	
Melitidin (naringenin 7-2''-α-rhamnosyl-6''-3''''-hydroxy-3''''-methylglutaryl)-β -glucoside) R_1=O-rhamnosyl-HMG, R_2=H, R_3=OH		X	X	X	
Neoeriocitrin HMG conjugated		X	X	X	

(Table 1) Contd….

Flavone O-glycosides and C-glycosides	Peel	Albedo	Flavedo	Juice	Industrial juice
	[59, 84, 92]	[59, 60, 79]	[59, 60, 79]	[58-60, 64-67, 79]	[58]
Apigenin **Mono-glucoside/mono-rhamnoside**	X				
Chrysoeriol 7-O-neohesperidoside R_1=H, R_2=H, R_3=O-neohesperidose, R_4=H, R_5=OCH_3, R_6=OH				X	X
Chrysoeriol 7-O-neohesperidoside-4'-glucoside R_1=H, R_2=H, R_3=O-neohesperidose, R_4=H, R_5=OCH_3, R_6=O-glucoside				X	X
Diosmetin mono glucoside	X				X
Diosmetin mono rhamnoside	X				
Diosmin (diosmetin 7-O-rutinoside) R_1=H, R_2=H, R_3=O-rutinose, R_4=H, R_5=OH, R_6=OCH_3	X	X	X	X	
Neodiosmin (diosmetin 7-O-neohesperidoside) R_1=H, R_2=H, R_3=O-neohesperidose, R_4=H, R_5=OH, R_6=OCH_3	X	X	X	X	X
Rhoifolin (apigenin 7-O-neohesperidoside) R_1=H, R_2=H, R_3=O-neohesperidose, R_4=H, R_5=OH, R_6=OH	X	X	X	X	X
Rhoifolin 4'-glucoside (apigenin 7-O-neohesperidoside 4'-glucoside) R_1=H, R_2=H, R_3=O-neohesperidose, R_4=H, R_5=OH, R_6=O-glucoside				X	X
Rutin (quercetin 3-O- rutinoside) R_1=O-rutinose, R_2=H, R_3=OH, R_4=H, R_5=OH, R_6=OH	X		X	X	
Isovitexin (apigenin 6-C-glucoside) R_1=H, R_2=glucoside R_3=OH, R_4=H, R_5=H, R_6=OH				X	X
Luteolin **Mono-glucoside/mono-rhamnoside**	X				
Lucenin-2 (luteolin 6,8-di-C-glucoside) R_1=H, R_2=glucoside, R_3=OH, R_4=glucoside, R_5=OH, R_6=OH				X	X
Lucenin-2 4'-methyl ether (diosmetin 6,8-di-C-glucoside) R_1=H, R_2=glucoside, R_3=OH, R_4=glucoside, R_5=OH, R_6=OCH_3	X			X	X
Stellarin-2 (chrysoeriol 6,8-di-C-glucoside) R_1=H, R_2=glucoside, R_3=OH, R_4=glucoside, R_5=OCH_3, R_6=OH				X	X
Scoparin (chrysoeriol 8-C-glucoside) R_1=H, R_2=H, R_3=OH, R_4=glucoside, R_5=OCH_3, R_6=OH				X	X
Orientin 4'-methyl ether (diosmetin 8-C-glucoside) R_1=H, R_2=H, R_3=OH, R_4=glucoside, R_5=OH, R_6=OCH_3				X	
Vicenin-2 (apigenin 6,8-di-C-glucoside) R_1=H, R_2=glucoside, R_3=OH, R_4=glucoside, R_5=H, R_6=OH	X			X	

(Table 1) Contd….

Polymethoxy flavones	Peel	Albedo	Flavedo	Juice	Industrial juice
	[59, 84, 92]	[59, 60, 79]	[59, 60, 79]	[58-60, 64-67, 79]	[58]
Sinensetin (3',4',5,6,7-pentamethoxyflavone) R_1=H, R_2=H, R_3=OCH_3	X		X		
Nobiletin (3',4',5,6,7,8,-esamethoxyflavone) R_1=H, R_2=OCH_3, R_3=OCH_3	X	X	X		
Tangeretin (4',5,6,7,8-pentamethoxyflavone) R_1=H, R_2=OCH_3, R_3=H	X		X		

Flavone-C-glycosides are mainly present in the juice, similarly to flavanone-O-glycosides some of these compounds lack in industrial juice [58, 63, 65-68]. In bergamot essential oil (data not showed) only two flavonoids have been detected: sinensetin and tetra-O-methylscutellarein. This latter has been detected in essential oil [69].

3. HYPOLIPIDEMIC AND ANTIATHEROSCLEROTIC PROPERTIES OF BERGAMOT DERIVATIVES

Hypolipidemic effects of *Citrus* species are due to several components, such as flavonoids, pectins and ascorbic acid. Flavonoids are believed to inhibit LDL oxidation and to increase LDL reuptake, furthermore, they can interfere with fecal excretion of bile acids and with HMGR, LDLR and FASN functions [14, 70-72]. In particular, naringin seems to be active on atherosclerosis, as demonstrated by animal studies [73], neoeriocitrin is believed to strongly inhibit LDL oxidation [74] whereas, HMG-flavonoids could be able to inhibit HMGR [60]. These observations have provided the rationale to investigate the protective hypolipidemic effect of bergamot extracts in animal models and in human patients.

Miceli *et al.* [75] demonstrated that daily administration of bergamot juice to hypercholesterolemic rats caused a significant reduction in TC, TG and LDL levels, an increase in serum HDL levels and a protective effect on hepatic parenchyma. In addition, fecal output of total bile acids and neutral sterols was enhanced in the bergamot juice treated group in comparison with the hyperlipidemic group. These results are in agree with previous studies, which hypothesized that pectins and flavonoids were able to lower serum cholesterol levels by modulating hepatic HMG-CoA concentration. It could be noted that in this study, the potential side-effect due to bergamottin presence in bergamot juice was not investigated. Bergamot juice is rich in bergamottin (ranging from 18 to 61 mg/L) [63, 66], a furanocoumarin compound that inhibits cytochrome P450 34A enzyme activity, significantly increasing the oral bioavailability of several drugs metabolized primarily by this cytochrome [74, 76].

This problem was overcome by Mollace *et al.* [77], that analyzed the hypolipidemic effect of a defurocoumarinizated bergamot-derived polyphenolic fraction supplemented with ascorbic acid on animal models of diet-induced hyperlipidemia and in patients suffering from metabolic syndrome [77]. They found that oral administration of this fraction both in animal and in patients, caused a significant reduction of TC, TG and glycemia with a concomitant increase of HDL levels. In particular, in 59 patients with metabolic syndrome a 30-days treatment period with bergamot-derived polyphenolic fraction, administred at the dose of 1 g/die, reduced the serum levels of TC, LDL and TG by 30%, 33% and 41%, respectively [26]. This effect was associated with a significant improvement in vascular reactivity in patients with both hyperlipidemia and hyperglycemia, suggesting a potential protective role for the use of bergamot-derived polyphenolic fraction in these patients.

Recent prospective studies, led on patients with hyperlipidemia demonstrated that administration of a defurocoumarinizated bergamot-derived polyphenolic fraction was able to reduce TC level by 31%. In the same conditions, rosuvastatin administration (10 mg/die) caused a similar reduction of TC content (30%). Their association produced a considerable enhancement of rosuvastatin hypolipidemic effect, causing a reduction of TC level by 38%, normalizing the serum lipid profile [78].

The authors suggested that the observed hypolipidemic effect could be mainly due to the presence in bergamot-derived polyphenolic fraction of melitidin, brutieridin and HMG-neoeriocitrin. This hypothesis was investigated by Di Donna *et al.* [79] in a hypercholesterolemic rat model, by measuring the effects on lipid profile of administration of HMG-flavanones enriched fraction (62% of brutieridin, 14% of melitidin and 15% of HMG-neoeriocitrin), extracted from bergamot fruit, in comparison with simvastatin. HMGR, LDLR and FASN transcription levels and their correlated protein amounts were evaluated.

In this study, simvastatin and HMG-flavanones enriched fraction singularly administrated reduced levels of TC (30%, 20% respectively), TG (32%, 20% respectively), VLDL (33%, 28% respectively) and LDL (24%, 40% respectively), whereas an increase of 20% in HDL content was observed exclusively in rats treated by HMG-flavanones enriched fraction [61]. Furthermore, according to the previously published data, HMGR, LDLR and FAS transcription levels were found up-regulated. An increased amount of their corresponding proteins was detected [80]. Genotoxicity and toxicity were not observed by testing HMG-flavanones enriched fraction *in vitro*. The authors hypothesized that HMGR inhibition leads to a reduction of endogenous cholesterol level which, in turn, is responsible of HMGR and LDLR transcriptional up-regulation, as well of the higher LDLR exposure within the hepatocytes membrane, through a compensatory mechanism based on SREBPs pathway. Furthermore, it was highlighted that cholesterol depletion below a certain threshold is known to be responsible for FASN genic transcription increase, via SREBPs activation, which is one of the observed effects. It was suggested that transcriptional up-regulation of these genes and the corresponding increased protein amounts could be occurred via SREBPs pathway (Fig. **2**).

Beside the already described hypolipidemic effect, flavonoids, in particular naringin, have received considerable attention because of their antioxidant/radical scavenging properties [15, 20]. Increasing clinical evidences support the hypothesis that phospholipid oxidation products may play a role in atherosclerosis. This was firstly suggested by demonstrating that mildly oxidized LDL proatherogenic activities were present in the fraction containing oxidized phospholipids. Subsequently, phospholipid oxidation products were reported to accumulate in hyperlipidemic plasma, atherosclerotic lesions and in several diseases that predispose to stroke [81-83].

Trombetta *et al.* [84] reported that two flavonoid-rich extracts from bergamot peel, endowed with radical-scavenging properties and lacking genotoxic activity, were able to prevent alterations induced by the pleiotropic inflammatory cytokine tumor necrosis factor-α (TNF-α) on human umbilical vein endothelial cells (HUVECs). This study was led by monitoring intracellular levels of malondialdehyde, reduced and oxidized glutathione levels, superoxide dismutase activity and the activation status of nuclear factor-κB. To clarify the mechanisms involved in flavonoid protective activity, flavonoid-rich extracts were tested in vitro for their ability to inhibit cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activity, in a human whole blood model. Conversely to literature data [85], authors excluded that the protective effect of bergamot peel extracts against TNF-α-induced changes in HUVECs might be due to their capability to inhibit COX-1 or COX-2 pathways, because these phytocomplexes were unable to modify prostaglandin E2 and tromboxan B2 release when they were tested on human whole blood.

Several investigations suggested that phospholipid oxidation products may play a pathogenic role in progressive renal damage [86, 87]. A prominent mechanism probably involved in the deleterious effects of hypercholesterolemia on the kidney was an increased formation of reactive oxygen species. In addition, oxidized LDL particles were injurious to renal tubular epithelial cells and they might contribute to tubulointerstitial damage and glomerulosclerosis [88]. In an experimental model of short-term diet-induced

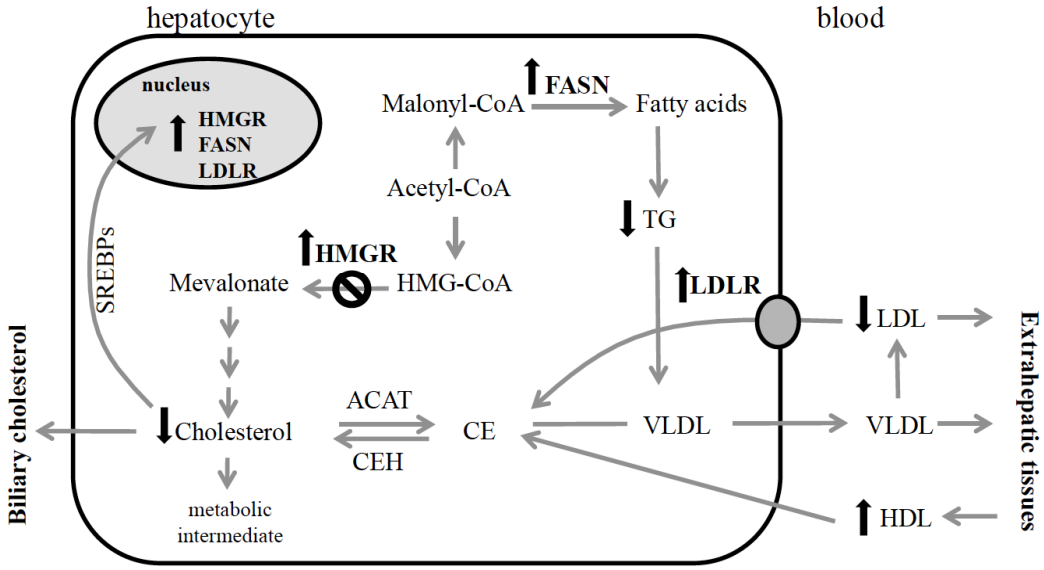


Fig. (2). Model depicting HMG flavanones enriched fraction effects on lipids metabolism elicited in rat hepatocytes (from Di Donna *et al* 2014 [61]). Black arrows indicate HMG flavanones enriched fraction effects on genes, enzymes and metabolites levels. ⃠ indicates enzymatic inhibition. ACAT: Acyl-CoA:cholesterol acyltransferase; CE: cholesteryl ester; CEH: cholesteryl ester hydrolase; FASN: fatty acid synthase; HDL: high-density lipoprotein; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase; LDL: low density lipoprotein; LDLR: low density lipoprotein receptor; SREBPs: sterol response element binding proteins; TG: triglycerides; VLDL: very low density lipoprotein.

hypercholesterolemia [89], a significant decrease in renal lipid peroxidation was observed after bergamot juice administration, as shown by the low malondialdehyde levels found. Furthermore, analysis of kidney histopathological sections supported the biochemical data, indicating a protective effect of bergamot juice on the development of kidney injury induced by the hypercholesterolemic diet. The authors hypothesized that the beneficial effect on renal parenchyma was due to the great abundance of flavonoids in bergamot juice, believed to reduce oxidative damage *in vivo*.

CONCLUSION

HMGR inhibitors (statins) are the most effective, practical and largely prescribed class of drugs for reducing LDL concentrations [10, 11]. Nevertheless some patients, especially those with metabolic syndrome do not achieve their recommended LDL targets with statin therapy [77, 90]. Moreover, statins may induce many side effects, including myalgia, myopathy, liver diseases and rhabdomyolysis [91]. Many studies demonstrated a relationship between the intake of flavonoid-rich foods and a reduced risk for cardiovascular disease [13]. Bergamot fruit is very rich in many peculiar bioactive flavonoids compared to other *Citrus* fruits [15-18], hence their evaluation as metabolic regulators might represent an attractive strategy in drug discovery. The aim of this review is to provide an overview on all flavonoids detected in *C. bergamia* Risso and on the current knowledge of their hypolipidemic effects [58-69], summarizing the results obtained from different *in vivo* studies [61, 75, 77, 89].

All data reported suggest that *C. bergamia* Risso flavonoids might be used in nutraceutical products or in functional foods, even if additional studies are needed to fully reveal their interaction with upstream mediators of lipid metabolism pathways. Furthermore, since only few studies on flavonoids administration in humans are available, further clinical studies are required to focus on dose, bioavailability, efficacy and safety of this class of flavonoids in humans.

LIST OF ABBREVIATIONS

ABC = ATP-Binding Cassette

AMPK = AMP- activated protein kinase

COX-1 = cyclooxygenase-1

COX-2 = cyclooxygenase-2

FASN = fatty acid synthase

HDL = high-density lipoprotein

HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA

HMGR = 3-hydroxy-3-methylglutaryl-CoA reductase

HUVECs = human umbilical vein endothelial cells

LDL = low-density lipoprotein

LDLR = low-density lipoprotein receptor

LXRs = liver X receptors

microRNAs = miRNAs

NPC1L1 = Niemann-Pick C1-Like 1

PPARs = peroxisome proliferator activated receptors

SREBPs = sterol regulatory element-binding proteins

TC = total cholesterol

TG = triglicerides

TNF-α = tumor necrosis factor-α

VLDL = very low-density lipoprotein

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIALS

Supplementary material is available on the publisher's web site along with the published article.

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