Regulation of PCSK9 by nutraceuticals

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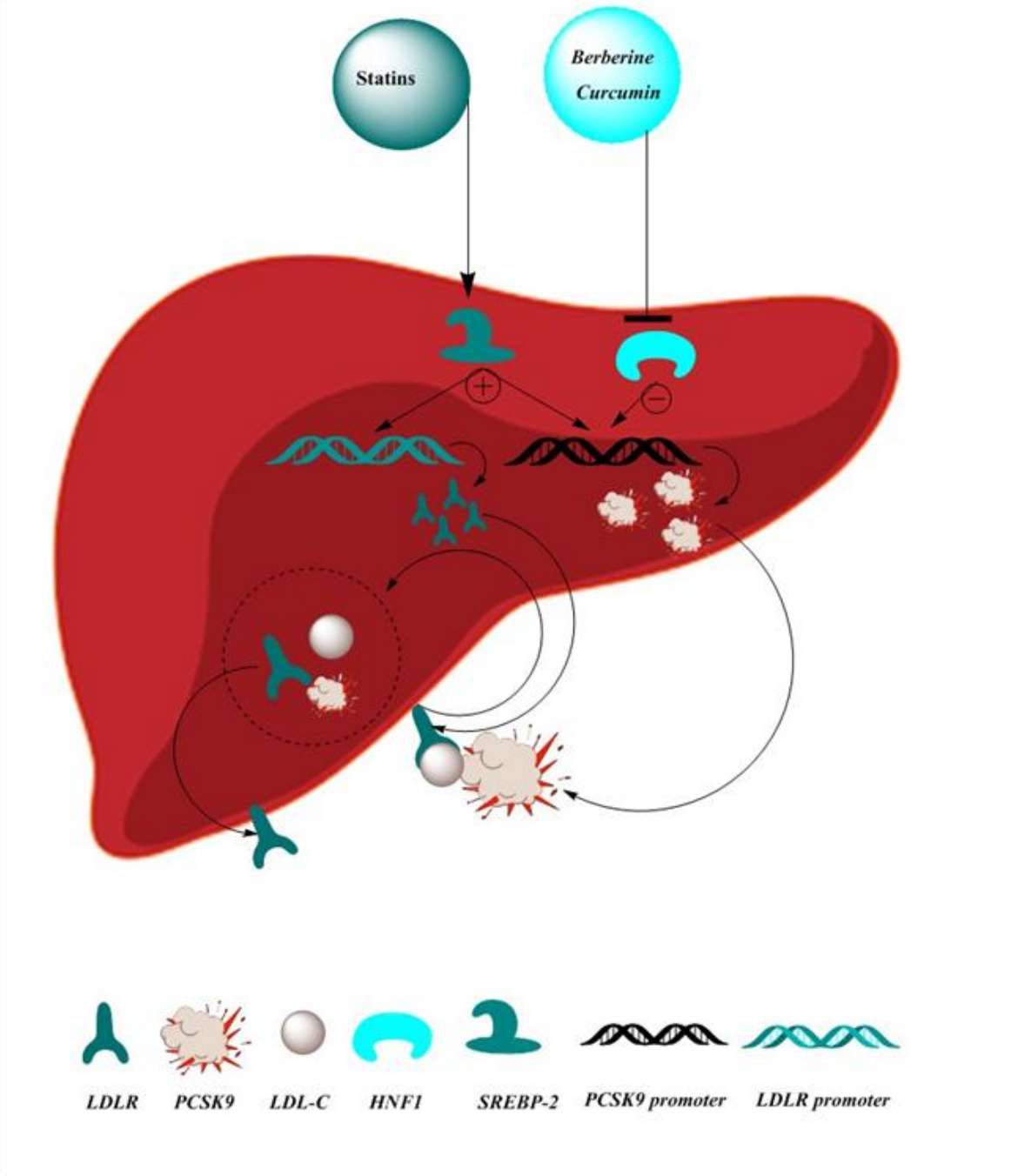
PCSK9, a critical inhibitor of LDLR, is up-regulated by both HNF1α and SREBP-2 transcription factors. Besides PCSK9, SREBP-2 up-regulates *LDLR* gene. Nutraceuticals, including curcumin and berberine, can decrease plasma LDL-C levels through elevation of the hepatic LDLR *via* inhibiting HNF1α which is a specific transcription factor for *PCSK9* gene. Statins increase the expression of both *PCSK9* and *LDLR* through the activation of SREBP-2, resulting in PCSK9-mediated attenuation of their effects.



Abstract

PCSK9 (proprotein convertase subtilisin kexin type 9) is a liver secretory enzyme that regulates plasma low-density lipoprotein (LDL) cholesterol (LDL-C) levels through modulation of LDL receptor (LDLR) density on the surface of hepatocytes. Inhibition of PCSK9 using monoclonal antibodies can efficiently lower plasma LDL-C, non-high-density lipoprotein cholesterol and lipoprotein (a). PCSK9 inhibition is also an effective adjunct to statin therapy; however, the cost-effectiveness of currently available PCSK9 inhibitors is under question. Nutraceuticals offer a safe and cost-effective option for PCSK9 inhibition. Several nutraceuticals have been reported to modulate PCSK9 levels and exert LDL-lowering activity. Mechanistically, those nutraceuticals that inhibit PCSK9 through a SREBP (sterol-responsive element binding protein)-independent pathway can be more effective in lowering plasma LDL-C levels compared with those inhibiting PCSK9 through the SREBP pathway. The present review aims to collect available data on the nutraceuticals with PCSK9-inhibitory effect and the underlying mechanisms.

Keywords: PCSK9, berberine, curcumin, phytochemical, hyperlipidemia

Abbreviations:
ALA: alpha-linolenic acid,
ApoB apolipoprotein B,
ApoER2: apolipoprotein E receptor2
APMF: aqueous extract of PM fruit
ASCVD: atherosclerotic cardiovascular disease,
COMIT: canola oil multicenter intervention trial,
CGN: cerebellar granule neurons,
DHA: docosahexaenoic acid;
DPA: Docosapentaenoic acid,
EPA: eicosapentaenoic acid,
ER: endoplasmic reticulum
EGF-A: epidermal growth factor-like repeat A
FH: familial hypercholesterolemia
GK: glucokinase
HeFH: heterozygous familial hypercholesterolemia
HNF1: hepatocyte nuclear factor1
HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A
LPS: lipopolysaccharide
LDL: low-density lipoprotein
LDL-C: low-density lipoprotein cholesterol
LDLR: low-density lipoprotein receptor
mAb: monoclonal antibodies
PUFA: polyunsaturated fatty acid
PM: *Phaleria macrocarpa*
PCSK9: proprotein convertase subtilisin kexin type 9
Q3G: quercetin-3-O-b-D-glucoside
SRE: sterol regulatory element
SREBP: sterol regulatory element-binding protein
VLDLR: very low-density lipoprotein receptor
LRP1: low-density lipoprotein receptor-related protein
XZK: Xuezhikang

PCSK9: Biogenesis and physiological function

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of cholesterol homeostasis that controls low-density lipoprotein (LDL) receptor (LDLR) density on the surface of hepatocytes. PCSK9 is a soluble member of the mammalian proprotein convertase family of serine proteases [1], which is synthesized and secreted mainly by the liver, and in lower extent by other tissues such as the kidney, the small intestine, the central nervous system, the pancreas, the colon epithelium and the vascular smooth muscle cells [2-6]. *PCSK9* gene, located in chromosome 1p33–34.3 close to the third genetic locus associated with familial hypercholesterolemia (FH)[2], encodes an inactive glycoprotein (i.e. pre-PCSK9) with 692 amino acids comprising a signal sequence followed by a subtilisin-like catalytic domain and a C-terminal domain [7-9]. Once the signal peptide is cleaved from pre-PCSK9 in the endoplasmic reticulum (ER), pro-PCSK9 (the soluble zymogen) is formed and then converted to mature secretory PCSK9 through autocatalytic cleavage of the prodomain in the Golgi apparatus [10, 11]. After PCSK9 maturation, prodomain stays noncovalently bound to the active site of the catalytic domain, obstructing further enzymatic activity of PCSK9, but serving as a chaperone [12, 13]. While the catalytic domain of mature PCSK9 binds to the extracellular epidermal growth factor-like repeat A (EGF-A) domain of LDLR, the C-terminal domain of PCSK9 is required to bind with cell surface proteins such as annexin A2 [14]. The best known function of PCSK9 is the post-translational regulation of LDLR in hepatocytes [15], representing the major route for LDL cholesterol (LDL-C) clearance from the blood circulation [16, 17]. Mechanistically, PCSK9 binds to the extracellular EGF-A domain of the hepatic LDLR and promotes lysosomal degradation of LDLRs through two independent intra- and extra-cellular ways. In the relatively faster mode, the intracellular pathway, PCSK9 binds to the EGF-A

domain of the newly formed LDLR in the *trans*-Golgi network, where the PCSK9-LDLR complex is targeted to the lysosome [18]. In the extracellular pathway, the secreted PCSK9 circulates in the bloodstream and binds to the EGF-A domain of the LDLR on the surface of hepatocytes, and escorts it into the lysosome compartment through clathrin-mediated endocytosis [19]. Given that normal trafficking of the LDLR back to the cell surface is dependent on the EGF-A domain [20-22], binding of PCSK9 to this domain inhibits recycling of the LDLR to the cell surface and enhances lysosomal degradation of LDLR [23, 24]. Consequently, there are not many LDLR remaining to clear LDL-C from the bloodstream when plasma PCSK9 levels are elevated as a result of gain-of-function mutations. Conversely, when there is low or no PCSK9 in the circulation as a result of loss-of function mutations, there will be more intact LDLR which in turn trap more LDL-C from the bloodstream [25].

PCSK9: Transcriptional regulation

It is known that the proximal promoters of either *PCSK9* or *LDLR* genes contain a functional sterol regulatory element (SRE) that is targeted by sterol-responsive element binding proteins (SREBPs) in response to alterations in intracellular levels of cholesterol [26]. Sterol-dependent regulation of both *PCSK9* and *LDLR* genes have been found to be mediated by SREBP-2 transcription factor [27, 28]. Specifically, SREBP-2 is able to upregulate the expression of both PCSK9 and LDLR in states of intracellular cholesterol depletion. Beside the SRE region, promoter region of *PCSK9* also involves a hepatocyte nuclear factor1 (HNF1) response site that binds predominantly to HNF1α, an essential transcription factor for basal expression of *PCSK9* [26]. HNF1α is also involved in SREBP-2-induced maximal *PCSK9* gene expression in response to intracellular cholesterol depletion in HepG2 cells [29]. Site-directed mutagenesis studies have indicated that the HNF1 regulatory site works cooperatively with the SRE and mutations of

HNF1 may reduce the sensitivity of the promoter to sterols and also transcriptional regulatory activity of SREBP-2 on the *PCSK9* promoter [29]. Expression of *PCSK9* is found to be abundant in the liver, possibly due to the rich content of HNF1α transcription factor in the hepatic tissue [29]. Considering the fact that *PCSK9* expression can also be regulated by HNF1 in an LDLR-independent manner, HNF1 inhibition may be an alternative strategy for specific reduction of circulating PCSK9 levels.

Statin therapy and PCSK9

Statins, the mainstay of pharmacotherapy for dyslipidemia, indirectly increase the expression of both *PCSK9* and *LDLR* through activation or nuclear translocation of SREBP-2 (Figure 1) [27]. Statins act mainly through inhibiting 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase which is the rate-limiting enzyme in the cholesterol biosynthesis pathway [30]. Statin-mediated HMG-CoA inhibition is paralleled by simultaneous intracellular cholesterol depletion. Mechanistically, intracellular cholesterol depletion activates the transcription factor SREBP-2 that can induce *LDLR* gene expression and improve hepatic uptake of plasma LDL-C. On the other hand, *PCSK9* expression has also been found to be strongly upregulated by SREBP-2 following cholesterol depletion [31-34]. This paradoxical effect of statins on *PCSK9* expression can limit their LDL-C-lowering capacity and partially explain the log-linear dose-response effect of these drugs. Moreover, increase in plasma PCSK9 levels during statin therapy has been associated with increased cardiovascular risk [35]. Therefore, the use of a PCSK9 inhibitor in combination with statins can be an effective strategy for reducing plasma LDL-C concentrations by counteracting the statin-induced *PCSK9* overexpression.

PCSK9 inhibition: a novel efficient cholesterol-lowering approach

The importance of PCSK9 inhibition as another lipid-lowering treatment has been anticipated by early studies that showed the causative effect of dominant gain-of-function mutations in the *PCSK9* gene on FH [36], while loss-of-function mutations were associated with hypocholesterolemia and protection against coronary artery disease [37-40]. Cohen et al. were the first to show the association of *PCSK9* polymorphism with both plasma LDL-C levels and cardiovascular risk in humans. They reported that loss-of-function mutations in the *PCSK9* gene are associated with 28-44% reduction in plasma LDL-C concentrations and up to 88% decrease in cardiovascular risk, showing the important role of *PCSK9* mutations in the regulation of cholesterol homeostasis [37, 38]. Furthermore, it was found that plasma PCSK9 levels are positively associated with circulating LDL-C levels in the ethnically diverse populations enrolled in the Dallas Heart Study and the JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) trial [41, 42].

The pivotal role of PCSK9 in the metabolism of LDL and LDLR as well as the verified safety of PCSK9 inhibition led to the development of PCSK9 inhibitors [43]. Novel PCSK9 inhibitors, including monoclonal antibodies (mAbs), active immunotherapy, small-molecule inhibitors, small interfering RNAs (siRNAs), and antisense oligonucleotides (ASOs) have been shown to profoundly reduce plasma LDL-C [43-45]. Although the development of two antisense oligonucleotides (BMS-84421 and SPC5001) and one humanized monoclonal antibody (bococizumab) was stopped, two FDA-approved human mAbs against circulating PCSK9 are already in the market:: evolocumab (Repatha®) and alirocumab (Praluent®). Both mAbs are indicated for FH or clinical atherosclerotic cardiovascular disease (ASCVD) requiring additional LDL-C lowering[46, 47][46, 47][46, 4746, 47][46,47]. The EMA has also approved both these drugs for the treatment of adults with primary hypercholesterolemia, such as those with HeFH and

mixed dyslipidemia who cannot achieve LDL-C levels with the maximally tolerated doses of statins and/or other lipid-lowering drugs as well as those who cannot tolerate statins or for whom statins are contra-indicated; evolocumab has also been approved for HoFH [48, 49]. These mAbs are the most potent cholesterol-lowering agents that can reduce LDL-C by up to 73% in patients with high LDL-C on maximally tolerated statin therapy and ezetimibe, including adult patients with heterozygous or homozygous FH, or statin-resistant patients with ASCVD [50-52]. Despite their efficacy, the cost-effectiveness of PCSK9 mAbs for the treatment of FH, which could be life-long, is questionable. According to a recent analysis, the use of PCSK9 inhibitors for heterozygous FH and ASCVD costs > $14,000 per patient per year, which is not cost-effective unless the annual costs can be reduced to $4,536 (the threshold to meet $100,000 per quality-adjusted life-year) [53]. This highlights the need for cheaper PCSK9 inhibitors that could serve either as alternative or as adjunct to PCSK9 mAbs in order to reduce the required dose and cost of treatment. PCSK9 inhibitors may also exert certain adverse events that may limit their clinical use. Therefore, we are waiting for the results of RCTs to demonstrate whether the PCSK9 inhibitors have any unexpected adverse effects due to their effectiveness (LDL-C levels < 40 mg/dL) and/or effects resulting from the loss of PCSK9 functions at other sites in the body, in particular regarding neurocognition [54-57].

Nutraceuticals as promising PCSK9 inhibitors

Nutraceuticals are either functional foods or dietary supplements with health benefits besides their basic nutritional value [58]. Several nutraceuticals may exert lipid-lowering, anti-atherosclerotic, anti-inflammatory and antioxidative properties [59]. Among lipid-lowering nutraceuticals, those inhibiting PCSK9 expression or activity can be considered as effective additions to the lipid-lowering armamentarium. As mentioned above, the paradoxical effects of

statins on *PCSK9* expression, leading to attenuated lipid-lowering effects at higher doses, can potentially be negated by PCSK9 inhibitors. Nutraceuticals with potential PCSK9 inhibitory effects can offer several advantages over current PCSK9 inhibitor drugs. First, nutraceuticals are usually widely available at considerably lower prices compared with agents such as mAbs, and second, most nutraceuticals generally possess lower toxicity compared with synthetic/chemical agents owing to their natural origin and the limited doses that are routinely consumed as a part of diet. On the other hand, it should be pointed out that at the doses currently allowed for human use, therapeutic effects of most nutraceuticals having PCSK9 inhibitory activity is far to be comparable to those obtained with mAbs. In the following sections, we will review nutraceuticals that have been shown to alter PCSK9 status, either through modulation of *PCSK9* expression or mature protein secretion.

Berberine

Berberine is a natural cholesterol-lowering agent and an alkaloid present in a number of plants such as *Berberis aristata*, *Berberis vulgaris*, *Coptis chinensis* [60]. Mechanistically, berberine increases the uptake of LDL-C by enhancing the stability of LDLR mRNA and increasing hepatic LDLR density [61]. Berberine stabilizes LDLR mRNA *via* activating regulatory proteins located downstream of the extracellular signal regulated kinase (ERK) pathway which interact with the proximal sequences in the 3′ untranslated region (UTR) of the LDLR mRNA [62]. Besides stabilization of LDLR mRNA, berberine increases the stability of LDLR protein in the surface of hepatocytes through regulation of *PCSK9* expression. Several *in vitro* and *in vivo* studies have shown berberine-mediated modulation of PCSK9 expression and are discussed in details as follows.

In vitro studies

Cameron et al. showed that at a non-toxic concentration (15µg/ml) berberine decreases *PCSK9* mRNA and protein levels in HepG2 cells by 77 and 87%, respectively, which were associated with a 3-fold increase in the mRNA expression of LDLR [63]. Furthermore, when HepG2 cells were concomitantly treated with berberine and mevastatin, the PCSK9 mRNA–raising effect of mevastatin was suppressed, whereas the LDLR-rising effect was enhanced [63]. Similarly, in another study on HepG2 cells, berberine (20 µM) was found to decrease mRNA expression of *PCSK9* by 23% at 24 h, while increase mRNA expression of LDLR by 1.8-3 folds at 4 and 24 h, respectively. In addition it was reported that berberine could contract the stimulatory effect of statins on *PCSK9* expression [29]. Consistent with the above-mentioned results, Dong et al. reported that berberine (40 µM) could significantly decrease protein and mRNA expression of *PCSK9* in HepG2 cells [64]. Beyond LDLR and cholesterol metabolism regulation, PCSK9 has been found to affect systemic and central nervous system through modulation of other lipoprotein receptor family members, including apolipoprotein E receptor2 (ApoER2), very-low-density lipoprotein receptor (VLDLR) and low-density lipoprotein receptor-related protein1 (LRP1) [65-67]. Berberine can exert both neuroprotective [68-70] and neurotoxic effects [70] depending on the applied concentration; this dual effect has been proposed to be mechanistically related to the regulation of neuronal lipoprotein receptor expression [71]. To evaluate the impact of stress on neuronal lipoprotein receptors and *PCSK9* expression in the nervous system, cerebellar granule neurons (CGN) were treated with a neuronal stressor but non-toxic concentration of berberine (1 µM) [71]. Results revealed that berberine at the concentration of 1 µM exerts differential effects on the expression of *PCSK9* and lipoprotein receptors. mRNA expression of *PCSK9* and *LDLR* were found to be reduced by 0.6 and 0.42 folds, respectively, while the expression of *APOER2* remained unaffected. In addition, mRNA levels of *VLDLR* and

LRP1 were upregulated by 2.64 and 1.94 folds, respectively [71], which can be justified by the downregulation of PCSK9 and subsequent reduction of receptor degradation. Berberine has been shown to affect expression of two important *PCSK9* transcription factors, SREBP-2 and HNF1α [29]. As reported above, despite *PCSK9* reduction in CGN treated cells, *LDLR* was also decreased which is not expected considering the observations in berberine-treated hepatocytes. This discrepancy may be attributed to the lack of hepatocyte-specific transcription factor HNF-1 in neurons [71]. Hence, the lack of HNF-1 might abrogate part of the inhibition of *PCSK9* expression and, consequently, part of the LDLR availability. Transcription of both *PCSK9* and *LDLR* are known to be controlled by SREBP, and berberine downregulates *LDLR* and *PCSK9* expression in a SREBP-dependent fashion in a CGN model, potentially through an increased lipid uptake via increased VLDLR and LRP1 levels [71].

In vivo studies

There is *in vivo* evidence on the effect of berberine treatment on PCSK9 levels in hypercholesterolemic animal models. Dong et al. evaluated the changes in plasma PCSK9 concentrations and hepatic LDLR expression in high fat diet-fed (HFD) mice and hamsters treated with 200 mg/kg/day and 100 mg/kg/day berberine, respectively [64]. Results from the berberine-treated mice indicated that serum PCSK9 levels and its liver mRNA expression were decreased by 50 and 46%, respectively, after 16 days of treatment; these changes were accompanied by increased protein levels of liver LDLR (67%) and improved lipid profile in HFD mice as compared with controls [64]. To evaluate the reproducibility of these results in other animal models, Dong et al. investigated the effect of 7-day treatment of HFD hamsters with 100 mg/kg/day berberine. Serum PCSK9 levels were reduced by 30% in the treated group as compared with control hamsters [64]. Similarly, De-liang et al. evaluated the effect of berberine

(156 mg/kg/day) and a structurally modified form of berberine, 8-hydroxydihydroberberine (78, 39 and 19.5 mg/kg/day), on HFD rats [72]. Both compounds significantly reduced liver PCSK9 protein levels, which were associated with increased levels of liver LDLR protein and subsequent reduction of plasma cholesterol levels [72]. On the other hand, it has been shown that lipopolysaccharide (LPS), an inflammatory agent, can induce PCSK9 expression and also reduce the protein levels of hepatic LDLR [73], leading to decreased hepatic clearance of circulating LDL-C and elevated plasma LDL-C levels [74, 75]. In this context, Xiao et al. reported that stimulation of PCSK9 expression, attenuation of liver LDLR and resultant elevation of plasma LDL-C can be prevented through a 4-week administration of 10 or 30 mg/kg/day berberine in C57BL/6 mice co-administrated with 5 mg/kg/day LPS [76]. The researchers also investigated the protective effect of berberine in HFD C57BL/6 mice pretreated with berberine (10 or 30 mg/kg/day) plus LPS (5 mg/kg/day). After 4 weeks of pretreatment, mice fed with the high-cholesterol diet showed decreased and increased mRNA expression of liver *PCSK9* and *LDLR*, respectively, which were associated with reduced levels of plasma LDL-C [76]. A clinical trial conducted by Pisciotta et al. investigated the impact of a nutraceutical pill containing berberine in heterozygote FH (HeFH) patients intolerant or resistant to statins [77]. The results showed that supplementation with the berberine-contained pill can reduce LDL-C by 10.5% in HeFH patients, which was suggested to be associated with an indirect berberine-mediated inhibitory effect on PCSK9 [77].

Notably, contradictory results were obtained when Jia et al. evaluated the effects of berberine treatment (400 mg/kg/day) for 6 weeks in HFD rats [34]. Interestingly, berberine was found to significantly increase plasma levels of PCSK9 in HFD rats, and increase levels of both mRNA and protein expression of hepatic LDLR, and decrease plasma LDL-C concentrations in HFD

rats when compared with control rats [34]. Such variations in *PCSK9* response to berberine could be due to the genetic polymorphisms in the promoter of *PCSK9* and *LDLR* genes that may affect interaction of berberine with the promoter and subsequently alter gene transcription [78].

Anti-PCSK9 mechanisms of berberine

The overall trend in *in vitro* and *in vivo* findings has been in favor of a PCSK9-lowering effect for berberine that could justify the lipid-lowering activity of this nutraceutical through enhanced LDLR density on the surface of hepatocytes. As mentioned above, PCSK9 transcription is mainly controlled by the two neighboring regulatory sites, SRE and HNF1 binding sites, which are 100% preserved sequences in the PCSK9 promoter in human, mouse and rat [29]. Mutagenesis studies in HepG2 cells have shown that: 1) HNF1 site is the key regulatory motif, 2) HNF1α is the essential cofactor for SREBP-2 in the transcriptional regulation of the *PCSK9* gene, and, 3) HNF1 is thought to be more effective than SREBP in lipid-mediated regulation of *PCSK9* [29]. Overall, berberine has been suggested to inhibit *PCSK9* transcription through mechanisms independent of sterol-mediated pathways [29, 63, 64, 72]. *In vitro* studies have shown that both PCSK9 and HNF1α protein levels are decreased in berberine-treated HepG2 cells. In contrast to the concomitant reduction of both proteins, mRNA expression of *HNF1α* and *SREBP-2* were found to remain unaltered in treated cells, whereas mRNA expression of *PCSK9* was reduced [29, 64]. These findings are underpinned by *in vivo* studies on berberine-treated mice and hamsters showing that the reduction of mRNA and circulating protein levels of PCSK9 is associated with decreased levels of liver HNF1α protein (42%) and increased levels of LDLR protein (67%) on the surface of hepatocytes, while mRNA expression of liver HNF1α, SREBP-1, SREBP-2, and LDLR, as a target gene of SREBP-2, were unaffected [64]. These findings are confirmed by another study that indicated SREBP-2 levels are unchanged in berberine-treated

rats [72]. It appears that berberine inhibits HNF1α-mediated *PCSK9* transcription by reducing hepatic HNF1α protein content but without affecting its mRNA levels. This hypothesis is consistent with data showing that berberine reduces cellular HNF1α protein in post-translational regulation via ubiquitin-induced proteasomal degradation [64]. Considering the fact that many lipid-lowering drugs, such as statins, up-regulate LDLR through stimulation of SREBP leading to PCSK9-mediated drug resistance in dyslipidemia, berberine could be regarded as a useful adjunct to statin therapy owing to its SREBP-independent inhibition of PCSK9.

Curcumin

Curcumin is a polyphenolic compound which is extracted from the rhizomes of *Curcuma longa* (turmeric). This nutraceutical is endowed with numerous pharmacological activities that are beneficial to human health including lipid-lowering, antitumor, immunomodulatory, anti-inflammatory, antioxidant, anti-ischemic, analgesic, anti-arthritic, anti-dyspeptic, anti-depressant and hepatoprotective effects [79-105]. This wide spectrum of pharmacological effects of curcumin is due to the multiple molecular targets of this compound [85, 106-111]. With respect to lipid metabolism, several experimental and clinical studies have shown that curcumin improves dyslipidemia and decreases serum lipid peroxides, cholesterol and triglycerides levels [112-117]. From the mechanistic standpoint, curcumin has been demonstrated to regulate several key targets involved in lipid metabolism and homeostasis including LDLR, Niemann-Pick C1-Like 1 protein, SREBP-1, apolipoprotein B-100, peroxisome proliferator-activated receptor-α and fatty acid synthase.

To further explore the molecular mechanisms underlying the lipid-lowering effects of curcumin, Tai et al. investigated the effect of 5-20 μM curcumin on the protein and gene expression of PCSK9 and LDLR in human hepatic HepG2 and Huh7 cell lines [118]. The results indicated that

curcumin, at 10 and 20 μM concentrations, could decrease *PCSK9* mRNA levels by up to 31 and 48%, respectively, in parallel with significant reductions in intracellular and secreted PCSK9 protein in both cell lines [118]. Furthermore, while curcumin had no significant effect on mRNA levels of *LDLR*, it enhanced the density (20-35%) and activity (LDL-C uptake) of LDLR protein on the surface of HepG2 cells. These findings revealed that curcumin can increase LDLR *via* post-translation regulation through inhibition of PCSK9 [118]. The aforementioned results are supported by two other studies that showed curcumin and its derivate, curcumin trinicotinate, to improve LDL-C uptake through inhibition of *PCSK9* expression and upregulation of LDLR density on the surface of HepG2 hepatocytes [118, 119]. Moreover, curcumin was found to reduce *PCSK9* expression in HepG2 cells *via* inhibition of HNF-1α transcription factor [118], an important PCSK9 regulator [120-122].

Polydatin

Polydatin (resveratrol-3-O-β-mono-d-glucoside), also called piceid, is a resveratrol glycoside and a main bioactive component of *Polygonum* cuspidatum**.** Polydatin possesses several pharmacological activities including anti-allergic [123], antioxidant [124, 125], anti-inflammatory [126-128], anti-tumor [129], anti-diabetic [130], hypolipidemic [129, 131] and cardioprotective effects [132, 133]. Polydatin has ameliorating effects on lipid and glucose metabolism in type 2 diabetes mellitus [130]. In this context, Wang et al. evaluated the role of *PCSK9* in the anti-diabetic effects of polydatin *in vitro* and *in vivo* [134]. The *in vitro* study was conducted on palmitic acid-induced insulin resistant HepG2 cells treated with polydatin (resveratrol-3-O-β-mono-d-glucoside). The results showed that the protein level of PCSK9, which was elevated in insulin resistant cells, is decreased by polydatin treatment (20 μM for 24 h) [134]. Mechanistically, polydatin inhibits both the protein expression and interaction of

PCSK9 with LDLR. Also, it was shown that polydatin increases protein expression of glucokinase (GK) in insulin resistant HepG2 cells through a mechanism involving PCSK9 inhibition [134]. GK plays an important role in glucose metabolism through phosphorylation of glucose to glucose 6-phosphate which is a key mediator in glycogen synthesis, glycolysis, and the pentose phosphate pathway [135]. It has been reported that GK expression is decreased in diabetic mice [136]. In accordance with *in vitro* studies, polydatin was found to improve glucose metabolism in female db/db C57BL/6 mice through *PCSK9*-dependent upregulation of GK [134]. It was also revealed that gene expression and protein levels of PCSK9 in the liver tissue and serum of db/db C57BL/6 mice treated with polydatin are significantly decreased and liver GK protein levels are increased [134]. Based on *in vitro* and *in vivo* studies, it was concluded that inhibition of PCSK9 could modify glucose metabolism and thereby ameliorate diabetic complications *via* increasing liver GK expression [134].

Xuezhikang and red yeast rice

Red yeast rice, widely marketed as cholestin, is a product of yeast that is grown on rice, and has documented cholesterol-lowering effects [137, 138] by inhibiting cholesterol synthesis via the suppression of HMG-CoA reductase [139, 140]. Xuezhikang (XZK) is a cholestin extract that contains a mixture of lovastatin (dominant compound), plant sterols and isoflavones. XZK has shown efficient lipid-lowering effects and it is well tolerated in patients with statin intolerance [141, 142]. Recently, Yan-jun et al. investigated the effect of short-term (1200 mg/kg/day for 3 days) and long-term (1200 mg/day for 8 weeks) XZK treatment in rats and dyslipidemic patients, respectively [143]. Experimental results showed that the short-term treatment with XZK could increase plasma PCSK9 levels by 70% in rats after 3 days, while there were no significant effects on lipid profile parameters such as LDL-C and HDL-C. It was also found that mRNA expression

of liver SREBP-2 and LDLR were markedly upregulated in XZK-treated rats. Furthermore, data from a clinical trial in XZL-treated patients indicated that plasma PCSK9 levels were increased by 34%, while LDL-C and total cholesterol were decreased by 28 and 22%, respectively, after 8 weeks of treatment [143]. Mechanistically, it appears that XZK induces PCSK9 through the SREBP-2 pathway but further investigations are required to unravel the exact mechanism. Based on the evidence showing that red yeast rice-induced *PCSK9* overexpression, combining red yeast rice preparations with one or more nutraceuticals with PCSK9-inhibitory activity might be of experimental and clinical value. In this regard, a nutraceutical pill containing both red yeast rice and berberine has demonstrated significant lipid-lowering activity [144-146].

Omega-3 fatty acids

Dietary intake of *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – that are frequently found in fish oil – and alpha-linolenic acid (ALA) – that is commonly found in plant oils – [147], is essential as *n*-3 PUFAs play numerous important roles in maintaining human health including regulation of blood pressure, coagulation and inflammation (through conversion into signaling molecules such as eicosanoids and docosanoids) [148]. *n*-3 PUFAs have also documented cholesterol-lowering and atheroprotective properties in animal models [59, 148]. Yuan et al. investigated the protective effect of *n*-3 PUFA-enriched fish oil consumption (10% in diet) in male rats fed with Western style high-fat and high-cholesterol diet (WD) [149]. The results showed that long-term intake (16 week) of *n*-3 PUFA-enriched fish oil can protect against WD-induced hypercholesterolemia *via* reducing hepatic *PCSK9* expression [149]. Reduced *PCSK9* expression was associated with a 84% reduction in plasma LDL-C levels in fish oil-fed rats compared with control rats [149]. Similarly, Sorokin et al. reported that both mRNA and circulating PCSK9 levels were

significantly reduced (by 70%) in ApoE$^{-/-}$ female mice treated with omega-3 FA-rich (OR) diet (1.8 g omega-3 FAs/kg diet per day) or omega-3 FA-rich plus aspirin (ORA) diet (0.1 g aspirin/kg diet per day) for 13 weeks [150]. Notably, circulating PCSK9 levels were found to be lower in the ORA diet group compared with the OR diet group, though treatment with aspirin alone showed no significant effects on plasma PCSK9 levels [150]. Furthermore, the reduction of PCSK9 levels in both OR and ORA diet groups was associated with 40% less plasma cholesterol in very low-density lipoprotein (VLDL) and LDL fractions as compared with the control group, whereas, as demonstrated by *en face* analysis and hematoxylin and Movat staining, the atherosclerotic plaque area was found to be reduced only in the ORA group [150].

The aforementioned findings are supported by those of Rodriguez-Perez et al. study that analyzed data from the Canola Oil Multicenter Intervention Trial (COMIT), a randomized double-blind controlled crossover feeding trial in which volunteers consumed diets with one of the following oil interventions: (1) canola oil; (2) canola oil rich in DHA; and (3) high-oleic acid canola oil [151]. It was shown that enrichment of canola oil with DHA (by 6%) lowers circulating PCSK9 and triacylglycerol levels compared with canola and canola oleic diets [151]. Furthermore, circulating PCSK9 levels were found to be significantly and positively associated with LDL-C, triacylglycerol and apolipoprotein B (apoB) levels, while no association was found between PCSK9 and HDL-C levels [151]. In addition, circulating PCSK9 levels were shown to be positively associated with plasma cholesterol synthesis markers, including lathosterol and desmosterol, in all three intervention groups, suggesting that circulating PCSK9 concentrations are sensitive to cholesterol synthesis [152]. Likewise, a *PCSK9* transcription factor, SREB1c is proposed to be inhibited by DHA, subsequently decreasing the expression of *PCSK9* [153]. In this context, Graversen et al. showed that the daily consumption of 2.2 g marine *n*-3 PUFAs

(containing 38.5% EPA, 25.9% DHA and 6.0% docosapentaenoic acid (DPA)) for 12 weeks can decrease circulating PCSK9 levels by 11.4 and 9.8% in premenopausal and postmenopausal women, respectively (120). In contrast, plasma LDL-C levels showed no significant changes [154]. Of note, it was shown that *n*-3 PUFAs can inhibit SREBP-2 through elevating hepatic cholesterol content which leads to decreased expression of *PCSK9* as a target gene of SREBP-2 [152]. It was also suggested that *n*-3 PUFAs can activate PPAR-α that downregulates *PCSK9* expression [155, 156].

Phytosterols

Phytosterols, including plant sterols and stanols, are steroid compounds similar to cholesterol which occur in plants [157]. Stanols are saturated forms of sterols lacking any double bond in the sterol ring. Phytosterols decrease the intestinal absorption of cholesterol in humans and can lower plasma LDL-C levels by up to 10% at daily intakes of 2-2.5 g [157]. While the exact mechanism of action is still uncertain, it has been suggested that phytosterols interfere with the intestinal and hepatic metabolism of sterols, thereby impeding incorporation of cholesterol in chylomicrons [158]. In a randomized controlled double-blind trial, Simonen et al. evaluated the effect of a 6-month consumption of plant stanol fatty acid esters (3 g/day) on circulating PCSK9 levels in normal and hypercholesterolemic subjects [159]. The results showed that long-term intake of plant stanol esters exerts no significant effect on circulating PCSK9 concentrations and hepatic LDLR levels in normo- to moderately hypercholesterolaemic subjects, but decrease plasma LDL-C by 7-10% [159]. This finding implies that long-term consumption of plant stanol esters can lower LDL-C through inhibition of cholesterol absorption, without interfering with PCSK9 metabolism and subsequently the LDL receptor-mediated cellular cholesterol uptake and clearance. However, conflicting results were obtained when the effect of the acute intake of 50

mg plant stanol esters (composed of 70% sitostanol and 30% campestanol esterified with a fatty acid blend containing 80% linoleic acid, 15 % oleic acid and 5% stearic and palmitic acids) was evaluated in C57BL/6J mice [158]. Smet et al. reported that acute oral intake of such a composition of plant sterol esters can up-regulate mRNA expression of intestinal *PCSK9* and *LDLR* and their main transcription factor, *SREBP-2*, whereas hepatic expression of these genes were down-regulated after 15 minutes following oral intake [158]. These alterations were associated with reduced intestinal cholesterol absorption and decreased plasma LDL-C levels [158]. While the data is still few to be conclusive, it appears that long-term and acute administration of plant stanol esters can exert different effects on PCSK9 and LDLR expression, whereas both routes efficiently decrease plasma LDL-C levels and intestinal cholesterol absorption.

Flavonoids

Flavonoids include a broad class of polyphenolic molecules which occur in vegetables, fruits and plant-derived juices, such as coffee, tea and wine. Several lines of evidence from clinical trials have verified the lipid-lowering and cardioprotective effects of flavonoids in humans [160-163]. Quercetin is a well-documented antioxidant flavonoid present in a wide range of vegetables and fruits. Several experimental and clinical studies have shown that quercetin can improve dyslipidemia, hypertension and atherosclerosis [163-165]. Mbikay et al. investigated the effect of 0-10 μM quercetin-3-O-b-D-glucoside (Q3G) on hepatic *PCSK9* and *LDLR* expression and also hepatic uptake of LDL-C in human Huh7 hepatocytes [166]. The results show that Q3G could decrease mRNA expression of *PCSK9* by 20-30%, while increase mRNA and protein expression of *LDLR* by 60% and 300-400%, respectively. Notably, it was found that Q3G gradually elevates the intracellular concentration of PCSK9 protein, but reduces its secretion.

Increased intracellular PCSK9 protein is due to its accumulation, which is attributed to the inhibition of sortilin, a sorting receptor found to enhance PCSK9 secretion. Overall, the aforementioned effects of Q3G were accompanied by increased uptake of LDL in Huh7 hepatocytes [166].

Phaleria macrocarpa fruit extract

Phaleria macrocarpa (PM) is an Indonesian medicinal plant belonging to Thymelaceae that grows in tropical areas of the Papua Island [167]. The extracts of PM have been found to possess several invaluable medicinal effects such as hypolipidemic, anticancer, antidiabetic, anti-inflammatory, antioxidant and antimicrobial effects [168]. As demonstrated by phytochemical analysis, PM fruits are rich in phenolic compounds, including benzophenone glycoside, icariside C3, magniferin and gallic acid [168]. As revealed by i*n vitro* and *in vivo* experiments, PM fruit can prevent arteriosclerosis and reduce cholesterol levels in Japanese quails and in primary cultures of rat hepatocytes [169, 170]. To evaluate the underlying mechanisms for the cholesterol-lowering effect of PM, Chong et al. investigated the effect of an aqueous extract of PM fruit (APMF) both *in vitro* and *in vivo* [170]. It was found that treatment with APMF at the dose of 20-40 mg/kg/day for 12 weeks can reduce plasma LDL-C levels by 32-38% in high-cholesterol (3%) diet-fed rats compared with normal diet-fed rats [170]. Although high-cholesterol diet decreased circulating PCSK9 concentrations and liver LDLR levels by 39 and 42%, respectively, compared with normal diet-rats, APMF treatment (20 mg/kg/day) restored these changes by increasing circulating PCSK9 and liver LDLR levels by 97 and 115%, respectively [170]. Notably, higher doses (30 and 40 mg/kg) of APMF showed no significant effects on circulating PCSK9 levels [170]. Similarly, an *in vitro* study on HepG2 cells showed that APMF treatment (0.1-1000 μg/mL) increases both mRNA and protein levels of PCSK9 and

LDLR [170]. These findings suggest that APMF ameliorates plasma LDL-C levels through inducing SRE pathway that leads to increased PCSK9 and LDLR levels, a mechanism that is similar to statin-induced effects [170].

Tanshinone IIA

Tanshinone IIA, known as "Danshen", is a nutraceutical compound isolated from the root of *Salvia miltiorrhiza* which is widely used in the Traditional Chinese Medicine [171]. As shown in experimental and clinical studies, tanshinone IIA elicits several biological and pharmaceutical activities such as anti-atherosclerotic, antihyperlipidemic, anti-adipogenic, antioxidant, anti-inflammatory and vasodilatory effects. Owing to its putative cardioprotective and anti-atherosclerotic effects, tanshinone IIA has been used to prevent and treat cardiovascular diseases [172-175]. Animal studies have shown that tanshinone IIA can reduce plasma LDL-C levels in HFD-C57BL/6J mice, and also regress atherosclerotic plaque without significant effect on other lipid markers [172, 175, 176]. Recently Jia et al. investigated the underlying mechanism for the lipid-lowering effects of tanshinone IIA *in vivo* [171]. The results revealed that intraperitoneal administration of tanshinone IIA at a daily dose of 10 mg/kg for 3 months can upregulate hepatic mRNA and protein expression of SREBP-2, PCSK9, and LDR in hyperlipidemic rats. These effects are translated into an overall enhanced hepatic clearance of LDL-C [171].

Conclusions

PCSK9 is an important regulator of lipid metabolism and an efficient target for plasma LDL-C reduction. Statins are known to elevate circulating PCSK9 levels and this may attenuate their lipid-lowering effects. Combining statins with a PCSK9 inhibitor is an optimal strategy for

reducing plasma LDL-C levels in severe dyslipidemias including FH. However, the widespread use of such a combination regimen is challenged by the cost of currently available PCSK9 inhibitors and in particular mAbs.

Several nutraceuticals exert lipid-lowering and atheroprotective properties. Berberine, curcumin, polydatin, *n*-3 PUFA-enriched fish oil, DHA-enriched canola oil, marine *n*-3 PUFAs and quercetin-3-O-b-D-glucoside have been identified to lower PCSK9 levels (**Table 1**) and the PCSK9-lowering effect of some of these agents is supported by data from human trials (**Table 2**). There are many other nutraceuticals with documented lipid-lowering properties [82-84,177-192]. However, there are no data with regard to their effects on PSCK9 or SRE.

SREBP-2, a common transcription factor for *PCSK9* and *LDLR* genes, is up-regulated by statins. Therefore, nutraceuticals that inhibit PCSK9 through SREBP-independent pathways can be considered as a useful adjunct to statins. Among the aforementioned nutraceuticals inhibiting PCSK9, berberine and curcumin may inhibit *PCSK9* expression through HNF1α suppression. However, mechanisms of the underlying *PCSK9* inhibition by other nutraceuticals described in this review (e.g. polydatin, *n*-3 PUFAs and quercetin-3-O-b-D-glucoside) have not yet been determined. Therefore, berberine and curcumin are suggested as useful adjuncts to statin therapy owing to their SREBP-independent inhibition of PCSK9 (**Figure 1**), safety and known anti-atherosclerotic and cardioprotective activities. Nevertheless, evidence from well-designed randomized controlled trials is required to support the added value of such a combination in reducing cardiovascular events compared with statin monotherapy.

Conflict of interests: MB has served on the speaker's bureau and as an advisory board member for Amgen, Sanofi, Aventis and Lilly. NK has given talks, attended conferences and participated

in trials sponsored by Amgen, Angelini, Astra Zeneca, Boehringer Ingelheim, Galenica, MSD, Novartis, Novo Nordisk, Sanofi and WinMedica. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

[1] K.N. Maxwell, E.A. Fisher, J.L. Breslow, Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment, Proceedings of the National Academy of Sciences of the United States of America 102(6) (2005) 2069-2074.
[2] N.G. Seidah, S. Benjannet, L. Wickham, J. Marcinkiewicz, S.B. Jasmin, S. Stifani, A. Basak, A. Prat, M. Chretien, The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation, Proceedings of the National Academy of Sciences of the United States of America 100(3) (2003) 928-33.
[3] C. Langhi, C. Le May, V. Gmyr, B. Vandewalle, J. Kerr-Conte, M. Krempf, F. Pattou, P. Costet, B. Cariou, PCSK9 is expressed in pancreatic δ-cells and does not alter insulin secretion, Biochemical and biophysical research communications 390(4) (2009) 1288-1293.
[4] N. Ferri, G. Tibolla, A. Pirillo, F. Cipollone, A. Mezzetti, S. Pacia, A. Corsini, A.L. Catapano, Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels, Atherosclerosis 220(2) (2012) 381-386.
[5] C. Le May, S. Kourimate, C. Langhi, M. Chétiveaux, A. Jarry, C. Comera, X. Collet, F. Kuipers, M. Krempf, B. Cariou, Proprotein convertase subtilisin kexin type 9 null mice are protected from postprandial triglyceridemia, Arteriosclerosis, thrombosis, and vascular biology 29(5) (2009) 684-690.
[6] N.G. Seidah, A. Prat, The biology and therapeutic targeting of the proprotein convertases, Nature reviews Drug discovery 11(5) (2012) 367-383.
[7] D. Cunningham, D.E. Danley, K.F. Geoghegan, M.C. Griffor, J.L. Hawkins, T.A. Subashi, A.H. Varghese, M.J. Ammirati, J.S. Culp, L.R. Hoth, Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia, Nature structural & molecular biology 14(5) (2007) 413-419.
[8] E.N. Hampton, M.W. Knuth, J. Li, J.L. Harris, S.A. Lesley, G. Spraggon, The self-inhibited structure of full-length PCSK9 at 1.9 Å reveals structural homology with resistin within the C-terminal domain, Proceedings of the National Academy of Sciences 104(37) (2007) 14604-14609.
[9] D.E. Piper, S. Jackson, Q. Liu, W.G. Romanow, S. Shetterly, S.T. Thibault, B. Shan, N.P. Walker, The crystal structure of PCSK9: a regulator of plasma LDL-cholesterol, Structure 15(5) (2007) 545-552.
[10] S. Benjannet, D. Rhainds, R. Essalmani, J. Mayne, L. Wickham, W. Jin, M.-C. Asselin, J. Hamelin, M. Varret, D. Allard, NARC-1/PCSK9 and its natural mutants zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol, Journal of Biological Chemistry 279(47) (2004) 48865-48875.
[11] N.G. Seidah, The proprotein convertases, 20 years later, Proprotein Convertases (2011) 23-57.
[12] M. Banach, M. Rizzo, M. Obradovic, G. Montalto, J. Rysz, D.P. Mikhailidis, E.R. Isenovic. PCSK9 inhibition - a novel mechanism to treat lipid disorders? Curr Pharm Des. 19(21) (2013) 3869-77.
[13] N.G. Seidah, S. Benjannet, L. Wickham, J. Marcinkiewicz, S.B. Jasmin, S. Stifani, A. Basak, A. Prat, M. Chrétien, The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation, Proceedings of the National Academy of Sciences 100(3) (2003) 928-933.
[14] M.C. Serban, M. Banach, D.P. Mikhailidis. Clinical implications of the IMPROVE-IT trial

in the light of current and future lipid-lowering treatment options. Expert Opin Pharmacother. 17(3) (2016) 369-80.
[15] G. Lambert, B. Sjouke, B. Choque, J.J. Kastelein, G.K. Hovingh, The PCSK9 decade Thematic Review Series: New Lipid and Lipoprotein Targets for the Treatment of Cardiometabolic Diseases, Journal of lipid research 53(12) (2012) 2515-2524.
[16] M.S. Brown, J.L. Goldstein, A receptor-mediated pathway for cholesterol homeostasis, Science 232(4746) (1986) 34-47.
[17] S. Ishibashi, M.S. Brown, J.L. Goldstein, R.D. Gerard, R.E. Hammer, J. Herz, Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery, Journal of Clinical Investigation 92(2) (1993) 883.
[18] S. Poirier, G. Mayer, V. Poupon, P.S. McPherson, R. Desjardins, K. Ly, M.-C. Asselin, R. Day, F.J. Duclos, M. Witmer, Dissection of the endogenous cellular pathways of PCSK9-induced low density lipoprotein receptor degradation evidence for an intracellular route, Journal of Biological Chemistry 284(42) (2009) 28856-28864.
[19] Y.-W. Qian, R.J. Schmidt, Y. Zhang, S. Chu, A. Lin, H. Wang, X. Wang, T.P. Beyer, W.R. Bensch, W. Li, Secreted PCSK9 downregulates low density lipoprotein receptor through receptor-mediated endocytosis, Journal of lipid research 48(7) (2007) 1488-1498.
[20] C.G. Davis, J.L. Goldstein, T.C. Südhof, R. Anderson, D.W. Russell, M.S. Brown, Acid-dependent ligand dissociation and recycling of LDL receptor mediated by growth factor homology region, Nature 326(6115) (1986) 760-765.
[21] G. Rudenko, L. Henry, K. Henderson, K. Ichtchenko, M.S. Brown, J.L. Goldstein, J. Deisenhofer, Structure of the LDL receptor extracellular domain at endosomal pH, Science 298(5602) (2002) 2353-2358.
[22] D. Van der Westhuyzen, M. Stein, H. Henderson, A. Marais, A. Fourie, G. Coetzee, Deletion of two growth-factor repeats from the low-density-lipoprotein receptor accelerates its degradation, Biochemical Journal 277(3) (1991) 677-682.
[23] D.-W. Zhang, T.A. Lagace, R. Garuti, Z. Zhao, M. McDonald, J.D. Horton, J.C. Cohen, H.H. Hobbs, Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation, Journal of Biological Chemistry 282(25) (2007) 18602-18612.
[24] H.J. Kwon, T.A. Lagace, M.C. McNutt, J.D. Horton, J. Deisenhofer, Molecular basis for LDL receptor recognition by PCSK9, Proceedings of the National Academy of Sciences 105(6) (2008) 1820-1825.
[25] J.D. Horton, J.C. Cohen, H.H. Hobbs, PCSK9: a convertase that coordinates LDL catabolism, Journal of lipid research 50(Supplement) (2009) S172-S177.
[26] R. Schulz, K.-D. Schlüter, U. Laufs, Molecular and cellular function of the proprotein convertase subtilisin/kexin type 9 (PCSK9), Basic research in cardiology 110(2) (2015) 1-19.
[27] J.L. Goldstein, M.S. Brown, R.G. Anderson, D.W. Russell, W.J. Schneider, Receptor-mediated endocytosis: concepts emerging from the LDL receptor system, Annual review of cell biology 1(1) (1985) 1-39.
[28] J.D. Horton, N.A. Shah, J.A. Warrington, N.N. Anderson, S.W. Park, M.S. Brown, J.L. Goldstein, Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes, Proceedings of the National Academy of Sciences 100(21) (2003) 12027-12032.

[29] H. Li, B. Dong, S.W. Park, H.-S. Lee, W. Chen, J. Liu, Hepatocyte nuclear factor 1α plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine, Journal of Biological Chemistry 284(42) (2009) 28885-28895.
[30] B. Haslinger-Löffler, Multiple effects of HMG-CoA reductase inhibitors (statins) besides their lipid-lowering function, Kidney international 74(5) (2008) 553-555.
[31] B. Dong, M. Wu, H. Li, F.B. Kraemer, K. Adeli, N.G. Seidah, S.W. Park, J. Liu, Strong induction of PCSK9 gene expression through HNF1α and SREBP2: mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters, Journal of lipid research 51(6) (2010) 1486-1495.
[32] N.G. Seidah, Z. Awan, M. Chrétien, M. Mbikay, PCSK9 a key modulator of cardiovascular health, Circulation research 114(6) (2014) 1022-1036.
[33] S. Rashid, D.E. Curtis, R. Garuti, N.N. Anderson, Y. Bashmakov, Y. Ho, R.E. Hammer, Y.-A. Moon, J.D. Horton, Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9, Proceedings of the National Academy of Sciences of the United States of America 102(15) (2005) 5374-5379.
[34] Y.-J. Jia, R.-X. Xu, J. Sun, Y. Tang, J.-J. Li, Enhanced circulating PCSK9 concentration by berberine through SREBP-2 pathway in high fat diet-fed rats, Journal of translational medicine 12(1) (2014) 1.
[35] C. Werner, M.M. Hoffmann, K. Winkler, M. Bohm, U. Laufs, Risk prediction with proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with stable coronary disease on statin treatment, Vascular pharmacology 62(2) (2014) 94-102.
[36] M. Abifadel, M. Varret, J.-P. Rabès, D. Allard, K. Ouguerram, M. Devillers, C. Cruaud, S. Benjannet, L. Wickham, D. Erlich, Mutations in PCSK9 cause autosomal dominant hypercholesterolemia, Nature genetics 34(2) (2003) 154-156.
[37] J. Cohen, A. Pertsemlidis, I.K. Kotowski, R. Graham, C.K. Garcia, H.H. Hobbs, Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9, Nature genetics 37(2) (2005) 161-165.
[38] J.C. Cohen, E. Boerwinkle, T.H. Mosley Jr, H.H. Hobbs, Sequence variations in PCSK9, low LDL, and protection against coronary heart disease, New England Journal of Medicine 354(12) (2006) 1264-1272.
[39] Z. Zhao, Y. Tuakli-Wosornu, T.A. Lagace, L. Kinch, N.V. Grishin, J.D. Horton, J.C. Cohen, H.H. Hobbs, Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote, The American Journal of Human Genetics 79(3) (2006) 514-523.
[40] A.J. Hooper, A.D. Marais, D.M. Tanyanyiwa, J.R. Burnett, The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population, Atherosclerosis 193(2) (2007) 445-448.
[41] S.G. Lakoski, T.A. Lagace, J.C. Cohen, J.D. Horton, H.H. Hobbs, Genetic and metabolic determinants of plasma PCSK9 levels, The Journal of Clinical Endocrinology & Metabolism 94(7) (2009) 2537-2543.
[42] Z. Awan, N.G. Seidah, J.G. MacFadyen, S. Benjannet, D.I. Chasman, P.M. Ridker, J. Genest, Rosuvastatin, proprotein convertase subtilisin/kexin type 9 concentrations, and LDL cholesterol response: the JUPITER trial, Clinical chemistry 58(1) (2012) 183-189.
[43] A. Catapano, N. Papadopoulos, The safety of therapeutic monoclonal antibodies: implications for cardiovascular disease and targeting the PCSK9 pathway, Atherosclerosis 228(1) (2013) 18-28.

[44] R.Q. Do, R.A. Vogel, G.G. Schwartz, PCSK9 Inhibitors: potential in cardiovascular therapeutics, Current cardiology reports 15(3) (2013) 1-12.
[45] S.S. Hall, A gene of rare effect, Nature 496(7444) (2013) 152-155.
[46] FDA approves Repatha to treat certain patients with high cholesterol, August 27, 2015. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm460082.htm.
[47] FDA approves Praluent to treat certain patients with high cholesterol, July 24, 2015. http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm455883.htm.
[48] First-in-class treatment to lower cholesterol, May 22, 2015. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/05/news_detail_002336.jsp&mid=WC0b01ac058004d5c1.
[49] Praluent recommended for approval to lower cholesterol, July 24, 2015 http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/07/news_detail_002377.jsp&mid=WC0b01ac058004d5c1.
[50] E.M. Roth, J.M. McKenney, C. Hanotin, G. Asset, E.A. Stein, Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia, New England Journal of Medicine 367(20) (2012) 1891-1900.
[51] M.J. Koren, R. Scott, J.B. Kim, B. Knusel, T. Liu, L. Lei, M. Bolognese, S.M. Wasserman, Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study, The Lancet 380(9858) (2012) 1995-2006.
[52] S. Mayor, NICE recommends PCSK9 inhibitors for patients not responding to statins, BMJ 353 (2016) i2609.
[53] D.S. Kazi, A.E. Moran, P.G. Coxson, J. Penko, D.A. Ollendorf, S.D. Pearson, J.A. Tice, D. Guzman, K. Bibbins-Domingo, Cost-effectiveness of PCSK9 Inhibitor Therapy in Patients With Heterozygous Familial Hypercholesterolemia or Atherosclerotic Cardiovascular Disease, JAMA 316(7) (2016) 743-753.
[54] N. Bergeron, B.A.P. Phan, Y. Ding, A. Fong, R.M. Krauss, Proprotein Convertase Subtilisin/Kexin type 9 inhibition a new therapeutic mechanism for reducing cardiovascular disease risk, Circulation 132(17) (2015) 1648-1666.
[55] J.G. Robinson, Nonstatins and Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors: Role in Non-Familial Hypercholesterolemia, Progress in Cardiovascular Diseases 59(2) (2016) 165-171.
[56] D. Paton, PCSK9 inhibitors: monoclonal antibodies for the treatment of hypercholesterolemia, Drugs of today (Barcelona, Spain: 1998) 52(3) (2016) 183-192.
[57] M.J. Chapman, J.K. Stock, H.N. Ginsberg, PCSK9 inhibitors and cardiovascular disease: heralding a new therapeutic era, Current opinion in lipidology 26(6) (2015) 511-520.
[58] E.K. Kalra, Nutraceutical-definition and introduction, Aaps Pharmsci 5(3) (2003) 27-28.
[59] A. Sahebkar, M.C. Serban, A. Gluba-Brzózka, D.P. Mikhailidis, A.F. Cicero, J. Rysz, M. Banach. Lipid-modifying effects of nutraceuticals: An evidence-based approach. Nutrition. 32(11-12) (2016) 1179-92.
[60] A. Pirillo, A.L. Catapano, Berberine, a plant alkaloid with lipid-and glucose-lowering properties: From in vitro evidence to clinical studies, Atherosclerosis 243(2) (2015) 449-461.
[61] W. Kong, J. Wei, P. Abidi, M. Lin, S. Inaba, C. Li, Y. Wang, Z. Wang, S. Si, H. Pan, Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins, Nature medicine 10(12) (2004) 1344-1351.

[62] P. Abidi, Y. Zhou, J.-D. Jiang, J. Liu, Extracellular signal-regulated kinase–dependent stabilization of hepatic low-density lipoprotein receptor mRNA by herbal medicine berberine, Arteriosclerosis, Thrombosis, and Vascular Biology 25(10) (2005) 2170-2176.
[63] J. Cameron, T. Ranheim, M.A. Kulseth, T.P. Leren, K.E. Berge, Berberine decreases PCSK9 expression in HepG2 cells, Atherosclerosis 201(2) (2008) 266-273.
[64] B. Dong, H. Li, A.B. Singh, A. Cao, J. Liu, Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1α protein expression through the ubiquitin-proteasome degradation pathway, Journal of Biological Chemistry 290(7) (2015) 4047-4058.
[65] M. Canuel, X. Sun, M.-C. Asselin, E. Paramithiotis, A. Prat, N.G. Seidah, Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1), PloS one 8(5) (2013) e64145.
[66] S. Poirier, G. Mayer, S. Benjannet, E. Bergeron, J. Marcinkiewicz, N. Nassoury, H. Mayer, J. Nimpf, A. Prat, N.G. Seidah, The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2, Journal of Biological Chemistry 283(4) (2008) 2363-2372.
[67] K. Kysenius, P. Muggalla, K. Mätlik, U. Arumäe, H.J. Huttunen, PCSK9 regulates neuronal apoptosis by adjusting ApoER2 levels and signaling, Cellular and molecular life sciences 69(11) (2012) 1903-1916.
[68] X.-Q. Zhou, X.-N. Zeng, H. Kong, X.-L. Sun, Neuroprotective effects of berberine on stroke models in vitro and in vivo, Neuroscience Letters 447(1) (2008) 31-36.
[69] K.S. Shin, H.S. Choi, T.T. Zhao, K.H. Suh, I.H. Kwon, S.O. Choi, M.K. Lee, Neurotoxic effects of berberine on long-term l-DOPA administration in 6-hydroxydopamine-lesioned rat model of Parkinson's disease, Archives of pharmacal research 36(6) (2013) 759-767.
[70] K. Kysenius, C.A. Brunello, H.J. Huttunen, Mitochondria and NMDA receptor-dependent toxicity of berberine sensitizes neurons to glutamate and rotenone injury, PloS one 9(9) (2014) e107129.
[71] K. Kysenius, H.J. Huttunen, Stress-induced upregulation of VLDL receptor alters Wnt-signaling in neurons, Experimental cell research 340(2) (2016) 238-247.
[72] D.-l. Liu, L.-j. Xu, H. Dong, G. Chen, Z.-y. Huang, X. Zou, K.-f. Wang, Y.-h. Luo, F.-e. Lu, Inhibition of proprotein convertase subtilisin/kexin type 9: A novel mechanism of berberine and 8-hydroxy dihydroberberine against hyperlipidemia, Chinese journal of integrative medicine 21 (2015) 132-138.
[73] K.R. Feingold, A.H. Moser, J.K. Shigenaga, S.M. Patzek, C. Grunfeld, Inflammation stimulates the expression of PCSK9, Biochemical and biophysical research communications 374(2) (2008) 341-344.
[74] L. Li, P.A. Thompson, R.L. Kitchens, Infection induces a positive acute phase apolipoprotein E response from a negative acute phase gene: role of hepatic LDL receptors, Journal of lipid research 49(8) (2008) 1782-1793.
[75] L. Wei, M. RUDLING, B. ANGELIN, Endotoxin suppresses rat hepatic low-density lipoprotein receptor expression, Biochemical Journal 313(3) (1996) 873-878.
[76] H.-B. Xiao, Z.-L. Sun, H.-B. Zhang, D.-S. Zhang, Berberine inhibits dyslipidemia in C57BL/6 mice with lipopolysaccharide induced inflammation, Pharmacological Reports 64(4) (2012) 889-895.
[77] L. Pisciotta, A. Bellocchio, S. Bertolini, Nutraceutical pill containing berberine versus ezetimibe on plasma lipid pattern in hypercholesterolemic subjects and its additive effect in

patients with familial hypercholesterolemia on stable cholesterol-lowering treatment, Lipids in health and disease 11(1) (2012) 1.
[78] I. De Castro-Orós, R. Solà, R.M. Valls, A. Brea, P. Mozas, J. Puzo, M. Pocoví, Genetic Variants of LDLR and PCSK9 Associated with Variations in Response to Antihypercholesterolemic Effects of Armolipid Plus with Berberine, PloS one 11(3) (2016) e0150785.
[79] A. Oppenheimer, Turmeric (curcumin) in biliary diseases, The Lancet 229(5924) (1937) 619-621.
[80] A. Momtazi, A. Amirhossein Sahebkar, Difluorinated curcumin: A promising curcumin analogue with improved anti-tumor activity and pharmacokinetic profile, Current Pharmaceutical Design 22.
[81] A. Sahebkar, Dual effect of curcumin in preventing atherosclerosis: the potential role of pro-oxidant–antioxidant mechanisms, Natural product research 29(6) (2015) 491-492.
[82] S. Rahmani, S. Asgary, G. Askari, M. Keshvari, M. Hatamipour, A. Feizi, A. Sahebkar, Treatment of Non-alcoholic Fatty Liver Disease with Curcumin: A Randomized Placebo-controlled Trial, Phytotherapy Research (2016).
[83] A. Sahebkar, G. Beccuti, L.E. Simental-Mendía, V. Nobili, S. Bo, Nigella sativa (black seed) effects on plasma lipid concentrations in humans: A systematic review and meta-analysis of randomized placebo-controlled trials, Pharmacological Research 106 (2016) 37-50.
[84] A. Sahebkar, G.T. Chew, G.F. Watts, Recent advances in pharmacotherapy for hypertriglyceridemia, Progress in Lipid Research 56(1) (2014) 47-66.
[85] M.S. Karimian, M. Pirro, M. Majeed, A. Sahebkar, Curcumin as a natural regulator of monocyte chemoattractant protein-1, Cytokine & growth factor reviews (2016).
[86] S. Ganjali, C.N. Blesso, M. Banach, M. Pirro, M. Majeed, A. Sahebkar. Effects of curcumin on HDL functionality. Pharmacol Res. 119 (2017) 208-218.
[87] Y. Panahi, M.S. Hosseini, N. Khalili, E. Naimi, L.E. Simental-Mendia, M. Majeed, A. Sahebkar, Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 82 (2016) 578-82.
[88] Y. Panahi, N. Khalili, E. Sahebi, S. Namazi, M.S. Karimian, M. Majeed, A. Sahebkar, Antioxidant effects of curcuminoids in patients with type 2 diabetes mellitus: a randomized controlled trial, Inflammopharmacology (2016).
[89] Y. Panahi, P. Kianpour, R. Mohtashami, R. Jafari, L.E. Simental-Mendia, A. Sahebkar, Curcumin Lowers Serum Lipids and Uric Acid in Subjects With Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial, Journal of cardiovascular pharmacology 68(3) (2016) 223-9.
[90] M. Teymouri, M. Pirro, T.P. Johnston, A. Sahebkar, Curcumin as a multifaceted compound against human papilloma virus infection and cervical cancers: A review of chemistry, cellular, molecular, and preclinical features, Biofactors (2016).
[91] A. Mohammadi, B. Fazeli, M. Taheri, A. Sahebkar, Z. Poursina, V. Vakili, S.Z. Yazdi, Z. Keramati, R. Boostani, I. Hampson, H. Rafatpanah, Modulatory effects of curcumin on apoptosis and cytotoxicity-related molecules in HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie (2016).

[92] A. Khonche, O. Biglarian, Y. Panahi, G. Valizadegan, S.S. Soflaei, M.E. Ghamarchehreh, M. Majeed, A. Sahebkar, Adjunctive Therapy with Curcumin for Peptic Ulcer: A Randomized Controlled Trial, Drug Research 66(8) (2016) 444-448.
[93] A. Mohammadi, A. Sahebkar, M. Iranshahi, M. Amini, R. Khojasteh, M. Ghayour-Mobarhan, G.A. Ferns, Effects of supplementation with curcuminoids on dyslipidemia in obese patients: A randomized crossover trial, Phytotherapy Research 27(3) (2013) 374-379.
[94] Y. Panahi, G.H. Alishiri, S. Parvin, A. Sahebkar, Mitigation of Systemic Oxidative Stress by Curcuminoids in Osteoarthritis: Results of a Randomized Controlled Trial, Journal of Dietary Supplements 13(2) (2016) 209-220.
[95] Y. Panahi, R. Badeli, G.R. Karami, A. Sahebkar, Investigation of the efficacy of adjunctive therapy with bioavailability-boosted curcuminoids in major depressive disorder, Phytotherapy Research 29(1) (2015) 17-21.
[96] Y. Panahi, M. Ghanei, S. Bashiri, A. Hajihashemi, A. Sahebkar, Short-term Curcuminoid Supplementation for Chronic Pulmonary Complications due to Sulfur Mustard Intoxication: Positive Results of a Randomized Double-blind Placebo-controlled Trial, Drug Research 65(11) (2014) 567-573.
[97] Y. Panahi, M. Ghanei, A. Hajhashemi, A. Sahebkar, Effects of Curcuminoids-Piperine Combination on Systemic Oxidative Stress, Clinical Symptoms and Quality of Life in Subjects with Chronic Pulmonary Complications Due to Sulfur Mustard: A Randomized Controlled Trial, Journal of Dietary Supplements 13(1) (2016) 93-105.
[98] Y. Panahi, M.S. Hosseini, N. Khalili, E. Naimi, L.E. Simental-Mendía, M. Majeed, A. Sahebkar, Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial, Biomedicine and Pharmacotherapy 82 (2016) 578-582.
[99] Y. Panahi, M.S. Hosseini, N. Khalili, E. Naimi, S.S. Soflaei, M. Majeed, A. Sahebkar, Effects of supplementation with curcumin on serum adipokine concentrations: A randomized controlled trial, Nutrition 32(10) (2016) 1116-22.
[100] Y. Panahi, A.R. Rahimnia, M. Sharafi, G. Alishiri, A. Saburi, A. Sahebkar, Curcuminoid treatment for knee osteoarthritis: A randomized double-blind placebo-controlled trial, Phytotherapy Research 28(11) (2014) 1625-1631.
[101] Y. Panahi, A. Sahebkar, M. Amiri, S.M. Davoudi, F. Beiraghdar, S.L. Hoseininejad, M. Kolivand, Improvement of sulphur mustard-induced chronic pruritus, quality of life and antioxidant status by curcumin: Results of a randomised, double-blind, placebo-controlled trial, British Journal of Nutrition 108(7) (2012) 1272-1279.
[102] Y. Panahi, A. Sahebkar, S. Parvin, A. Saadat, A randomized controlled trial on the anti-inflammatory effects of curcumin in patients with chronic sulphur mustard-induced cutaneous complications, Annals of Clinical Biochemistry 49(6) (2012) 580-588.
[103] A. Sahebkar, Molecular mechanisms for curcumin benefits against ischemic injury, Fertility and Sterility 94(5) (2010).
[104] A. Sahebkar, Why it is necessary to translate curcumin into clinical practice for the prevention and treatment of metabolic syndrome?, Biofactors 39(2) (2013) 197-208.
[105] A. Sahebkar, M.C. Serban, S. Ursoniu, M. Banach, Effect of curcuminoids on oxidative stress: A systematic review and meta-analysis of randomized controlled trials, Journal of Functional Foods 18 (2015) 898-909.

[106] G. Derosa, P. Maffioli, L.E. Simental-Mendia, S. Bo, A. Sahebkar, Effect of curcumin on circulating interleukin-6 concentrations: A systematic review and meta-analysis of randomized controlled trials, Pharmacological research 111 (2016) 394-404.
[107] M. Ghandadi, A. Sahebkar, Curcumin: An effective inhibitor of interleukin-6, Current pharmaceutical design (2016).
[108] A.A. Momtazi, G. Derosa, P. Maffioli, M. Banach, A. Sahebkar, Role of microRNAs in the Therapeutic Effects of Curcumin in Non-Cancer Diseases, Molecular diagnosis & therapy 20(4) (2016) 335-45.
[109] A.A. Momtazi, F. Shahabipour, S. Khatibi, T.P. Johnston, M. Pirro, A. Sahebkar, Curcumin as a MicroRNA Regulator in Cancer: A Review, Reviews of physiology, biochemistry and pharmacology 171 (2016) 1-38.
[110] A. Sahebkar, Low-density lipoprotein is a potential target for curcumin: novel mechanistic insights, Basic & clinical pharmacology & toxicology 114(6) (2014) 437-8.
[111] A. Sahebkar, Are curcuminoids effective C-reactive protein-lowering agents in clinical practice? Evidence from a meta-analysis, Phytotherapy research : PTR 28(5) (2014) 633-42.
[112] J.M. Zingg, S.T. Hasan, D. Cowan, R. Ricciarelli, A. Azzi, M. Meydani, Regulatory effects of curcumin on lipid accumulation in monocytes/macrophages, Journal of cellular biochemistry 113(3) (2012) 833-840.
[113] M.C. Kou, S.Y. Chiou, C.Y. Weng, L. Wang, C.T. Ho, M.J. Wu, Curcuminoids distinctly exhibit antioxidant activities and regulate expression of scavenger receptors and heme oxygenase-1, Molecular nutrition & food research 57(9) (2013) 1598-1610.
[114] M. Ramırez-Tortosa, M. Mesa, M. Aguilera, J. Quiles, L. Baro, C. Ramirez-Tortosa, E. Martinez-Victoria, A. Gil, Oral administration of a turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis, Atherosclerosis 147(2) (1999) 371-378.
[115] A. Sahebkar, A. Mohammadi, A. Atabati, S. Rahiman, S. Tavallaie, M. Iranshahi, S. Akhlaghi, G.A. Ferns, M. Ghayour-Mobarhan, Curcuminoids Modulate Pro-Oxidant–Antioxidant Balance but not the Immune Response to Heat Shock Protein 27 and Oxidized LDL in Obese Individuals, Phytotherapy research 27(12) (2013) 1883-1888.
[116] A. Sahebkar, N. Saboni, M. Pirro, M. Banach. Curcumin: An effective adjunct in patients with statin-associated muscle symptoms? J Cachexia Sarcopenia Muscle. 8(1) (2017) 19-24.
[117] A. Ramırez-Boscá, A. Soler, M.A. Carrion, J. Dıaz-Alperi, A. Bernd, C. Quintanilla, E.Q. Almagro, J. Miquel, An hydroalcoholic extract of Curcuma longa lowers the apo B/apo A ratio: implications for atherogenesis prevention, Mechanisms of ageing and development 119(1) (2000) 41-47.
[118] M.H. Tai, P.K. Chen, P.Y. Chen, M.J. Wu, C.T. Ho, J.H. Yen, Curcumin enhances cell-surface LDLR level and promotes LDL uptake through downregulation of PCSK9 gene expression in HepG2 cells, Molecular nutrition & food research 58(11) (2014) 2133-2145.
[119] Z. Cai-Ping, S. Shao-Wei, G. Yong-Zhen, O. Lu, L. Li-Mei, Z. Xing, T. Qin-Hui, L. Xiao-Yong, L. Duan-Fang, PCSK9/LDLR Pathway Mediates Curcumin Trinicotinate Promoting Lipid Uptake of HepG2, Progress in Biochemistry and Biophysics 42(9) (2015) 825-832.
[120] G. Dubuc, A. Chamberland, H. Wassef, J. Davignon, N.G. Seidah, L. Bernier, A. Prat, Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia, Arteriosclerosis, thrombosis, and vascular biology 24(8) (2004) 1454-1459.

[121] V.R. Shende, M. Wu, A.B. Singh, B. Dong, C.F.K. Kan, J. Liu, Reduction of circulating PCSK9 and LDL-C levels by liver-specific knockdown of HNF1α in normolipidemic mice, Journal of lipid research 56(4) (2015) 801-809.
[122] M. Ruscica, C. Ricci, C. Macchi, P. Magni, R. Cristofani, J. Liu, A. Corsini, N. Ferri, Suppressor of Cytokine Signaling-3 (SOCS-3) induces Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) expression in hepatic HepG2 cell line, Journal of Biological Chemistry (2015) jbc. M115. 664706.
[123] B. Yang, J.-J. Li, J.-J. Cao, C.-B. Yang, J. Liu, Q.-M. Ji, Z.-G. Liu, Polydatin attenuated food allergy via store-operated calcium channels in mast cell, World journal of gastroenterology: WJG 19(25) (2013) 3980.
[124] X.-h. Li, X. Gong, L. Zhang, R. Jiang, H.-z. Li, M.-j. Wu, J.-y. Wan, Protective effects of polydatin on septic lung injury in mice via upregulation of HO-1, Mediators of inflammation 2013 (2013).
[125] K. Huang, C. Chen, J. Hao, J. Huang, S. Wang, P. Liu, H. Huang, Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced upregulation of fibronetin and transforming growth factor-β1 in rat glomerular messangial cells, Molecular and cellular endocrinology 399 (2015) 178-189.
[126] X. Xie, J. Peng, K. Huang, J. Huang, X. Shen, P. Liu, H. Huang, Polydatin ameliorates experimental diabetes-induced fibronectin through inhibiting the activation of NF-κB signaling pathway in rat glomerular mesangial cells, Molecular and cellular endocrinology 362(1) (2012) 183-193.
[127] J. Zhang, Y. Tan, F. Yao, Q. Zhang, Polydatin alleviates non-alcoholic fatty liver disease in rats by inhibiting the expression of TNF-α and SREBP-1c, Molecular medicine reports 6(4) (2012) 815-820.
[128] T. Li, Y. Liu, G. Li, X. Wang, Z. Zeng, S. Cai, F. Li, Z. Chen, Polydatin attenuates ipopolysaccharide-induced acute lung injury in rats, Int J Clin Exp Pathol 7(12) (2014) 8401-10.
[129] Y. Zhang, Z. Zhuang, Q. Meng, Y. Jiao, J. Xu, S. Fan, Polydatin inhibits growth of lung cancer cells by inducing apoptosis and causing cell cycle arrest, Oncology letters 7(1) (2014) 295-301.
[130] J. Hao, C. Chen, K. Huang, J. Huang, J. Li, P. Liu, H. Huang, Polydatin improves glucose and lipid metabolism in experimental diabetes through activating the Akt signaling pathway, European journal of pharmacology 745 (2014) 152-165.
[131] W.-W. Xing, J.-Z. Wu, M. Jia, J. Du, H. Zhang, L.-P. Qin, Effects of polydatin from Polygonum cuspidatum on lipid profile in hyperlipidemic rabbits, Biomedicine & Pharmacotherapy 63(7) (2009) 457-462.
[132] Q. Miao, X.-P. Shi, M.-X. Ye, J. Zhang, S. Miao, S.-W. Wang, B. Li, X.-X. Jiang, S. Zhang, N. Hu, Polydatin attenuates hypoxic pulmonary hypertension and reverses remodeling through protein kinase C mechanisms, International journal of molecular sciences 13(6) (2012) 7776-7787.
[133] R. Zhang, Y. Liu, Z. Yang, Y. Li, X. Rong, L. Wang, C. Guo, S. Li, J. Liu, M. Li, Y. Wu, Construction of nanoparticles based on amphiphilic PEI-PA polymers for bortezomib and paclitaxel co-delivery, RSC Advances 5(20) (2015) 15453-15460.
[134] E. Topchiy, M. Cirstea, H.J. Kong, J.H. Boyd, Y. Wang, J.A. Russell, K.R. Walley, Lipopolysaccharide is cleared from the circulation by hepatocytes via the low density lipoprotein receptor, PLoS ONE 11(5) (2016).

[135] R. Dentin, J.-P. Pégorier, F. Benhamed, F. Foufelle, P. Ferré, V. Fauveau, M.A. Magnuson, J. Girard, C. Postic, Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression, Journal of Biological Chemistry 279(19) (2004) 20314-20326.
[136] X. Xie, W. Li, T. Lan, W. Liu, J. Peng, K. Huang, J. Huang, X. Shen, P. Liu, H. Huang, Berberine ameliorates hyperglycemia in alloxan-induced diabetic C57BL/6 mice through activation of Akt signaling pathway, Endocrine journal 58(9) (2011) 761-768.
[137] J. Liu, J. Zhang, Y. Shi, S. Grimsgaard, T. Alraek, V. Fønnebø, Chinese red yeast rice (Monascus purpureus) for primary hyperlipidemia: a meta-analysis of randomized controlled trials, Chinese medicine 1(1) (2006) 1.
[138] B. Trimarco, C. Benvenuti, F. Rozza, C.S. Cimmino, R. Giudice, S. Crispo, Clinical evidence of efficacy of red yeast rice and berberine in a large controlled study versus diet, Mediterranean journal of nutrition and metabolism 4(2) (2011) 133-139.
[139] R. Menéndez, A.M. Amor, I. Rodeiro, R.M. González, P.C. González, J.L. Alfonso, R. Más, Policosanol modulates HMG-CoA reductase activity in cultured fibroblasts, Archives of medical research 32(1) (2001) 8-12.
[140] D.K. Singh, L. Li, T.D. Porter, Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase, Journal of Pharmacology and Experimental Therapeutics 318(3) (2006) 1020-1026.
[141] Z. Lu, W. Kou, B. Du, Y. Wu, S. Zhao, O.A. Brusco, J.M. Morgan, D.M. Capuzzi, C.C.S.P.S. Group, Effect of Xuezhikang, an extract from red yeast Chinese rice, on coronary events in a Chinese population with previous myocardial infarction, The American journal of cardiology 101(12) (2008) 1689-1693.
[142] D.J. Becker, R.Y. Gordon, S.C. Halbert, B. French, P.B. Morris, D.J. Rader, Red yeast rice for dyslipidemia in statin-intolerant patients: a randomized trial, Annals of internal medicine 150(12) (2009) 830-839.
[143] Y.-j. Jia, Y. Zhang, J. Liu, Y.-l. Guo, R.-x. Xu, J.-j. Li, Short-and long-term effects of Xuezhikang (血脂康), an extract of cholestin, on serum proprotein convertase subtilisin/kexin type 9 levels, Chinese journal of integrative medicine 22 (2016) 96-100.
[144] M. Pirro, M.R. Mannarino, V. Bianconi, L.E. Simental-Mendia, F. Bagaglia, E. Mannarino, A. Sahebkar, The effects of a nutraceutical combination on plasma lipids and glucose: A systematic review and meta-analysis of randomized controlled trials, Pharmacol Res 110 (2016) 76-88.
[145] M. Pirro, M.R. Mannarino, S. Ministrini, F. Fallarino, G. Lupattelli, V. Bianconi, F. Bagaglia, E. Mannarino, Effects of a nutraceutical combination on lipids, inflammation and endothelial integrity in patients with subclinical inflammation: a randomized clinical trial, Scientific reports 6 (2016) 23587.
[146] M.R. Mannarino, S. Ministrini, M. Pirro, Nutraceuticals for the treatment of hypercholesterolemia, European journal of internal medicine 25(7) (2014) 592-9.
[147] R. Wall, R.P. Ross, G.F. Fitzgerald, C. Stanton, Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids, Nutrition reviews 68(5) (2010) 280-289.
[148] C.L. Chang, R.J. Deckelbaum, Omega-3 fatty acids: mechanisms underlying “protective effects” in atherosclerosis, Current opinion in lipidology 24(4) (2013) 345.

[149] F. Yuan, H. Wang, Y. Tian, Q. Li, L. He, N. Li, Z. Liu, Fish oil alleviated high-fat diet–induced non-alcoholic fatty liver disease via regulating hepatic lipids metabolism and metaflammation: a transcriptomic study, Lipids in health and disease 15(1) (2016) 1.
[150] A.V. Sorokin, Z.-H. Yang, B.L. Vaisman, S. Thacker, Z.-X. Yu, M. Sampson, C.N. Serhan, A.T. Remaley, Addition of aspirin to a fish oil-rich diet decreases inflammation and atherosclerosis in ApoE-null mice, The Journal of Nutritional Biochemistry 35 (2016) 58-65.
[151] C. Rodríguez-Pérez, V.R. Ramprasath, S. Pu, A. Sabra, R. Quirantes-Piné, A. Segura-Carretero, P.J. Jones, Docosahexaenoic acid attenuates cardiovascular risk factors via a decline in proprotein convertase subtilisin/kexin type 9 (PCSK9) plasma levels, Lipids 51(1) (2016) 75-83.
[152] H. Bjermo, D. Iggman, J. Kullberg, I. Dahlman, L. Johansson, L. Persson, J. Berglund, K. Pulkki, S. Basu, M. Uusitupa, Effects of n− 6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial, The American journal of clinical nutrition 95(5) (2012) 1003-1012.
[153] D.B. Jump, N-3 polyunsaturated fatty acid regulation of hepatic gene transcription, Current opinion in lipidology 19(3) (2008) 242.
[154] C.B. Graversen, S. Lundbye-Christensen, B. Thomsen, J.H. Christensen, E.B. Schmidt, Marine n-3 polyunsaturated fatty acids lower plasma proprotein convertase subtilisin kexin type 9 levels in pre-and postmenopausal women: A randomised study, Vascular pharmacology 76 (2016) 37-41.
[155] S. Kourimate, C. Le May, C. Langhi, A.L. Jarnoux, K. Ouguerram, Y. Zaïr, P. Nguyen, M. Krempf, B. Cariou, P. Costet, Dual mechanisms for the fibrate-mediated repression of proprotein convertase subtilisin/kexin type 9, Journal of Biological Chemistry 283(15) (2008) 9666-9673.
[156] G. Lambert, A.-L. Jarnoux, T. Pineau, O. Pape, M. Chetiveaux, C. Laboisse, M. Krempf, P. Costet, Fasting induces hyperlipidemia in mice overexpressing proprotein convertase subtilisin kexin type 9: lack of modulation of very-low-density lipoprotein hepatic output by the low-density lipoprotein receptor, Endocrinology 147(10) (2006) 4985-4995.
[157] M.S. Lauer, P.B. Fontanarosa, Updated guidelines for cholesterol management, Jama 285(19) (2001) 2508-2509.
[158] E. De Smet, R.P. Mensink, M. Konings, G. Brufau, A.K. Groen, R. Havinga, M. Schonewille, A. Kerksiek, D. Lütjohann, J. Plat, Acute intake of plant stanol esters induces changes in lipid and lipoprotein metabolism-related gene expression in the liver and intestines of mice, Lipids 50(6) (2015) 529-541.
[159] P. Simonen, U.-H. Stenman, H. Gylling, Serum proprotein convertase subtilisin/kexin type 9 concentration is not increased by plant stanol ester consumption in normo-to moderately hypercholesterolaemic non-obese subjects. The BLOOD FLOW intervention study, Clinical Science 129(5) (2015) 439-446.
[160] J.M. Assini, E.E. Mulvihill, M.W. Huff, Citrus flavonoids and lipid metabolism, Current opinion in lipidology 24(1) (2013) 34-40.
[161] E. E Mulvihill, M. W Huff, Citrus flavonoids and the prevention of atherosclerosis, Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders) 12(2) (2012) 84-91.
[162] E.E. Mulvihill, A.C. Burke, M.W. Huff, Citrus Flavonoids as Regulators of Lipoprotein Metabolism and Atherosclerosis, Annual review of nutrition (0) (2016).
[163] M. A Islam, R. W Schmidt, S. Gunaseelan, A. Sanchez, An update on the cardiovascular effects of quercetin, a plant flavonoid, Current Nutrition & Food Science 10(1) (2014) 36-48.

[164] A.R. Rahimnia, Y. Panahi, G. Alishiri, M. Sharafi, A. Sahebkar, Impact of Supplementation with Curcuminoids on Systemic Inflammation in Patients with Knee Osteoarthritis: Findings from a Randomized Double-Blind Placebo-Controlled Trial, Drug research 65(10) (2015) 521-5.
[165] M.C. Serban, A. Sahebkar, A. Zanchetti, D.P. Mikhailidis, G. Howard, D. Antal, F. Andrica, A. Ahmed, W.S. Aronow, P. Muntner, G.Y. Lip, I. Graham, N. Wong, J. Rysz, M. Banach, Lipid, G. Blood Pressure Meta-analysis Collaboration, Effects of Quercetin on Blood Pressure: A Systematic Review and Meta-Analysis of Randomized Controlled Trials, J Am Heart Assoc 5(7) (2016).
[166] M. Mbikay, F. Sirois, S. Simoes, J. Mayne, M. Chrétien, Quercetin-3-glucoside increases low-density lipoprotein receptor (LDLR) expression, attenuates proprotein convertase subtilisin/kexin 9 (PCSK9) secretion, and stimulates LDL uptake by Huh7 human hepatocytes in culture, FEBS open bio 4(1) (2014) 755-762.
[167] V. Savolainen, M.W. Chase, S.B. Hoot, C.M. Morton, D.E. Soltis, C. Bayer, M.F. Fay, A.Y. De Bruijn, S. Sullivan, Y.-L. Qiu, Phylogenetics of flowering plants based on combined analysis of plastid atpB and rbcL gene sequences, Systematic Biology 49(2) (2000) 306-362.
[168] R. Altaf, M.Z.B. Asmawi, A. Dewa, A. Sadikun, M.I. Umar, Phytochemistry and medicinal properties of Phaleria macrocarpa (Scheff.) Boerl. extracts, Pharmacognosy reviews 7(13) (2013) 73.
[169] E. Armenia, R. Widya, D. Rusdi, M. Netty, Anti-Atherosclerotic effect and liver toxicity of ethanolic extract of Phaleria macrocarpa (Scheff. Boerl) fruit on Japanese Quail, Asian Symposium on Medicinal Plants, Spices and other natural product XII (ASOMP), Padang, Indonesia, 2006, pp. 13-18.
[170] S.C. Chong, M.A. Dollah, P.P. Chong, A. Maha, Phaleria macrocarpa (Scheff.) Boerl fruit aqueous extract enhances LDL receptor and PCSK9 expression in vivo and in vitro, Journal of ethnopharmacology 137(1) (2011) 817-827.
[171] L. Jia, N. Song, G. Yang, Y. Ma, X. Li, R. Lu, H. Cao, N. Zhang, M. Zhu, J. Wang, Effects of Tanshinone IIA on the modulation of miR‐33a and the SREBP‐2/Pcsk9 signaling pathway in hyperlipidemic rats, Molecular medicine reports 13(6) (2016) 4627-4635.
[172] F. Tang, X. Wu, T. Wang, P. Wang, R. Li, H. Zhang, J. Gao, S. Chen, L. Bao, H. Huang, Tanshinone II A attenuates atherosclerotic calcification in rat model by inhibition of oxidative stress, Vascular pharmacology 46(6) (2007) 427-438.
[173] S. Gao, Z. Liu, H. Li, P.J. Little, P. Liu, S. Xu, Cardiovascular actions and therapeutic potential of tanshinone IIA, Atherosclerosis 220(1) (2012) 3-10.
[174] W. Xu, J. Yang, L.-M. Wu, Cardioprotective effects of tanshinone IIA on myocardial ischemia injury in rats, Die Pharmazie-An International Journal of Pharmaceutical Sciences 64(5) (2009) 332-336.
[175] Z. Gong, C. Huang, X. Sheng, Y. Zhang, Q. Li, M.-W. Wang, L. Peng, Y.Q. Zang, The role of Tanshinone IIA in the treatment of obesity through peroxisome proliferator-activated receptor γ antagonism, Endocrinology 150(1) (2009) 104-113.
[176] F.-T. Tang, Y. Cao, T.-Q. Wang, L.-J. Wang, J. Guo, X.-S. Zhou, S.-w. Xu, W.-H. Liu, P.-Q. Liu, H.-Q. Huang, Tanshinone IIA attenuates atherosclerosis in ApoE−/− mice through down-regulation of scavenger receptor expression, European journal of pharmacology 650(1) (2011) 275-284.
[177] A.M. Patti, N. Katsiki, D. Nikolic, K. Al-Rasadi, M. Rizzo, Nutraceuticals in lipid-lowering treatment a narrative review on the role of chitosan, Angiology 66(5) (2015) 416-421.

[178] M. Rizzo, R.V. Giglio, D. Nikolic, A.M. Patti, C. Campanella, M. Cocchi, N. Katsiki, G. Montalto, Effects of Chitosan on Plasma Lipids and Lipoproteins A 4-Month Prospective Pilot Study, Angiology (2013) 0003319713493126.
[179] S. Asgary, A. Ghannadi, G. Dashti, A. Helalat, A. Sahebkar, S. Najafi, Nigella sativa L. improves lipid profile and prevents atherosclerosis: Evidence from an experimental study on hypercholesterolemic rabbits, Journal of Functional Foods 5(1) (2013) 228-234.
[180] S. Asgary, R. Kelishadi, M. Rafieian-Kopaei, S. Najafi, M. Najafi, A. Sahebkar, Investigation of the lipid-modifying and antiinflammatory effects of cornus mas L. supplementation on dyslipidemic children and adolescents, Pediatric Cardiology 34(7) (2013) 1729-1735.
[181] S. Asgary, M. Rafieian-Kopaei, S. Najafi, E. Heidarian, A. Sahebkar, Antihyperlipidemic Effects of Sesamum indicum L. in Rabbits Fed a High-Fat Diet, The Scientific World Journal 2013 (2013).
[182] S. Asgary, M. Rafieiankopaei, A. Sahebkar, F. Shamsi, N. Goli-malekabadi, Anti-hyperglycemic and anti-hyperlipidemic effects of Vaccinium myrtillus fruit in experimentally induced diabetes (antidiabetic effect of Vaccinium myrtillus fruit), Journal of the Science of Food and Agriculture 96(3) (2016) 764-768.
[183] S. Asgary, M. Rafieian-Kopaei, F. Shamsi, S. Najafi, A. Sahebkar, Biochemical and histopathological study of the anti-hyperglycemic and anti-hyperlipidemic effects of cornelian cherry (Cornus mas L.) in alloxan-induced diabetic rats, Journal of Complementary and Integrative Medicine 11(2) (2014) 63-69.
[184] S. Asgary, A. Sahebkar, N. Goli-Malekabadi, Ameliorative effects of Nigella sativa on dyslipidemia, Journal of Endocrinological Investigation 38(10) (2015) 1039-1046.
[185] N. Goli-Malekabadi, S. Asgary, B. Rashidi, M. Rafieian-Kopaei, M. Ghannadian, S. Hajian, A. Sahebkar, The protective effects of Ziziphus vulgaris L. Fruits on biochemical and histological abnormalities induced by diabetes in rats, Journal of Complementary and Integrative Medicine 11(3) (2014) 171-177.
[186] Y. Panahi, M.E. Ghamarchehreh, F. Beiraghdar, M. Zare, H.R. Jalalian, A. Sahebkar, Investigation of the effects of Chlorella vulgaris supplementation in patients with non-alcoholic fatty liver disease: A randomized clinical trial, Hepato-Gastroenterology 59(119) (2012) 2099-2103.
[187] Y. Panahi, B. Pishgoo, F. Beiraghdar, Z.M. Araghi, A. Sahebkar, E. Abolhasani, Results of a randomized, open-label, clinical trial investigating the effects of supplementation with Heracleum persicum extract as an adjunctive therapy for dyslipidemia, TheScientificWorldJournal 11 (2011) 592-601.
[188] M. Pirro, M.R. Mannarino, V. Bianconi, L.E. Simental-Mendía, F. Bagaglia, E. Mannarino, A. Sahebkar, The effects of a nutraceutical combination on plasma lipids and glucose: A systematic review and meta-analysis of randomized controlled trials, Pharmacological Research 110 (2016) 76-88.
[189] M.C. Serban, A. Sahebkar, S. Dragan, G. Stoichescu-Hogea, S. Ursoniu, F. Andrica, M. Banach, A systematic review and meta-analysis of the impact of Spirulina supplementation on plasma lipid concentrations, Clinical Nutrition 35(4) (2016) 842-851.

[190] S. Ursoniu, A. Sahebkar, M.C. Serban, M. Banach. Lipid profile and glucose changes after supplementation with astaxanthin: a systematic review and meta-analysis of randomized controlled trials. Arch Med Sci. 11(2) (2015) 253-66.

[191] A.M. Patti, P.P. Toth, R.V. Giglio, M. Banach, M. Noto, D. Nikolic, G. Montalto, M. Rizzo. Nutraceuticals as an Important Part of Combination Therapy in Dyslipidaemia. Curr Pharm Des. (2017) doi: 10.2174/1381612823666170317145851.

[192]. Barrios V, Escobar C, Cicero AF, Burke D, Fasching P, Banach M, Bruckert E. A nutraceutical approach (Armolipid Plus) to reduce total and LDL cholesterol in individuals with mild to moderate dyslipidemia: Review of the clinical evidence. Atheroscler Suppl. 24 (2017) 1-15.

Figure legends

Figure1. PCSK9, a critical inhibitor of LDLR, is up-regulated by both HNF1α and SREBP-2 transcription factors. Besides PCSK9, SREBP-2 up-regulates *LDLR* gene. Nutraceuticals, including curcumin and berberine, can decrease plasma LDL-C levels through elevation of the hepatic LDLR *via* inhibiting HNF1α which is a specific transcription factor for *PCSK9* gene. Statins increase the expression of both *PCSK9* and *LDLR* through the activation of SREBP-2, resulting in PCSK9-mediated attenuation of their effects.

Table1. Effects of nutraceuticals on PCSK9 levels in experimental studies.

Nutraceutical	Study type	Dose	Change in PCSK9 mRNA or protein levels	Change in LDLR mRNA or protein levels	Ref
Berberine	*In vitro*/ HepG2 cell line	15 μg/ml	-77% (mRNA) -87% (protein)	+3folds (mRNA)	[63]
		20 μM	-23% (mRNA)	+1.8-3 folds (mRNA)	[29]
	In vivo/ high fat diet-fed mouse	200 mg/kg	-46%(mRNA) -50% (protein)	+67% (protein)	[64]
	In vivo/ high fat diet fed hamster	100 mg/kg	-30% (protein)	ND	[64]
	In vivo/ high fat diet fed rat	156 mg/kg	Significant decrease of protein level	Significant increase of protein level	[72]
	In vivo/ LPS- induced dyslipidemic mice	30 mg/kg	Significant decrease of protein level	Significant increase of protein level	[76]
	In vivo/ LPS- induced dyslipidemic mice fed with high-cholesterol diet	30 mg/kg	Significant decrease of protein level	Significant increase of protein level	[76]
Curcumin	*In vitro*/ HepG2 and Huh7 cell lines	10 μM	-31% (mRNA) and Significant decrease of protein level	+20-35% (protein)	[118]
		20 μM	-48% (mRNA) and significant decrease of protein level		
Polydatin	*In vitro*/ HepG2 cell line	20 μM	significant decrease of mRNA and protein level	ND	[134]
	In vivo/ db/db C57BL/6 mice				
Xuezhikang	*In vivo*/ Rat	1200 mg/kg for 3days	+70% (protein)	ND	[143]
***n*-3 PUFA-enriched fish oil**	*In vivo*/ high-fat and high-cholesterol diet- fed rat	10% in diet (16 week)	Significant reduction of *PCSK9* expression	ND	[150]
***n*-3 PUFA** **or** ***n*-3 PUFA plus aspirin**	ApoE$^{-/-}$ female mice	1.8 g *n*-3 PUFA /kg diet per day	Significant reduction of		
		0.1 g aspirin/kg diet per day			

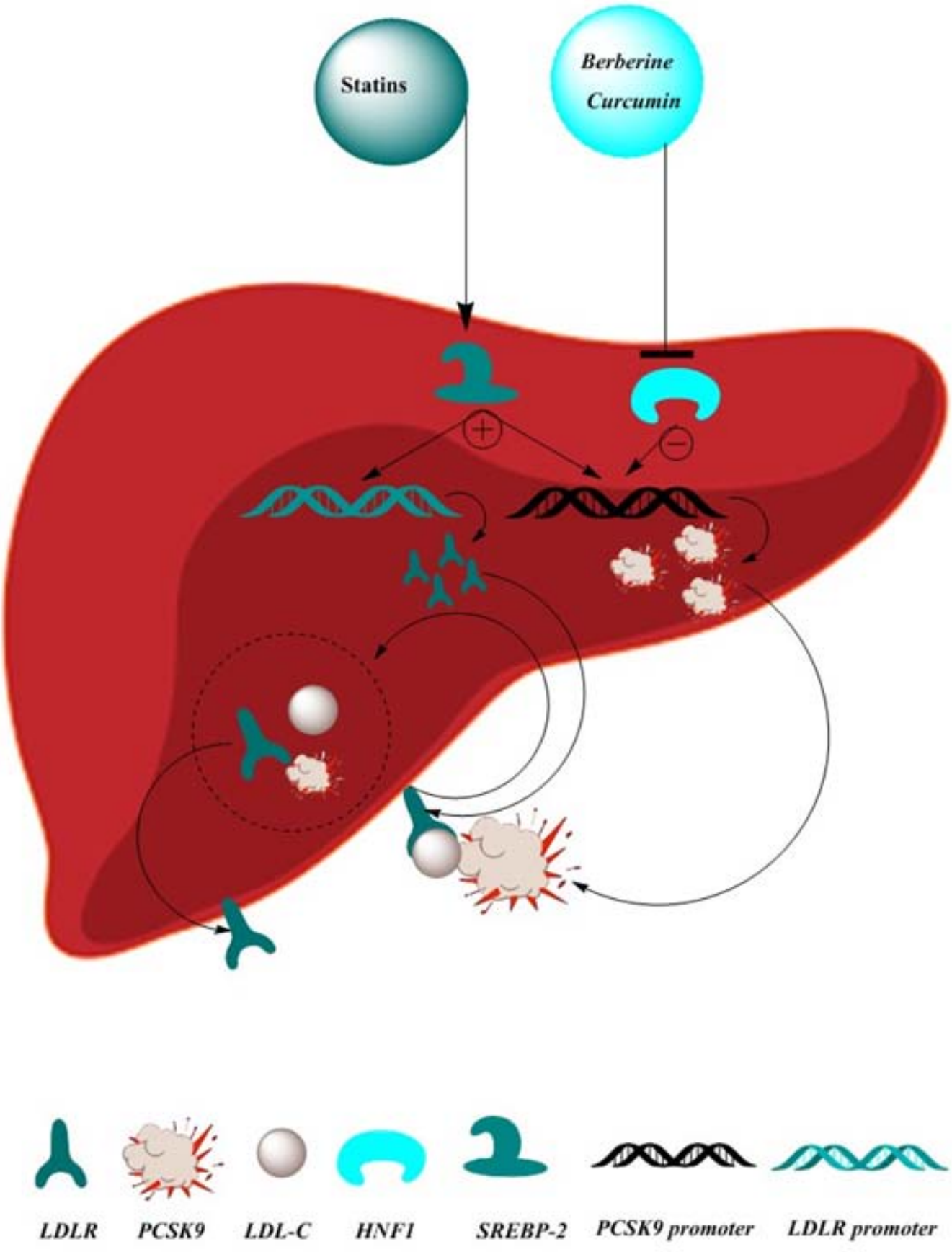
			protein		
Plant stanol esters	*In vivo*/ C57BL/6J mice	50 mg	Significant increase of intestinal mRNA	Significant increase of intestinal mRNA	[158]
Quercetin-3-O-b-D-glucoside	*In vitro*/ Huh7	0-10 µM	-20 to -30% (mRNA) and decreased secreted protein	+60% (mRNA) +300 to 400 % (protein)	[166]
Aqueous extract of *Phaleria macrocarpa*	*In vivo*/ high-cholesterol (3%) diet-fed rat	20 mg/kg	+97% (protein)	+115%(protein)	[170]
	In vitro/ HepG2 cell line	0.1-1000 µg/mL	Significant increase of both mRNA and protein	Significant increase of both mRNA and protein	[170]
Tanshinone IIA	*In vivo*/ high fat diet-fed C57BL/6J mouse	10 mg/kg for 3 months	Significant increase of both mRNA and protein	Significant increase of both mRNA and protein	[171]

ND: not defined.

Table2. Effect of nutraceuticals on PCSK9 levels in human trials.

Nutraceutical	Subjects	Dose	Plasma PCSK9 changes	LDL-C decrease	Ref
Berberine	HeFH patients (n=30)	500 mg for 6 months	Suggested to be decreased	Significant decrease by 10.5%	[77]
Xuezhikang	Dyslipidemic patients (n=16)	1200 mg for 8 weeks	Significant increase by 34%	Significant decrease by 28%	[143]
Docosahexaenoic acid-enriched canola oil	Patients with least one condition related to the metabolic syndrome (n=54)	ND	Significant decrease	Significant decrease	[151]
Marine *n*-3 PUFAs	premenopausal women (n=23)	2200 mg for 12 weeks	Significant decrease by 11.4%	Significant decrease	[154]
	postmenopausal women (n=22)		Significant decrease by 9.8%	Significant decrease	

ND: not defined.


Statins
Berberine
Curcumin
LDLR
PCSK9
LDL-C
HNF1
SREBP-2
PCSK9 promoter
LDLR promoter