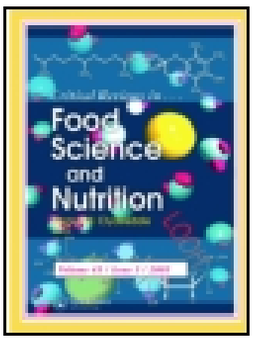

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REVIEW

The role and mechanism of citrus flavonoids in cardiovascular diseases prevention and treatment

Yudi Deng, Yali Tu, Shenghui Lao, Mengting Wu, Hantong Yin, Linqing Wang, and Wenzhen Liao

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ABSTRACT

Cardiovascular diseases (CVDs) have been ranked as the leading cause of death in the world, whose global incidence is increasing year by year. Citrus, one of the most popular fruits in the world, is rich in flavonoids. Citrus flavonoids attract special attention due to a variety of biological activities, especially in the prevention and treatment of CVDs. The research progress of citrus flavonoids on CVDs have been constantly updated, but relatively fragmented, which needed to be systematically summarized. Hence, the recent research about citrus flavonoids and CVDs were reviewed, including the types and in vivo processes of citrus flavonoids, epidemiology study and mechanism on prevention and treatment of CVDs by citrus flavonoids. This review would provide a theoretical basis for the citrus flavonoids research and a new idea in the citrus industry development and application.

KEYWORDS

Cardiovascular diseases; citrus flavonoids; mechanism; prevention; treatment

Introduction

Cardiovascular diseases (CVDs), a group of diseases mainly including coronary heart disease, hypertension, cerebrovascular disease, rheumatic heart disease, congenital heart disease, aortic aneurysm, atherosclerosis, etc., are regarded as a major public health challenge with the highest mortality rate in the world (Pala et al. 2020). Evidences from clinical trials indicated that dietary and pharmacological means could reduce the mortality rate of CVDs (Hu et al. 2014). The drugs currently used for the treatment of CVDs are mainly included antiarrhythmic agents, hypolipidemic drugs, antihypertensive medicines, cardiotonic medications, and antithrombotic medicaments. However, these common medicines can bring toxicity and side effects such as myalgia, liver disease, and rhabdomyolysis to the patients while treating diseases. Thus, finding more effective and safer natural products to prevent and treat CVDs has become one of the most important scientific issues.

Citrus fruits, the most productive fruit species all over the world, belong to the Rutaceae family (Gbaj et al. 2019). The world's average annual output of citrus is about 100 million tons, while China ranks first in the world (Mahato et al. 2019; FAO 2017). Citrus mainly include sweet oranges, grapefruits, pomeloes, lemons, citruses and kumquats. As one of the most popular fruits in the whole world, citrus is rich in nutrients and biologically active substances (flavonoids, limonoids, coumarin, dietary fiber and etc.). Among them, citrus flavonoids have various biological activities, including antioxidant, antiviral, anti-inflammatory, anticancer activity and etc. In the existing literature reports, flavonoids in citrus are found to have an effect on preventing and treating CVDs (Lee et al. 2003; Yu et al. 2005). However, information about the mechanism of flavonoids in citrus related to the prevention and treatment of CVDs was fragmented. Hence, recent research on citrus flavonoids for CVDs was reviewed systematacially, including the types, in-vivo biotransformation and mechanism of citrus flavonoids. This reviewed could provide instructions for the intervention strategies of citrus flavonoids in CVDs and a new direction in the citrus industry development and application.

Overview of citrus flavonoids

Structural characterization of citrus flavonoids

More than 60 kinds of flavonoids have been identified in citrus fruits for the time being, including four major categories of substances, flavanones, flavones, flavonols and anthocyanins (Table 1) (Tripoli et al. 2007). Among them, flavonols merely exist in lemon, and anthocyanins just exist in blood orange. Among most citrus fruits of different varieties, flavanone content is the most abundant, accounting for 80% of the total flavonoids. But in non-lignified parts of other plants, flavanone content is extremely low. The majority of flavanones exist in the form of glycosides, and a small amount exist in the form of aglycones. Flavanones in the form of glycosides can be divided into rutin glycoside and neohesperidin. The former includes bitter-tasting naringin, citrin, etc., mainly in grapefruit and bergamot. The latter

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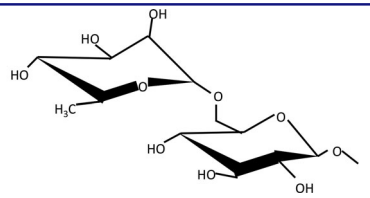
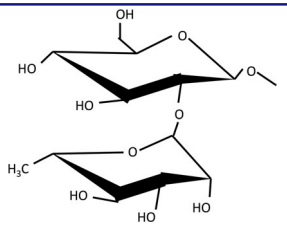
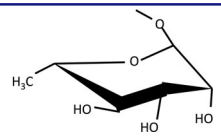
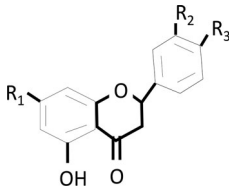
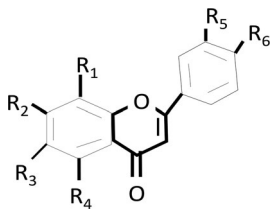
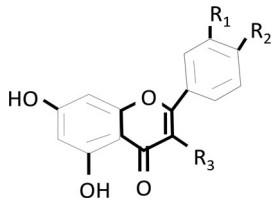
Table 1. Structure and characteristics of main flavonoids in citrus fruits.

Flavonoids	Chemical name	Structural formula	Molecular formula	Molecular weight	Characteristic
Rutinoside = Ru; Neohesperidin = Nh; Rhamnoside = Rh	Ru= 	Nh= 			Rh= 
Flavanones					Sensitive to oxidation, light and pH, a bitter taste, aglycone is insoluble in water and glycosides are soluble in water
Naringin	4′, 5,7-trihydroxyflavanone-7-new hesperidin	R_1 = Nh ; R_2 = H ; R_3 = OH	$C_{27}H_{32}O_{14}$	580.54	
Hesperidin	3′, 5,7-trihydroxy-4′-methoxyflavanone-7-rutinoside	R_1 = Ru ; R_2 = OH ; R_3 = OCH_3	$C_{28}H_{34}O_{15}$	610.56	
Neohesperidin	3′, 5,7-trihydroxy-4′-methoxyflavanone-7-new hesperidin	R_1 = Nh ; R_2 = OH ; R_3 = OCH_3	$C_{28}H_{34}O_{15}$	610.56	
Eriocitrin	3′, 4′, 5,7-tetrahydroxyflavanone-7-rutinoside	R_1 = Ru ; R_2 = R_3 = OH	$C_{27}H_{32}O_{15}$	596.53	
Narirutin	4′,5,7-trihydroxyflavanone-7-rutinoside	R_1 = Ru ; R_2 = H ; R_3 = OH	$C_{27}H_{32}O_{14}$	580.53	
Didymin	5,7-dihydroxy-4′-methoxyflavanone-7-rutinoside	R_1 = Ru ; R_2 = H ; R_3 = OCH_3	$C_{28}H_{34}O_{14}$	594.56	
Poncirin	5,7-dihydroxy-4′-methoxyflavanone-7-neosperoside	R_1 = Nh ; R_2 = H ; R_3 = OCH_3	$C_{28}H_{34}O_{14}$	594.56	
Eriodictyol	3′, 4′, 5,7-tetrahydroxyflavanone	R_1 = OH ; R_2 = R_3 = OH	$C_{15}H_{12}O_6$	288.25	
(±)-Naringenin	4′, 5,7-trihydroxyflavanone	R_1 = OH ; R_2 = H ; R_3 = OH	$C_{15}H_{12}O_5$	272.25	
Hesperetin	3′, 5,7-trihydroxy-4′-methoxyflavanone	R_1 = OH ; R_2 = OH ; R_3 = OCH_3	$C_{16}H_{14}O_6$	302.28	
Flavones					Sensitive to oxidation and pH, aglycone is slightly soluble in water and glycosides soluble in water
Tangeretin	4′, 5,6,7,8-pentamethoxyflavone	R_5 = H ; R_1 = R_2 = R_3 = R_4 = R6 = OCH_3	$C_{20}H_{20}O_7$	372.37	
Nobiletin	3′,4′,5,6,7,8-hexamethoxyflavonoid	R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3	$C_{21}H_{22}O_8$	402.39	

Sinensetin	3′, 4′, 5,6,7-pentamethoxyflavone	$R_1 = H$; $R_2 = R_3 = R_4 = R_5 = R6 = OCH_3$	$C_{20}H_{20}O_7$	372.37	
Rhoifolin	4′, 5,7-trihydroxyflavone-7-new hesperidin	$R_1 = R_3 = R_5 = H$; $R_4 = R_6 = OH$; R2 = Nh	$C_{27}H_{30}O_{14}$	578.52	
Luteolin	3′,4′,5,7-tetrahydroxyflavone	$R_1 = R_3 = H$; $R_2 = R_4 = R_5 = R_6 = OH$	$C_{15}H_{10}O_6$	286.24	
Diosmetin	3′, 5,7-trihydroxy-4′-methoxyflavone	$R_1 = R_3 = H$; $R_2 = R_4 = R_5 = OH$; $R_6 = OCH_3$	$C_{16}H_{12}O_6$	300.26	
Apigenin	4′, 5,7-trihydroxyflavone	$R_1 = R_3 = R_5 = H$; $R_2 = R_4 = R_6 = OH$	$C_{15}H_{10}O_5$	270.24	
–	3′,4′, 6,7-tetramethoxyflavone	$R_1{=}R_5{=}H$; $R_2{=}R_3{=}R_4{=}R_6{=}OCH_3$	$C_{19}H_{18}O_6$	342.34	
Isosinensetin	3′, 4′, 5,7,8-pentamethoxyflavone	$R_1{=}R_2{=}R_4{=}R_5{=}R_6{=}OCH_3$; $R_3{=}H$	$C_{20}H_{20}O_7$	372.37	
5-DemethylTangeretin	5-DemethylTangeretin 5-hydroxy-6,7,8,4′-tetramethoxyflavone	$R_1{=}R_2{=}R_3{=}R_6{=}OCH_3$; $R_4{=}OH$; $R_5{=}H$	$C_{19}H_{18}O_7$	358.35	
5-Demethylnobiletin	5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone	$R_1{=}R_2{=}R_3{=}R_5{=}R_6{=}OCH_3$; $R_4{=}OH$	$C_{20}H_{20}O_8$	388.37	
Sudachitin	–	$R_1{=}R_3{=}R_5{=}OCH_3$; $R_2{=}R_4{=}R_6{=}OH$	$C_{18}H_{16}O_8$	360.32	
Flavonols					Sensitive to oxidation, light and pH, aglycone is slightly soluble in water and glycosides are soluble in water.
Quercetin	3, 3′, 4′, 5,7-pentahydroxyflavone	$R_1 = R_2 = R_3 = OH$	$C_{15}H_{10}O_7$	302.24	
Kaempferol	3, 4′, 5,7-tetrahydroxyflavone	$R_1 = H$; $R_2 = R_3 = OH$	$C_{15}H_{10}O_6$	286.24	
Quercitrin	3, 3′, 4′, 5,7-pentahydroxyflavone-3-L-rhamnoside	$R_1 = R_2 = OH$; $R_3 = Rh$	$C_{21}H_{20}O_{11}$	448.38	
Rutin	3, 3′, 4′, 5,7-pentahydroxyflavone-3-rutinoside	$R_1 = R_2 = OH$; $R_3 = Ru$	$C_{27}H_{30}O_{16}$	610.52	

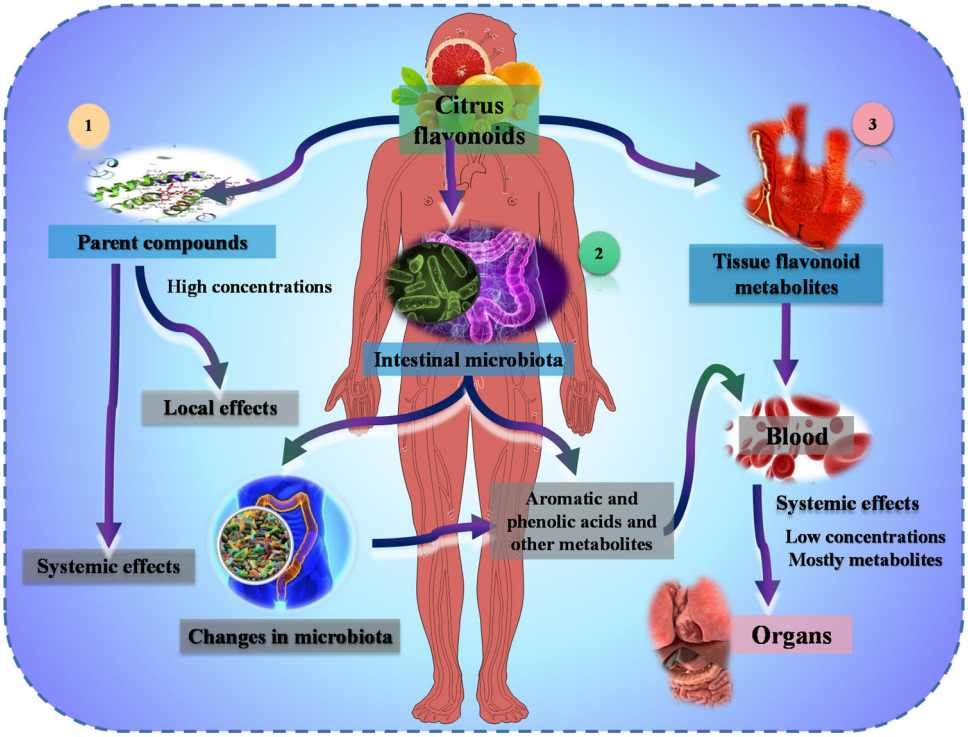


Figure 1. The fate of citrus flavonoids in the gastrointestinal tract. In the GI, citrus flavonoids can as parent compounds exert local effects favored by their high concentrations, interact with the microbiota changing its profile and/or be metabolized mainly to aromatic and phenolic acids which can be absorbed (systemic effects) and/or exert (local effects). They can be metabolized, usually to conjugates of the parent compounds by intestinal epithelial cells; subsequently metabolites may be absorbed (systemic effects) or excreted to the gut lumen (local effects). Local effects can also extend systemically, e. g. when they lead to the secretion of gut hormones with systemic actions.

includes hesperidin and naringin without bitterness, which are mainly found in broad-skinned citrus and oranges. The flavanones in the form of aglycone mainly include hesperetin and naringenin. Additionally, there are a series of polymethoxyflavones (PMFs) in citrus. PMFs are found in citrus fruits, especially in the oil cell layer of wide-skinned citrus and sweet orange, and their basic structural characteristics are 2-phenyl chromogen (C6-C3-C6 structure) as the skeleton, C4 position as the carbonyl group, with two or more methoxy groups. (Gaydou, Bianchini, and Randriamiharisoa, 1987). To date, more than 20 kinds of PMFs have been isolated and identified. Among them, nobiletin, tangeretin and sinensetin are the most common among citrus fruits as to their repeated biological activities, which has attracted increasing attention.

The process of citrus flavonoids in vivo

Gastrointestinal tract (GI) performs a central function in the absorption, distribution, metabolism and excretion of citrus flavonoids, which ultimately determines the impact of these bioactive substances on health for humanity (Ding et al. 2021; Oteiza et al. 2018; Wang, Liu, et al. 2020). Nevertheless, the interaction of citrus flavonoids with other dietary constituents, environmental factors, hosts and microorganisms affects the in vivo process of them in GI (Odenwald and Turner 2016; Williamson and Clifford 2017) (Figure 1). To better appreciate the health effects of citrus fruits, it is significant to understand the *in-vivo* biotransformation of citrus flavonoids. The biotransformation of citrus flavonoids in the body from their intake to excretion was reviewed.

Absorption of citrus flavonoids in vivo

Citrus flavonoids can be absorbed in the stomach, small intestine and large intestine. But different citrus flavonoids have exceedingly different absorption speeds and locations due to their different chemical structures (Gerard et al. 2015).

The absorption of citrus flavonoids in the stomach

The stomach has a special acidic environment and a small absorption area of the gastric mucosa. Due to phenolic hydroxyl groups, citrus flavonoids are weakly acidic and can be absorbed in the stomach. The flavonoid aglycone that can be absorbed through the stomach includes quercetin, but the flavonoid glycoside can't be absorbed. Crespy et al. (2002) showed that after intragastric administration (i.g.) of quercetin, isoquercitrin and rutin to rats for 30 minutes, 38% of quercetin disappeared, indicating that quercetin was rapidly absorbed, while rutin and isoquercetin glycosides were hydrolyzed into aglycones and absorbed in the rat stomach. Anthocyanin compounds, which belong to glycosides, can be rapidly absorbed in the stomach (Taiming and Xuehua 2006). An organic anion transporter, bilitranslocase, which is present in gastric wall epithelial cells, may participate in this absorption. Felgines et al. (2006) perfused red orange juice in situ in rats to study the absorption of

Table 2. Comparison of oral absorption characteristics of citrus flavonoids with different structures.

Compound	Type	Experimental model	Absorption method
Naringenin	Flavanone	BCRP cell model	Vector-mediated
Naringin		BCRP cell model	Vector-mediated
Hesperetin		BCRP cell model	Vector-mediated
Hesperidin		Rat intestinal sac valgus model	Passive transport, carrier-mediated
Neohesperidin		Rat intestinal sac valgus model	Passive transport, carrier-mediated
Kaempferol	Flavonol	In vivo intestinal absorption model	Passive transport
Rutin		Caco-2 cell model	Passive transport
Quercetin		Caco-2 cell model	Passive transport
Luteolin	Flavonoid	Caco-2 cell model	Passive transport
Nobiletin		Caco-2 cell model	Vector-mediated

Abbreviations: BCRP, breast cancer resistance protein.

anthocyanins. The results demonstrated that red orange anthocyanins could be quickly absorbed in the stomach, as well as in the small intestine, and about 20% could be absorbed in the stomach.

The absorption of citrus flavonoids in the small intestine

Flavonoid glycosides, which have greater hydrophilicity and larger relative molecular mass, are generally considered to be difficult to be absorbed by the small intestine in their original form. Murota et al. (2000) showed that compared with quercetin, 4-O-β-D-glucoside and quercetin-3-O-β-D-glucoside couldn't effectively penetrate the monolayer cell membrane. Their absorption might be relevant to the transport of Na^+-dependent glucose transporter (SGLT1) at the cell membrane of the small intestinal wall epithelial cells or the hydrolysis of lactose-phlorizin hydrolase (LPH), which was affected by its own structure (such as number, type and location connection of sugar-linked sugar), pH and other factors (Tammela et al. 2004). Whether it is the participation of SGLT1 or the role of LPH, flavonoid glycosides enter the body mainly through hydrolytic metabolism, rather than being absorbed by the original drug. Most flavonoid glycosides are usually hydrolyzed to aglycones. Since flavonoid aglycones are relatively hydrophobic, they can be absorbed by passive diffusion through biofilms. Therefore, the absorption of flavonoid aglycone is usually superior to flavonoid glycoside. But Andlauer, Stumpf, and Fürst (2001) found that the flavonoid glycoside compound rutin was mainly absorbed in the rat small intestine in the form of glycosides, which revealed that some flavonoid glycoside compounds could also be used in the original form and absorbed into the body through the small intestine.

The specific citrus flavonoids have different absorption capacities, and the absorption mechanisms are quite different as shown in Table 2 (Kaeko et al. 2002; Kimura et al. 2014). The Caco-2 cell model and phospholipid carrier were used to study the membrane transport process of citrus flavonoids and its affinity to the membrane (Tammela et al. 2004). It was found that the alkyl chain length, hydroxyl substitution, molecular conformation and structure greatly affected the transportation and affinity of citrus flavonoids to cells.

The absorption of citrus flavonoids in the large intestine

After selective absorption by the stomach and small intestine, unabsorbed citrus flavonoids and combined metabolites discharged from the small intestine wall are back into the intestinal cavity, what is more, enter the large intestine through the small intestine peristalsis. There are large quantities of intestinal bacterias in the large intestine (approximately 7 and 11 orders of magnitude higher than those in the stomach and small intestine, respectively), especially in the colon, which obtains carbon sources and energy from food residues. Therefore, the citrus flavonoids entering the large intestine could be hydrolyzed by various enzymes produced by these intestinal bacteria, and metabolic products could be absorbed into the blood (Hollman 2004).

The majority of anaerobic bacterias in the large intestine produce α-rhamnosidase, β-glucosidase, endo-β-glucosidase, β-glucuronidase and other glycosidases, which involved in the hydrolysis of flavonoids apparent glycosidic bonds of glycoside compounds. Glycosidases produced by bacteria of different genera have obvious substrate selectivity. In addition to the type of sugar group, they may also be related to the connection position on the flavonoid skeleton and other factors (Gwiazdowska et al. 2015). Kim et al. (1999) isolated fusobacteri-um K-60, which produced alpha-rhamnosidase to hydrolyze quercitrin. Additionally, the combined products that are excreted into the intestine via bile can also be hydrolyzed in the large intestine, regenerate aglycones and be absorbed into the blood, forming a hepatointestinal circulation (Aura et al. 2002).

The effects of efflux transporters on the absorption of citrus flavonoids

P-glycoprotein (P-gp), multi-drug resistance-related protein (MRP) and breast cancer resistance protein (BCRP) are the main drug efflux proteins currently studied. These types of proteins, which are highly expressed in stomach and intestine, are the main parts of oral drug absorption. Drugs absorbed through intestinal wall cells may be pumped out of the membrane by these efflux proteins and returned to the intestinal cavity (Passamonti et al. 2009). P-gp substrates are more than broad, and most P-gp substrates are lipophilic, neutral or weakly basic compounds. There are quite a few types of substrates for MRP-2, mainly organic anionic compounds, and many anionic amphoteric substances are their substrates, including glucuronic acid conjugates and sulfuric

acid conjugates. BCRP has been identified from human breast cancer cells, colon cancer cells and placenta in recent years. It belongs to the semi-ABC transporter, and needs to form a homodimer before it can function as a transporter. These efflux proteins are the main factors affecting the intestinal absorption of citrus flavonoids.

Wang, Cao, and Zeng (2005) used Bcap37/MDR1 cells transfected with P-gp gene as a model. When the P-gp inhibitor verapamil was added, the transport of quercetin and kaempferol increased significantly, indicating that P-gp mediated absorption of cortex and kaempferol in the small intestine. The absorption of quercetin was investigated by intestinal perfusion experiment in rats, and it was found that while the breast cancer resistance protein (BCRP-1/ABCG2) inhibitor fumitremorgin (FTC) was added, quercetin and its main the plasma concentration of the methylated metabolite isorhamnetin was twice higher than that of the control group. It could be indicated that the inhibition of BCRP-1 reduced the efflux of quercetin and its metabolites, and thus that BCRP-1 could efflux the ileal cavity, which limited its intestinal absorption (Sesink et al. 2005).

In addition, citrus flavonoids are weakly acidic and alkaline, so their solubility is poor, which affects their absorption in the gastrointestinal tract. They belong to a class of compounds with poor absorption, but chemical modification or dosage form modification can improve their absorption. Wen and Walle (2006) studied the difference between the absorption characteristics of methylated and non-methylated flavonoids, and found that in comparison with non-methylated flavonoids, the apparent penetration of methylated flavonoids was higher. Additionally, citrus flavonoids made into solid lipid nanoparticles could also improve the absorption properties of flavonoids in the GI tract, and effectively improve their bioavailability (Li et al. 2009).

Distribution of citrus flavonoids in vivo

The distribution of citrus flavonoids to a certain extent shows the targeting of citrus flavonoids to the body. Studying the distribution of citrus flavonoids in vivo can provide a reasonable explanation for clarifying the mechanism of citrus flavonoids, their effectiveness, toxic and side effects. Hence, it is necessary to study the distribution of citrus flavonoids in vivo. After oral administration of quercetin, the majority of the contents in the GI were not metabolized, and 30% to 100% of quercetin entering the tissues were its metabolites. When pigs continued to consume quercetin-containing food for 3 d, the concentration of quercetin and its metabolites in the liver and kidney was the highest. After pigs ingested different doses of quercetin (25, 50 $mg \cdot kg^{-1} \cdot d^{-1}$) for 4 consecutive weeks, the concentration of it and its metabolites in the liver was higher, and the concentration in the high-dose group was higher in the kidney and small intestine. After 11 weeks of continuous intake of food containing quercetin in rats, quercetin and its metabolites were widely distributed, with the highest content in the lungs, and short-term intake of quercetin showed no signs of such distribution. Thus, the distribution of citrus flavonoids in the body is mainly in the form of metabolites (Bieger et al. 2008; Graf et al. 2006). As a matter of fact, the time, dose and structure can affect the distribution of citrus flavonoids.

Metabolism of citrus flavonoids in vivo

The main metabolic methods of citrus flavonoids are hydrolysis, binding, cracking and oxidation, and the main organs of metabolism are the intestine and liver. Firstly, the hydrolysis reaction mainly occurs in the intestine. The flavonoid glycoside is hydrolyzed into aglycone by the intestinal mucosa lactase phlorizin hydrolase and passively diffused or directly transferred into intestinal epithelial cells by sodium-dependent sugar transporters (Graf et al. 2006). Under the action of glucosidase, it is hydrolyzed to aglycon, and subsequently under the action of phase II enzymes including glucuronosyltransferase (UDPGT), glucuronide conjugate, sulfate and methylate are produced (Figure 2) (Gradolatto et al. 2004; Van der Woude et al. 2004). Phase II metabolism may also occur in the liver. Most of the phase II metabolites are anionic metabolites such as UDPGT or sulfatase, which are generally not substrates of P-gp, but may be substrates of MRP2 and BCRP.

Secondly, the combined reaction mainly occurs in the small intestine and liver. Some of the polar functional groups (such as hydroxyl groups) contained in the original drug entering the liver or metabolites formed after the first phase reaction such as oxidation, are coupled with some endogenous substances under the action of various catalytic enzymes or combine to produce various binding products to achieve the purpose of removing activity, reducing toxicity, increasing water solubility and easily excluding in vitro (Van der Woude et al. 2004). The binding reactions of citrus flavonoids are mainly methylation, sulfation, and glucuronidation (Gradolatto et al. 2004).

Thirdly, the cleavage reaction is unique to the large intestine. Part of the flavonoid aglycone produced by the hydrolysis of the intestinal flora is directly absorbed by the large intestine. The other part is further cracked to produce small molecules of phenolic acids, which can be absorbed into the blood. Citrus flavonoids can be roughly divided into 4 types: (i) flavonoids and flavanones produce C6-C3 phenolic acid; (ii) flavonols produce C6-C2 phenolic acid; (iii) flavanols pass through phenyl-β -Intermediate of valerolactone produces C6-C3 phenolic acid; (iv) isoflavones produce ethylphenol derivatives.

Finally, the oxidation reaction proceeds in the liver and is metabolized by liver cytochrome P450 enzymes (CYPs). The metabolic pathway of citrus flavonoids in the liver has both a binding reaction and an oxidation reaction, but the binding reaction is dominant. Flavonoid glycosides are susceptible to hydrolysis in the intestine, while flavonoid aglycones are susceptible to oxidation in the liver.

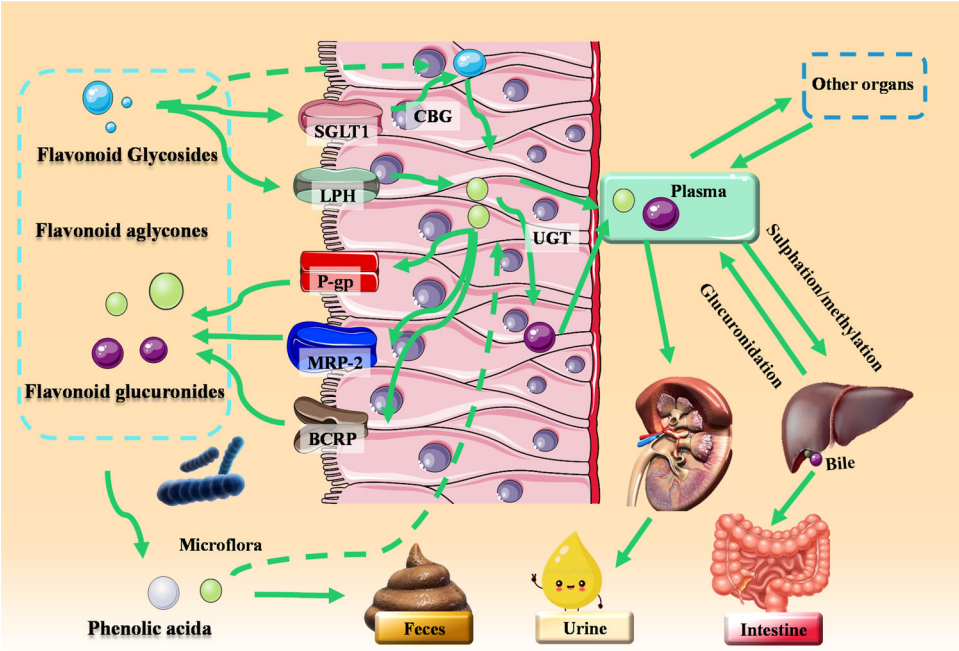


Figure 2. The metabolism of citrus flavonoids. Abbreviations: LPH, lactase phlorizin hydrolase; SGLT-1, sodium dependent glucose transporter; MRP-2, multidrug resistance associated protein-2; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; CBG: Cytosolic b-glucosidase; UGT: UDP-glucuronosyltransferase.

Excretion of citrus flavonoids in vivo

The excretion pathways of citrus flavonoids in the body are mainly renal excretion and bile excretion. Most excretions are metabolites of citrus flavonoids, and a few which are excreted, are their original form.

At the moment, the pharmacokinetic studies about citrus flavonoids are few and incomplete. The in vivo process of most citrus flavonoid monomers and the conversion mechanism of absorption and metabolic amount in the body is not yet clear, the role of citrus flavonoids and their interaction with other substances is also a research focus, thus further research is needed.

Epidemiological study on the prevention and treatment of cardiovascular diseases by citrus flavonoids

In recent years, observing the conversion and transport laws of biologically active substances in the human body, as well as the therapeutic effects and toxic and side effects on humans, have become an important form of evaluating the activities of biologically active substances. Flavonoids are common medicines for clinical treatment of CVDs, with cardiotonic, dilated coronary blood vessels, increased coronary blood flow, anti-arrhythmia, blood pressure reduction, lowered blood cholesterol and normalized the ratio of phospholipids to normal, reduced capillaries permeability and other effects.

A large number of epidemiological data indicated that eating more fruits and vegetables could reduce the risk of CVDs. A survey of the relationship between food and coronary heart disease in seven European countries found that comparing the 25-year coronary heart disease mortality at the same blood cholesterol level (5.2 mmol/L), the nordic countries were over 5 times than the Mediterranean coastal countries (Kromhout 1999). The analysis believed that this was not only related to the low content of saturated fat in foods in mediterranean countries, and also closely related to the consumption of red wine and the intake of large amounts of fruits and vegetables. The flavonoids in these foods might exert a strong antioxidant effect and reduce morbidity and mortality of coronary heart disease.

With the deepening of research, the researchers gradually found that flavonoids in fruits and vegetables were at work. In an epidemiological survey study, it was found that the intake of flavonoids in human daily diet was negatively correlated with the occurrence of CVDs, and increasing the intake of dietary flavonoids could significantly reduce the occurrence of CVDs rate (Chun et al. 2008). Zutphen Elderly Study, an epidemiological study, explored the correlation between the intake of dietary flavonoid and the risk of coronary heart disease (Hertog et al. 1993). The study by evaluating the intake of flavonoids in men aged 65 to 84 years, illustrated that dietary flavonoid intake was significantly negatively correlated with death due to coronary heart disease and negatively weakly correlated with the incidence of myocardial infarction. A survey in the Netherlands also showed that the intake of flavonols and flavonoids was negatively correlated with the mortality of coronary heart disease and the incidence of stroke (Geleijnse et al. 1999). The survey excluded the influence of blood cholesterol, blood pressure, smoking, drinking and other antioxidant substances.

Moreover, the consumption of citrus fruits or their juices is associated with the reduction of cardiovascular events, which suggests that the ingestion of flavonoids unique to citrus fruits may have cardioprotective effects. The crowd investigation experiments of the biological activity evaluation of citrus fruits were mainly conducted among volunteers, and facts have proved that drinking a

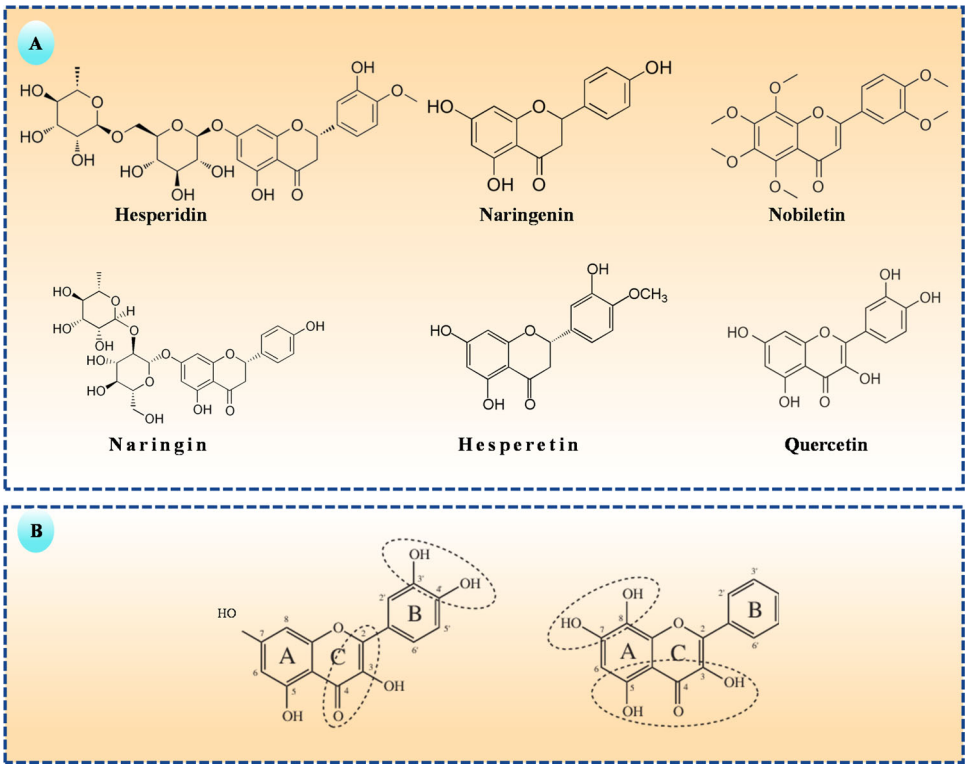


Figure 3. The common citrus flavonoids related to the prevention and treatment of cardiovascular diseases (A), and the structure of citrus flavonoids exerting antioxidant activity (B).

glass of orange juice every day could reduce the risk of stroke in men by 25% (Joshipura 1999), while the intake of grapefruit significantly reduced the mortality resulted from coronary heart disease (Mink et al. 2007). In a larger study from Japanese subjects (10,623 participants: 4,147 men and 6,476 women), citrus fruit intake (6-7 times a week) and CVDs events (especially ischemic stroke) also showed a negative correlation (Yamada et al. 2011). Similarly, after 14 years of follow-up (69,622 women) in the Nurses' Health study, ingesting large amounts of flavanones through orange juice and grapefruit juice could reduce the 19% risk of ischemic stroke. Cassidy et al. (2012) found through epidemiological investigations that the amounts of citrus flavonoids consumed by women was inversely proportional to the incidence of stroke. Eating citrus or orange juice could reduce the risk of stroke and protect the cardiovascular and cerebrovascular. In a population trial of drinking orange juice ($500\,mL \cdot d^{-1}$), it was found that drinking orange juice for 4 weeks could affect the expression of 3,422 genes, including a large number of anti-inflammatory and anti-atherosclerosis related genes, involving mononuclear macrophages physiological and biochemical processes of cell chemotaxis, adhesion, infiltration and lipid transport (Milenkovic et al. 2011). Drinking 500 mL of red orange juice every day could significantly improve the function of human vascular endothelial cells and reduce the level of inflammatory factors in the blood vessel wall (Buscemi et al. 2012). The above studies only confirmed that orange juice containing citrus flavonoids regulated cardiovascular risk factors and had cardiovascular protection effects. Many cell culture and animal studies that would be summarized below to provide more strong evidence for elucidating the mechanism of purified citrus flavonoids in the prevention and treatment of CVDs.

Mechanism of citrus flavonoids in the prevention and treatment of cardiovascular diseases

In citrus, the flavonoids related to the prevention and treatment of CVDs mainly include hesperidin, naringenin, nobiletin, naringin, hesperetin, quercetin, etc. (Figure 3A) Numerous studies have found that citrus flavonoids could prevent the occurrence and development of CVDs through multiple mechanisms and different pathways, such as inhibition of oxidative stress (Lin et al. 2017) and platelet aggregation, reduction of inflammation (Manthey, Guthrie, and Grohmann 2001), improvement of lipid metabolism disorders (Kurowska et al. 2004), regulation of apoptosis, autophagy (Carresi et al. 2018) and intestinal flora (Stevens et al. 2019), and etc. (Figure 4)

Inhibition of oxidative stress

Under normal physiological conditions, the body maintains a dynamic balance between oxidation and anti-oxidation. Once the balance is broken, it may lead to reactive oxygen species (ROS) and reactive nitrogen produced by oxidative stress, resulting in the formation of a large number of free radicals and non-free radicals, which in turn damage cells. The mechanism and internal connection of free radicals interfering with cell function are not fully understood, but lipid peroxidation plays a pivotal role in it, which can lead

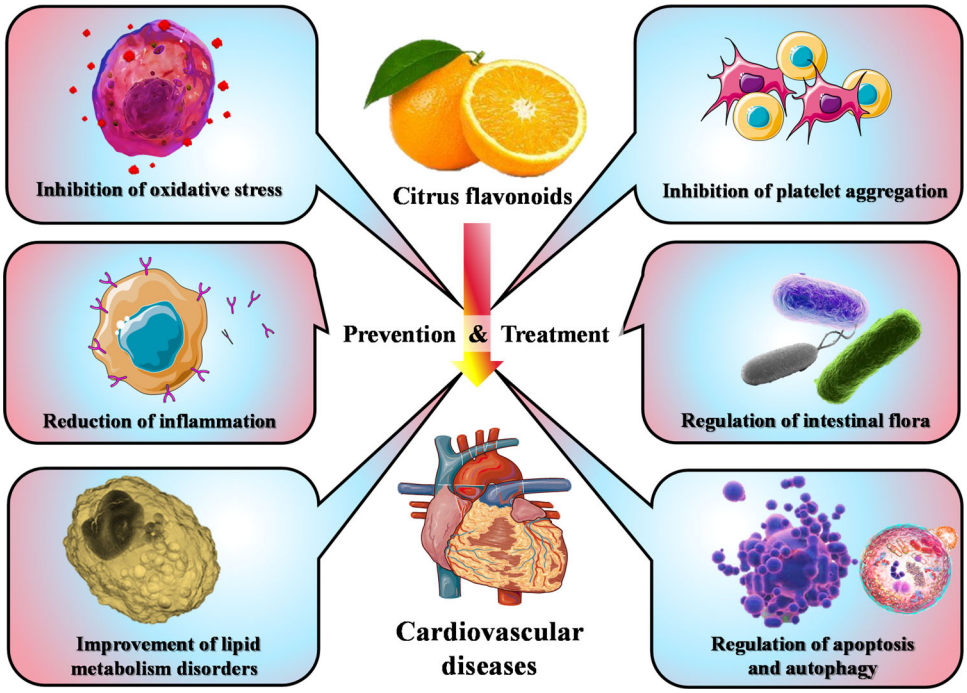


Figure 4. Mechanism of citrus flavonoids in the prevention and treatment of cardiovascular diseases.

to cell membrane damage, in turn lead to changes in the net charge and osmotic pressure of cells, cell swelling and eventually cell death (Panche, Diwan, and Chandra, 2016).

Almost every flavonoid has antioxidant activity. According to reports, flavonoids are the most powerful flavonoids that fight ROS and protect the body. Citrus flavonoids have extensive antioxidant effects and are also scavengers for various oxidation reactions, including superoxide anions, hydroxyl radicals and peroxyl radicals (Lin et al. 2017). This has aroused researchers' interests in cardiovascular protection. Due to the antioxidant and chelating properties of citrus flavonoids, they can inactivate ROS and scavenge free radicals, thereby counteracting low-density lipoprotein (LDL) oxidation and improving vascular endothelial inflammation (Davide Grassi 2009). Additionally, they can also reduce the activity of xanthine oxidase (XO), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and lipoxygenase (LOX), reduce the generation of ROS, and thus protect myocardial function.

In a comparative experiment on the protective effect of citrus flavonoids on cardiomyocytes injured by hypoxia/reoxygenation, it was found that quercetin has a good cardiomyocyte protection, which might be relevant to its strong antioxidant activity. The oxidation activity is weak, which may be related to the structure-activity relationship of antioxidant activity. The antioxidant activity of citrus flavonoids was mainly related to their structure (Figure 3B), incSTluding three structural groups: (i) the hydroxyl group at the 3,5 position on the B ring; (ii) 3 ', 4′-o-two phenolic hydroxyl group can participate in the electronic translocation and stabilize the aroyl radical through hydrogen bonding; (iii) The carbon-carbon double bond at the 2,3 position on the C ring can translocate the electrons on the B ring (Chen, Tait, and Kitts 2017; Khan and Dangles 2014).

Citrus flavonoids can also exert antioxidant effects by influencing gene expression, leading to changes in the transmission of information between cells. For instance, they can regulate IκBα protein through nuclear factor-κB (NF-κB) signaling pathway, so that NF-κB directly binds to DNA, thereby exerting antioxidant effect. A recent study found that hesperidin could increase the expression of nuclear factor erythroid-2 related factor 2 (Nrf2), improve antioxidant defense, and have a certain protective effect on the myocardium of aging rats. Similar results were obtained in vitro cell experiments, naringenin could significantly inhibit the generation of β-galactosidase in the aging model induced by doxorubicin (Da et al. 2017). It can be seen that the signaling pathways mediated by these signaling molecules provide a strong basis for further determining the direct targets of the antioxidant mechanism of citrus flavonoids (Figure 5).

Reduction of inflammation

There is a certain relationship between CVDs and inflammation (Wang, Liu, et al. 2020). Mahmoudi, Curzen, and Gallagher (2007) have confirmed that macrophages that entered the blood vessel wall could be activated by oxidized low-density lipoprotein (ox-LDL), gram-negative bacteria and their lipopolysaccharides, and produce tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nitric oxide (NO), prosta-glandins (PGs) and other inflammatory mediators that promoted the formation and development of atherosclerosis plaques, while inhibiting inflammation could improve the degree of atherosclerosis lesions. Many small molecules can give rise to inflammation, including cytokines (IL-6, IL-1, TNF) and adhesion molecules (ICAM-1, VCAM-1). Cyclooxygenase 2 (COX-2) and lipoxygenase are enzymes necessary for the metabolism of arachidonic acid,

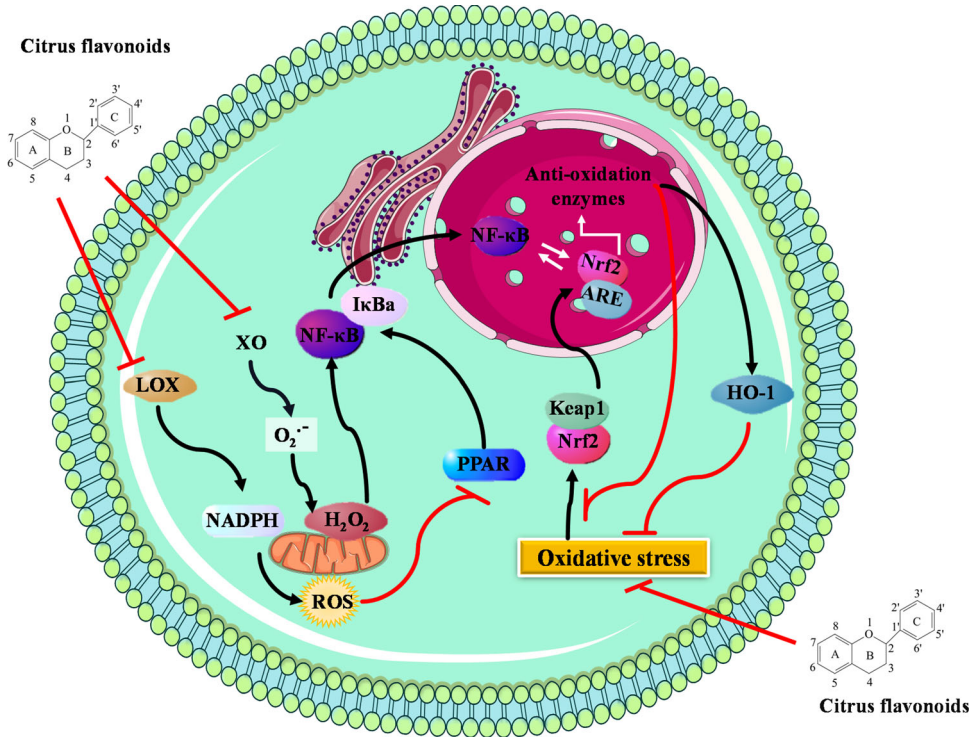


Figure 5. Mechanism of citrus flavonoids on inhibiting oxidative stress. Abbreviations: LOX, lipoxygenase; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear transcription factor-kappa B; IκB a, I kappa B alpha; PPAR, peroxisome proliferator-activated receptor; Nrf2, nuclear factor erythroid-2-related factor 2; Keap1, kelch-like ECH-associated protein 1; HO-1, heme oxygenase-1; XO, xanthine oxidase.

as well as the enzymes necessary for the synthesis of PG, leukotriene, and thromboxane. They can all aggravate the inflammatory response.

Citrus flavonoids have good anti-inflammatory effects. The mechanism is mainly achieved by eliminating ROS, down-regulating the activity of COX and lipoxygenase, up-regulating the activity of endothelial nitric oxide synshase (eNOS) and shifting the expression of related genes (Lemos et al. 1999; Manthey, Guthrie, and Grohmann 2001). The anti-inflammatory activity of citrus flavonoids is also inseparable from its structure. For instance, citrus flavonoids with 3′, 4′-OH or 4′-OH substituents on ring B can be used as selective lipoxygenase inhibitors and containing flavone compounds with five or more methoxy groups have higher phosphodiesterase inhibitory activity (Manthey, Guthrie, and Grohmann 2001; Testai and Calderone 2017).

In vitro researches

Chen, Tait, and Kitts (2017) have shown that in RAW 264.7 cells induced by lipopolysaccharide (LPS) and interferon-γ (IL-γ), orange peel extracts with high content of hesperetin and chuannan peelin could effectively inhibit the mRNA and protein expression of proinflammatory factors nitric oxide synthase (iNOS) and COX-2. Further experiments with pure monomers confirmed that nobiletin and tangeretin could inhibit the gene expression of pro-inflammatory cytokines including IL-6, interleukin-α (IL-α), and interleukin-β (IL-β) in mouse macrophages (Li et al. 2007; Lin et al. 2003; Tripoli et al. 2007). This might be due to the role of methoxy groups in hesperetin and ligustrazine. Liu et al. (2016) used LPS induced murine macrophages BMDMs or RAW 264.7 model, found that naringenin could inhibit the expression of mRNA and protein of TNF-α and IL-6, and showed a dose-dependent. Additionally, by inhibiting the activation of the two signaling pathways mitogen-activated protein kinases (MAPK) and NF-κB, the expression levels of inducible iNOS, COX2, nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2) and toll-like receptor 4 (TLR4) caused by LPS could also be significantly reduced. Hirai et al. (2007) have found that both naringenin chalcone and naringenin significantly reduced the production of inflammatory cytokines such as TNF-α and NO, and inhibited their activity, while naringenin had a stronger effect.

In vivo researches

In the high-fat diet-induced obesity model of C57BL/6 mice, after feeding with 1% naringenin for 16 weeks, naringenin could inhibit the expression of toll-like receptor 2 (TLR2) in adipose tissue by mediating peroxisome proliferator-activated receptor γ (PPARγ) activation, then inhibit the expression of inflammatory factor genes such as TNF-α and monocyte chemoattractant protein-1 (MCP-1) (Yoshida et al. 2013). Peritoneal macrophages from $Ldlr^{-/-}$ mice fed a high-fat, high-cholesterol diet is considered a great model of foam cells in that they accumulate more cholesterol esters than macrophages from cholesterol-fed mice. After intervention, naringenin (3%) would contribute to the cholesterol ester content of peritoneal macrophages to be reduced by more than 50%, which was related to the apparent reduction of neutral lipid droplets. Analysis of the expression of

Table 3. Citrus flavonoids prevent and treat cardiovascular diseases by reducing inflammation.

Compound	Model of study	Treatment protocol	Underlying mechanisms of the observed results	References
Orange peel extract with high content of hesperetin and chuanxietin	RAW 264.7 cells induced by lipopolysaccharide and interferon-γ	1, 2, 4 mg/mL, 24 h	Inhibition of the expression of mRNA and pro-inflammatory factors iNOS and COX-2	Chen, Tait, and Kitts (2017)
Nobiletin and its metabolites	LPS-induced RAW264.7 macrophages	10 and 30 μM, 24 h	Inhibition of NO production and iNOS, COX-2 protein and gene expression to exert its anti-inflammatory	Li et al. (2007)
Nobiletin	Human synovial fibroblasts and mouse macrophages J774A.1 cells	4, 8, 16, 32 and 64 μM, 24 h	Down-regulation of the COX-2 gene in human synovial fibroblasts to interfere with the production of PGE_2; reduction of the expression of IL-1α, IL-1β, TNF-α and IL-6 mRNA in mice macrophages	Lin et al. (2003)
Naringenin	LPS induced murine macrophages BMDMs or RAW 264.7	2.5, 5, 10, 20, 40 and 80 μM, 12 h	Inhibition of the expression of TNF-α, IL-6, TLR4, iNOS, COX-2 and NOX2	Liu et al. (2016)
Naringenin, naringenin chalcone	LPS stimulated RAW 264.7 macrophages	25, 50, 100, 200 μM, 24 h	Inhibition of cell production of TNF-α, MCP-1 and NO	Hirai et al. (2007)
Naringenin	HFD male C57BL/6J mice	1% naringenin for 16 weeks	Inhibition of the expression of TLR2 in adipose tissue by mediating the activation of PPARγ, and inflammatory gene expression (TNF-α and MCP-1, etc.)	Yoshida et al. (2013)
Naringenin	$Ldlr^{-/-}$ mice with high-fat and high-cholesterol diet	3% wt/wt naringenin was fed to mice for 12 weeks	Decrease of lipid content of peritoneal macrophages and expression of TNF-a, CCL2 and CCL3	Julia et al. (2012)

Abbreviations: NO, nitric oxide; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase 2; PGE2, prostaglandin E2; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-6, interleukin-6; TLR4, toll like receptor-4; TLR2, toll like receptor-2; MCP-1, monocyte chemotactic protein 1; TNF-α, tumor necrosis factor α; PPARγ, peroxisome proliferator-activated receptor gamma; CCL2, C-C motif ligand 2; CCL3, C-C motif ligand 3; LPS, lipopolysaccharide; HFD, high-fat diet.

inflammatory cytokines demonstrated that naringin significantly attenuated the expression of TNF-a and chemokine (C-C motif) ligand 2 (CCL2) (Julia et al. 2012). It can be seen that citrus flavonoids can inhibit the inflammatory response by inhibiting the gene expression pathway of inflammatory cytokines and realize the prevention and treatment of CVDs (Table 3).

Improvement of lipid metabolism disorders

Lipid-lowering activity of citrus flavonoids

Abnormal blood lipid metabolism is a predisposing factor for CVDs (Kohno et al. 2018). Fatty acids, triglycerides (TG) and cholesterol are extremely important substances to maintain the steady state of the cardiovascular system in the body, and they jointly maintain the dynamic balance of lipid levels in the body. Once the lipid metabolism is abnormal, it may result in the occurrence and development of lipid accumulation and atherosclerosis, and eventually lead to severe CVDs. In citrus fruits, the flavonoids related to the prevention and treatment of CVDs mainly include hesperidin, hesperetin, naringin, naringenin, quercetin and etc. (Baselga-Escudero et al. 2015; Kou et al. 2012; Mari et al. 2014; Nambiar et al. 2014) These flavonoids, as a class of natural active substances with high safety, can improve dyslipidemia, alleviate metabolic disorders induced by high-fat diet, and have good prevention, relief, and even therapeutic effects on CVDs.

For the lipid-lowering activity of citrus flavonoids, researchers have done an army of research from cell experiments. Kurowska and Manthey (2004) found thathesperetin, a kind of PMFs in citrus, could inhibit cholesterol ester (CH), triglyceride (TG) synthesis and ApoB secretion in HepG2 cells. Whitman et al. (2005) reported that ligustilide could reduce the accumulation of cholesterol in mouse macrophages to prevent atherosclerosis.

The lipid-lowering activity of citrus flavonoids has also been confirmed by plenty of animal experiments. In the female zebrafish research model, the orange peel extract significantly inhibited plasma triglyceride rising and liver lipid accumulation (Zang et al. 2014). Feeding zebrafish with saponin ($32\,mg \cdot kg^{-1}$) for 28 days could improve steatosis induced by high-fat diet (Hiramitsu et al. 2014).

The study found that quercetin, epicatechin, hesperetin, apigenin, and anthocyanin could also significantly reduce the weight gain and TG content in the liver of mice fed high-fat diet, and the lipid-lowering effect of quercetin the best (Hoek-van den Hil et al. 2015). Kurowska and Manthey (2004) studied the hypolipidemic effects of hesperidin, naringin, PMFs and hesperetin formula on diet-induced hypercholesterolemia in hamsters. The study found that a diet containing 1% PMFs could significantly reduce serum total ultra-low-density lipoprotein, LDL, and serum triacylglycerol, indicating that high levels of PMFs metabolites in the liver might in vivo directly produce a lipid-lowering effect. Kurowska et al. (2004) reported that containing 1% of citrus

PMFs (mainly red tangerine) reduced the cholesterol content of hamsters induced by diet-induced hyperlipidemia. Hesperidin (0.2 g · kg^{-1}) could significantly reduce the levels of plasma free fatty acids (FFA), TG and plasma total cholesterol (TC) in liver of db/db mice (Jung et al. 2006). Using the C57BL/6 mouse model, Lee and others have also found that chuancetin could reduce the weight of mice, white adipose tissue in the body, plasma triglyceride and adiponectin levels, and improve symptoms such as obesity and dyslipidemia (Lee et al. 2014; Lee, Cha, et al. 2013a). Kim et al. (2003) reported that hesperetin and its metabolites could attenuate the synthesis and esterification of cholesterol in high-fat male rats, thereby reducing cholesterol levels. Alam, Kauter, and Brown (2013) have illustrated that feeding naringin (100 mg · kg^{-1}) significantly reduced plasma lipid accumulation, TC and free fatty acid levels in rats, but had no effect on body weight and plasma triglyceride levels. Naringin also showed significant lipid-lowering effects in rabbits and other experimental animal models (Baselga-Escudero et al. 2015). Feeding rabbits with a high cholesterol diet and 0.05% naringin could significantly reduce TC and low-density lipoprotein cholesterol (LDL-C) concentrations, and liver triglyceride and cholesterol levels (Jeon, Yong, and Choi, 2004).

Some researchers have conducted crowd experiments on citrus flavonoids. Roza et al. (2007) recruited men and women with hypercholesterolemia between 19 and 65 years old (cholesterol level> 230 mg/dL), given PMFs extracted from orange peels to intervene, and found that it reduced LDL-C, but the content of triglyceride had no significant effect on the content of high-density lipoprotein-cholesterol (HDL-C). In another clinical trial, after taking naringin (400 mg · d^{-1}) for 8 weeks in patients with high cholesterol, their TC, LDL-C and apolipoprotein B (Apo B) levels decreased by more than 14% (Jung et al. 2003). Nevertheless, in other clinical study, naringin (500 mg · d^{-1}) did not affect TC, LDL-C, or triglyceride levels in patients with high cholesterol after taking it for 4 weeks (Demonty et al. 2010). Therefore, the wider clinical application of citrus flavonoids depends on factors including type, dosage, dosage cycle and individual differences among the population.

The lipid-lowering effect of citrus flavonoids is related to its structure. Lin et al. (2011) compared the lipid-lowering effect and structure-activity relationship of 19 citrus flavonoids with different structures. The results showed that these compounds could inhibit the secretion of apoB and the synthesis of TG and CH in liver cells to varying degrees. Compounds with a high degree of oxygenation have a better inhibitory effect on the synthesis and secretion of apoB. This revealed that the citrus flavonoids might have different lipid-lowering activities due to their slightly different structures.

The mechanism of citrus flavonoids in reducing lipid

The intestine is the only way for exogenous lipid absorption and endogenous lipid excretion, meanwhile, the liver is the main organ of lipid metabolism in the body, since it has many key enzymes and regulatory factors related to lipid synthesis and decomposition. It means that the small intestine and liver are important target sites for citrus flavonoids and their metabolites to regulate lipid metabolism (Musso, Gambino, and Cassader, 2009). Researchers have found that citrus flavonoids might improve lipid metabolism disorders in CVDs by regulating the absorption and excretion of lipids in the intestine, lipid synthesis and decomposition in the liver, and etc. (Baselga-Escudero et al. 2015)

Reduction of lipid absorption and increase of lipid excretion

Dietary fat is broken up by pancreatic lipase into FFA, monoglycerides, cholesterol, phospholipids and so on. They are emulsified into small micelles by bile acid salts, which are absorbed by small intestinal mucosal epithelial cells. The CD36 receptor and niemann-pick C1 like 1 (NPC1L1) protein located on the brush border of epithelial cells play a significant role in intestinal lipid uptake and intracellular transport. Citrus flavonoids can reduce lipid absorption and increase lipid excretion by inhibiting the activity of lipid-binding receptors and transporters on small intestinal epithelial cells. Quercetin inhibits the absorption of cholesterol by regulating specific transporters on the brush border membrane of intestinal epithelial cells, showing a significant dose dependence, at the same time, it also increases the release of cholesterol in feces (Mari et al. 2014; Orhan et al. 2015). Shin et al. (1999) experimented with feeding male rats with high cholesterol feed and naringin, and found that naringin could significantly reduce the cholesterol levels in plasma and liver, while the amounts of neutral steroids excreted in the fecal excretion of mice was reduced. Kim et al. (2004) found that naringin could increase the excretion of coprosterol and neutral steroids to reduce serum cholesterol levels.

Inhibition to the production of oxidatively modified low-density lipoprotein

LDL is the main carrier for transporting cholesterol. When the body's free radical metabolism is out of balance, the polyunsaturated fatty acids in LDL undergo lipid peroxidation to form ox-LDL, an atherosclerotic substance (Kattoor, Kanuri, and Mehta 2018). Ox-LDL is toxic to arterial endothelial cells and enters the inner wall of the blood vessel. Meanwhile, it also increases the adhesion activity of monocytes to the endothelial cells of the blood vessel (Ahotupa 2017). Ox-LDL can be recognized by scavenger receptors on macrophages and engulfed in large quantities, resulting in the accumulation of intracellular cholesterol and cholesterol lipids, then macrophages continue to accumulate in the lower endothelium of the arterial wall to form foam cells, on this basis further development forms atheromatous plaque (Maxwell 1997). Research by Lo et al. (2010) found that the main metabolite of chuancetin, 3′, 4′-hydroxy-5,6,7,8-tetramethoxyflavone could significantly inhibit Cu^{2+}-mediated LDL oxidation, the gene expression of CD36 and scavenger

receptor A (SR-A), and weak the absorption of ox-LDL by macrophages, thereby playing the role of anti-atherosclerosis. D & Aquino (2005) reported that citrus PMFs could inhibit LDL cholesterol synthesis by inhibiting the secretion of Apo B. Cesar et al. (2010) reported that PMFs in orange juice might reduce the content of LDL by regulating the synthesis of hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase and transferase in the liver.

On the control of cholesterol synthesis

Bok et al. (1999) extracted two bioflavonoids, naringin and hesperidin, and added them to the feed in an amount of 0.5 g in 100 g to feed the mice. The mice were fed with cholesterol feed (1 g cholesterol/100 g) and after 42 d, a model of high cholesterol was formed. The experimental results showed that the above citrus flavonoids could significantly reduce the content of lipids, liver cholesterol and liver TG ($P < 0.05$) in the plasma and liver of mice, and the activities of HMG-CoA reductase and a-cholesterol acyltransferase (ACAT) enzymes also decreased significantly. Lee et al. (2003) and Jung et al. (2006) reported that naringenin and hesperidin could reduce HMG-CoA reductase and acyl-coenzyme by feeding rats with high cholesterol. The activity of ACAT reduced the content of cholesterol in plasma and liver, and had the effect of improving hyperlipidemia and atherosclerosis. Lee et al. (2001) reported that naringenin reduced the content of cholesterol in the plasma and liver of rabbits fed high cholesterol by inhibiting the activities of HMG-CoA reductase and ACAT. This showed that citrus flavonoids could reduce cholesterol levels by inhibiting the body's participation in cholesterol synthase activity and other ways.

Inhibition of fatty acid synthesis and promotion of lipolysis

Citrus flavonoids can activate the signal pathway of energy metabolism, adenosine-5′-monophosphate (AMP)-activated protein kinase (AMPK), to initiate the decomposition pathway of lipid metabolism or inhibit the lipogenic genes and regulate the fatty acid use. The catabolism pathway of lipid metabolism is initiated, and the synthesis of fatty acid catabolism enzymes including carnitine palmitoyl transferase, acetyl-CoA oxidase, etc. is up-regulate (Kou et al. 2012). Feeding 0.3% chuancetin in $ldlr^{-/-}$ mice on a high-fat diet could promote liver fatty acid β oxidation, reduce VLDL-TG secretion and liver triglyceride significantly, which might be associated with activation of liver PGC-1α and the expression of genes such as carnitine palmitoyl transferase-1a (CPT-1a) (Mulvihill et al. 2011).

Reducing lipid synthesis is achieved by inhibiting lipogenic genes, such as inhibiting the expression of acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS), etc. This process involves three important transcription factors sterol-regulatory element binding proteins (SREBPs), peroxisome proliferator-activated receptor γ (PPARγ), liver X receptors (LXRs) and their downstream target genes (Kou et al. 2012).

SREBPs are cholesterol sensors located in the endoplasmic reticulum of cells, while mature SREBPs cleaved by S1P (site 1 protease) and S2P (site 2 protease) are transferred to the nucleus, thereby realizing transcriptional regulation of target genes. SREBPs have three subtypes, including SREBP-1a, SREBP-1c, and SREBP-2, which can up-regulate the genes of all enzymes in the lipid synthesis pathway and are essential for maintaining the body's lipid metabolism balance (Duan et al. 2017). Morin et al. (2008) used HepG2 cell to find that citrus flavonoids might enhance the expression of LDL receptor (LDLR) by activating the operon of the mature SREBP and the LDLR gene, and promote the absorption of endogenous cholesterol by the liver at the same concentration. The latter was more active than the former, which might be because the polymethoxy group (5,6,7,8,3′,4′-position) in the structure of chuanchensuin helps its interaction with cell membrane proteins. However, Nichols et al. (2011) used a mouse primary cell model study to find that although hesperetin and ligustrazine could inhibit the expression of SREBP targeting gene-SCD1, the effect on SREBPs themselves was not yet more than certain and needed further study and verification.

PPARs are a class of nuclear transcription factors activated by ligands, and can be divided into three subtypes of PPARα, PPARβ and PPARγ according to their structure and function. The most studied currently is the PPARα subtype, which can regulate the expression of many mitochondrial fatty acid oxidases and stimulate the oxidation of fatty acid β. In oleic acid-loaded human liver cancer cells, rutin can induce AMPK phosphorylation and activate PPARα to promote lipid breakdown, while also inhibiting the activation of SREBP-1, which in turn downregulates FAS, ACC, recombinant 3-hydroxy-3-methylglutaryl coenzyme a reductase (HMGCR) and other lipid synthesis-related gene expression to reduce lipid synthesis (C. H. Wu et al. 2011). Naringenin could promote gene expression of PPARγ and adiponectin receptor 2 (AdipoR2) in 3T3-L1 adipocytes and secretion of proteins such as adiponectin, and improve the metabolic function of adipocytes (Horiba et al. 2010). Pomelo peel extract could prevent metabolic disorders induced by high-fat diet in C57BL/6 mice, which might play a role by activating PPARα and glucose transporter 4 (GLUT4) signaling pathways (Ding et al. 2013). Del Río et al. (2004) reported that PMFs could reduce the activity of diglyceride acetyltransferase (DGAT) necessary for the synthesis of TG and enhance the liver PPAR, while enhancing the oxidation activity of fatty acids, thereby reducing the level of TG in the blood. Feeding $Ldlr^{-/-}$ mice with a western-style diet showed many features of insulin resistance, including excessive production of very low-density lipoprotein (VLDL), dyslipidemia, and obesity. In this study, naringenin (1% or 3%) relieved hyperinsulinism and inhibited the production of lipid production mediated by SREBP-1c in the liver and muscle, which could be regulated peroxisome proliferator receptor gamma coactivator 1α (PGC1α)/PPARα and other transcription factors increase liver fatty acid oxidation (Mulvihill et al. 2009). Long-Evans hooded rats fed 0.006% naringenin had significantly lower total cholesterol levels in

liver and plasma, and 0.012% naringenin increased significantly the expressions of genes of liver PPARα, CPT-1 and uncoupling protein 2 (UCP-2) (Cho et al. 2011). Naringin ($0.2\,g \cdot kg^{-1}$) could effectively reduce the levels of serum FFA, TG and liver TG in db/db mice by regulating the expression of genes related to fatty acid, cholesterol and glucose metabolism (Jung et al. 2006). In the wistar rat research model of high-fat diet, after 28 days of supplemental feeding with naringin ($50, 100\,mg \cdot kg^{-1}$), the expression of PPARγ protein in rat liver and kidney was significantly increased, and naringin improved insulin resistance, abnormal blood lipids and steatosis of liver cells (Kumar Sharma et al. 2011).

LXRs are members of the nuclear receptor superfamily, including LXRα and LXRβ homologous subtypes (Kalaany and Mangelsdorf 2006). The downstream target gene of LXRs, ATP-binding cassette transporter A1 (ABCA1), can transfer the free cholesterol in peripheral tissues and cells to HDL, and has the role of regulating reverse cholesterol transport (RCT) in cholesterol crucial role. Activated LXRs can also up-regulate the expression of cholesterol 7α hydroxylase (CYP7A1), the rate-limiting enzyme of the classic bile acid synthesis pathway, and accelerate the conversion of cholesterol to bile acid (Wójcicka et al. 2007). Ohara et al. (2013) and Lee, Cha, et al. (2013b) found through a series of studies from in vivo experiments to cell culture and in vitro LXRα activation experiments that quercetin and its metabolite quercetin-3-O-glucuronide were activators of LXRα which bound and activated it. Activated LXRα upregulates the expression of ABCA1, which promotes cholesterol and tissue outflow to ApoAI to synthesize HDL, which is transported to the liver via reverse cholesterol transport (RCT) for metabolic transformation, thereby exerting its lipid-lowering and anti-atherosclerotic effects.

Regulation of microRNAs with lipid metabolism regulation function

In recent years, it was revealed that the critical role of microRNAs (miRNAs) in a variety of CVDs (Zhu et al. 2013). miRNAs played an important role in regulating lipid metabolism. Among them, miR-122 and miR-33 are reported more frequently. This provides a new perspective for the study of lipid-lowering mechanism of citrus flavonoids. Some researchers have found that quercetin could improve lipid metabolism by regulating the expression level of related miRNA, reducing cholesterol content (Garelnabi and Mahini 2014).

In brief, different citrus flavonoids can regulate lipid metabolism through different or the same targets and intracellular signaling pathways, therapy playing a part in the prevention and treatment of CVDs (Table 4, Figure 6), but the mechanism of action of some citrus flavonoids is still not very clear. Hence, more research is still required to determine the initial targets of these compounds, and to understand the direct relationship between active substances and cell signaling molecules and pathways.

Regulation of apoptosis and autophagy

Apoptosis, also known as programmed cell death, is a pathological process regulated by multiple genes and mediated through signal pathways, involving gene regulation, signal transduction, immune response, etc. It is also one of the important pathology mechanisms of myocardial ischemia-reperfusion injury. Hence, blocking the signaling pathways related to pro-apoptosis can block the occurrence of apoptosis, then prevent the reduction of the number of cardiomyocytes, and thus can maintain or improve cardiac function. Hesperidin had a protective effect on cardiomyocyte apoptosis induced by H_2O_2; hesperidin pretreatment exerted a certain protective effect on myocardial injury after coronary artery ligation in rats (Minchin 1987).

Cellular autophagy is a physiological phenomenon that achieves the decomposition, metabolism, reuse, and recycling of cells and organelles. It plays a "double-edged sword" role in myocardial ischemia-reperfusion, that is, during ischemia, myocardial ischemia activates, cells autophagy plays a protective role in ischemic myocardium, but in the course of the reperfusion period, excessive autophagy leads to myocardial cell death and aggravates myocardial damage (Dong et al. 2010; Ma et al. 2015). In the model of atherosclerosis induced by LDL, the treatment with luteolin increased autophagosome formation and Beclin-1 activity, reduced intracellular lipid accumulation and the rate of apoptosis, moreover, these effects were abolished by 3-MA. (Zhang et al. 2016). In the cell model of cerebral ischemia-reperfusion, naringin could reduce the formation of 3-nitrotyrosine and the ratio of microtubule-associated protein 1 light chain 3II (LC3II) and microtubule-associated protein 1 light chain 3I (LC3I). Thus, it could inhibit the transport of parkin protein to mitochondria, and weaken the excessive autophagy in the cell, thereby reducing the infarct size and cell dying mortality rate to prevent cerebral ischemia-reperfusion injury (Feng et al. 2018). Moreover, hesperidin could inhibit myocardial cell autophagy induced by myocardial ischemia/reperfusion injury (I/R), thereby reducing myocardial ischemia/reperfusion injury. Ligating the left anterior descending branch (LAD) coronary artery could induce acute myocardial infarction (AMI). It was confirmed that nobiletin (a citrus PMFs) could restore autophagic flux and protect against AMI (X. Wu et al., 2017). In the heart injury induced by doxorubicin (DOXO) in rats, since bergamot polyphenolic fraction could restore protective autophagy and weaken cardiomyocyte apoptosis, it had cardioprotective effects on DOXO acute toxicity (Carresi et al. 2018).

The phosphatidylinositol-3 kinase/protein kinase B (PI3K/AKT) signaling pathway is an important signal transduction pathway for cardiomyocyte survival, and plays a crucial biological role in the process of cell metabolism, proliferation and apoptosis, which can inhibit the process of cell apoptosis (Figure 7). Activated AKT has anti-myocardial ischemia and inhibits cardiomyocyte apoptosis (Song et al. 2016; Yu et al. 2016). Li et al. (2018) found that hesperidin mainly activated the

Table 4. Citrus flavonoids prevent and treat cardiovascular diseases by regulating disorders of lipid metabolism.

Compound	Model of study	Treatment protocol	Underlying mechanism of the observed result	References
Naringin	High cholesterol male rats	0.1% (wt/wt), fed for 42 days	The combination of inhibited HMG-CoA reductase and ACAT activity to reduce fecal sterol excretion	Shin et al. (1999)
Naringin	LDL receptor knockout (LDLR-KO) mice	0.02 g/100 g for 6 weeks	Inhibition of liver HMG-CoA reductase activity, reduce of plasma cholesterol levels and increase of fecal sterol excretion	Kim et al. (2004)
The main metabolite of ligustilide is 3′, 4′-hydroxy-5, 6, 7, 8-tetramethoxyflavone	THP-1 monocytes and macrophages	10-20 μM, 0-24 h	Inhibition of Cu^{2+}-mediated oxidation of LDL, the gene expression of CD36 and SR-A, and the differentiation of monocytes into macrophages and reduction of macrophage uptake of OX-LDL	Lo et al. (2010)
Concentrated orange juice	Subjects with hypercholesterolemia (HCH)	1: 6 orange juice/water, lasted 60 days	Regulation of the synthesis of HMG-CoA reductase and transferase in the liver	Cesar et al. (2010)
Naringenin and its synthetic derivatives	High cholesterol rat	0.073 mmol/ 100 g, 6 weeks of diet	HMG-CoA reductase and ACAT activity to reduce plasma and liver cholesterol levels	Lee et al. (2003)
Hesperidin and Naringin	C57BL/KsJ-db/db male mice	Hesperidin supplement (0.2 g/kg diet), Naringenin supplement (0.2 g/kg diet), feeding for 5 weeks	Decrease of liver HMG-CoA reductase and ACAT activity and increase of fecal cholesterol, GLUT4, liver and fat cell oxidase PARP γ expression	Jung et al. (2006)
Naringin and naringenin	Rabbits fed with high cholesterol Male New Zealand White	0.1% naringenin or 0.05% naringenin for 8 weeks	Related to decreased liver ACAT activity and down-regulation of VCAM-1 and MCP-1 gene expression	Lee et al. (2001)
Hesperetin and Nobiletin	HepG2 cells	0-200 μmol/L, 24 h	Through SRE binding protein in the upstream region of the LDLR gene	Morin et al. (2008)
Hesperetin and Nobiletin	Rat primary hepatocytes	5-150 μM, 18-20 h	Reduction of liver level SCD1 mRNA	Nichols et al. (2011)
Rutin	Oleic acid-loaded human liver cancer cells	100-200 μM, 24 h	Induction of AMPK phosphorylation and activation of PPARα to promote lipid decomposition, inhibition of the activation of SREBP-1, and down-regulation of the expression of FAS, ACC, HMGCR and other lipid synthesis related genes to reduce lipid synthesis	C. H. Wu et al. (2011)
Naringenin	3T3-L1 fat cells	25-100 μM, 8 days	Promotion of gene expression of PPARγ and AdipoR2 in cells and secretion of proteins such as adiponectin, improvement of the metabolic function of adipocytes	Horiba et al. (2010)
Pomelo peel extract	C57BL/6 female mice with high fat diet	1% for 8 weeks	Increase of mRNA expression of PPARα and its target genes such as FAS, PGC-1α and PGC-1β and GLUT4 in liver and white fat cell tissue	Ding et al. (2013)
Naringenin	LDL receptor ineffective ($Ldlr^{-/-}$) male mice with a high-fat (Western) diet	1% or 3% added to western diet, feeding for 4 weeks	i) Through PPAR gamma coactivator 1alpha / PPAR alpha-mediated transcription program, increased liver fatty acid oxidation; ii) prevention of liver and muscle by reducing fasting	Mulvihill et al. (2009)

(*continued*)

Table 4. Continued.

Compound	Model of study	Treatment protocol	Underlying mechanism of the observed result	References
			hyperinsulinemia medium sterol regulatory element binding protein 1c-mediated fat production; ii) reduction of liver cholesterol, cholesterol ester synthesis, VLDL derived and endogenously synthesized fatty acids, prevention of muscle triglyceride accumulation; iv) improvement of overall insulin sensitivity and glucose tolerance	
Naringenin	Long-evans hooded male rat	0.003, 0.006 and 0.012% diet for 6 weeks	Increase of PPARα protein expression in liver; enhancement of expression of CPT-1 and UCP2	Cho et al. (2011)
Naringin, Hesperidin	C57BL/KSJ-db/db male mice	Hesperidin 0.2 g/kg diet, Naringenin 0.2 g/kg diet, feeding for 5 weeks	Decrease of Liver HMG-CoA reductase and ACAT activity and increase of fecal cholesterol, and expression of GLUT4 and PPAR-γ	Jung et al. (2006)
Naringin	High-fat diet Wistar rats	50, 100 mg · kg^{-1}, 28 d	Increase of adiponectin and β cell function and PPARγ expression in liver and kidney	Kumar Sharma et al. (2011)
Quercetin and its metabolite quercetin-3-O-glucuronide	Mouse macrophage-like cell line RAW264.7; male BALB/c mice	0.5 mg/mL, 0-24 h; 5% NNE, feeding AIN-93G for two weeks	Directly bound to LXRα, activation of LXRα up-regulated ABCA1 expression, which promoted cholesterol outflow from tissues and cells to Apo AI to synthesize HDL	Ohara et al. (2013)
Quercetin	THP-1 cells	0.15, 0.3 μM, 4-8 h	Induction of expression of PPARγ and LXRα, up-regulation of the target gene ABCA1, improvement of macrophage cholesterol outflow	Lee, Moon, et al. (2013)
Quercetin	C57BL6 LDL$^{-/-}$ mice	Oral 100 μg for 30 days	Regulation of the expression of miR-21, 125 b and 451	Garelnabi and Mahini (2014)

Abbreviations: HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; ACAT, Acyl coenzyme A-cholesterol acyltransferase; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; ox-LDL, oxidized low density lipoprotein; LDLR, low-density lipoprotein receptor; CD36, vertebrate platelet glycoprotein 4; SR-A, scavenger receptor type A; GLUT4, glucose transporter 4; PPAR, peroxisome proliferator-activated receptor; PPARα, peroxisome proliferator-activated receptor α; PPARγ, peroxisome proliferator-activated receptor γ; VCAM-1, vascular cell adhesion protein 1; MCP-1, monocyte chemotactic protein 1; SRE, sterol regulatory element; SCD1, stearoyl-CoA desaturase-1; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; SREBP-1, sterol regulatory element-binding protein 1; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; AdipoR2, adiponectin receptor 2; PGC-1α, peroxisome proliferator-activated receptor-γ co-activator 1α; PGC-1β, peroxisome proliferator-activated receptor-γ co-activator 1β; WAT, white fat cell tissue; CPT-1, carnitine palmitoyl transterase-1; UCP2, uncoupling protein 2; LXRα, Liver X Receptor α; NNE, nelumbo nucifera leaf extracts; ABCA1, ATP-binding cassette transporter 1; HDL, high density lipoprotein; miR-21, microRNA 21; miR-125b, microRNA 125 b; miR-451, microRNA 451.

mammalian target of PI3K/AKT/mTOR to inhibit excessive autophagy to reduce I/R.

Antithrombotic activity

Platelet aggregation after activation is considered to be a vital factor in cardiovascular, brain and peripheral vascular diseases. Platelet aggregation and thrombosis usually occur in the middle and late stages of atherosclerosis, which promotes and worsens CVDs. At the same time, some active substances released such as thromboxane, serotonin, and adenosine diphosphate will aggravate the progress of atherosclerosis and cause various complications. Citrus flavones could inhibit platelet aggregation and thrombosis caused by adenosine diphosphate (ADP), collagen or thrombin, thereby reducing the risk of blood clot formation (Nabavi et al. 2017). In the in vitro experiment of rats induced by ADP, seven flavonoids all showed the effect of inhibiting platelet aggregation, and the effect size was quercetin > rutin > isoquercetin > hesperidin > naringenin > naringin (Guo et al. 2005). Beretz and Cazenave (1988) found that ligustilin and tangerine in the concentration range of 0-30 μM could inhibit platelet aggregation induced by ADP and collagen. Ligusticin had potent anti-platelet activity, initially inhibiting the cascade of PLCγ2/PKC and the formation of hydroxyl radicals, subsequently inhibiting the activation of Akt and MAPKs and ultimately to platelet

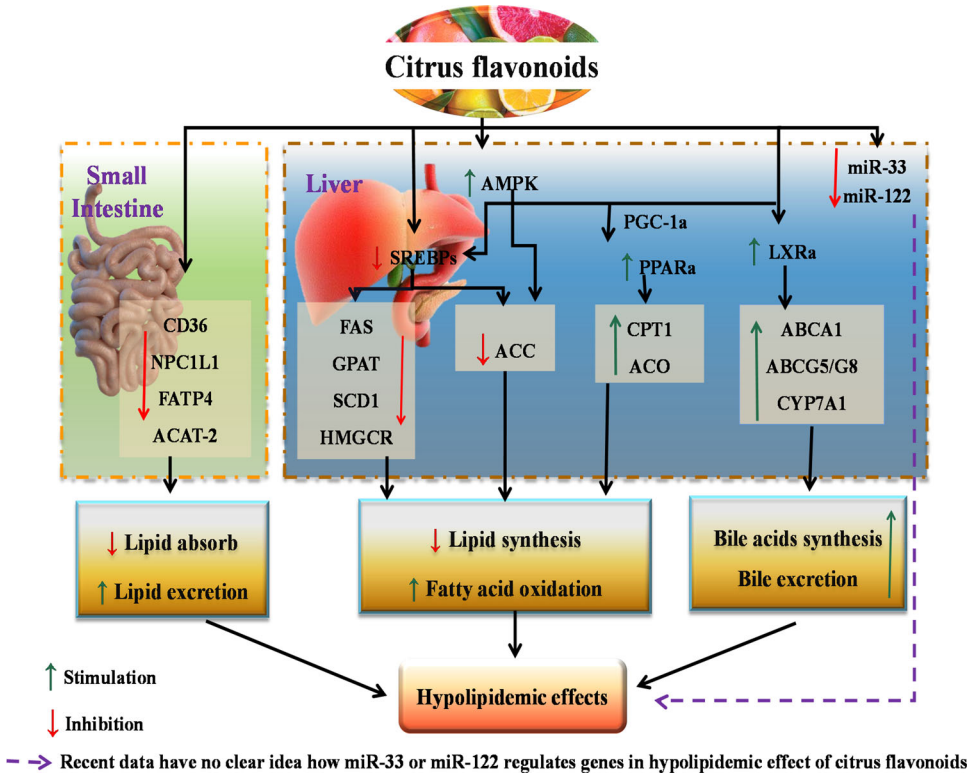


Figure 6. Potential molecular mechanism of citrus flavonoids for lipid-lowering effect. Abbreviations: SCD1, stearoyl-CoA desaturase-1; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; SREBP-1, sterol regulatory element-binding protein 1; PPAR a, peroxisome proliferator-activated receptor a; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; PGC-1α, peroxisome proliferator-activated receptor-γ co-activator 1α; CPT-1, carnitine palmitoyl transterase-1; LXRα, Liver X Receptor α; ABCA1, ATP-binding cassette transporter 1; miR-21, microRNA 21; miR-33, microRNA 33; NPC1L1, Niemann-Pick C1-like protein 1; ABCG5, ATP-binding cassette transporter 5; ABCG8, ATP-binding cassette (ABC) transporter 8; CYP7A1, cholesterol 7α-hydroxylase; ACO, aconitase closing odor; FATP4, fatty acid transport protein4; ACAT-2, acyl coenzyme A-cholesterol acyltransferase.

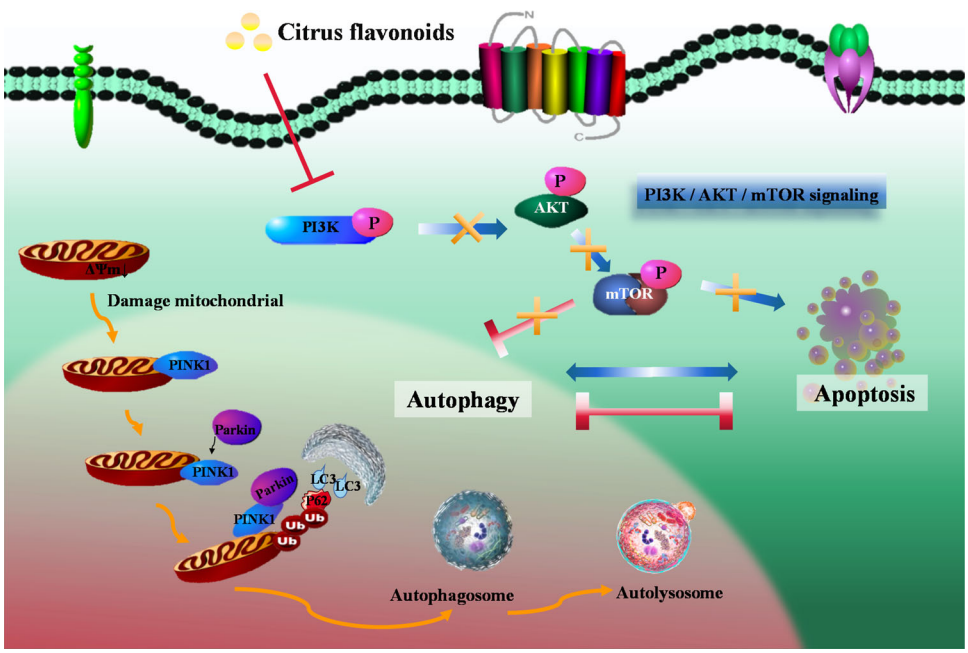


Figure 7. The regulation of citrus flavonoids on apoptosis and autophagy. Abbreviations: PI3K, phosphatidylinositol 3 kinase; Akt/PKB protein kinase B; mTOR, mammalian target of rapamycin; Ub, ubiquitin; PINK1, PTEN induced putative kinase 1.

activation (Lu et al. 2015). Calcium promotes platelet aggregation. Flavonoids can inhibit the binding between fibrinogen GPIIb/IIIa by activating Ca^{2+}-ATPase and inhibiting phospholipase C and inositol triphosphate, thereby exerting an antiplatelet effect. Collagen-induced platelet aggregation is also related to the increase and activation of calcium ions. The study found that serotonin also participated in the platelet aggregation process, hesperetin could inhibit arachidonic acid secretion of serotonin, anti-platelet aggregation. The effect of hesperetin and hesperidin on coagulation

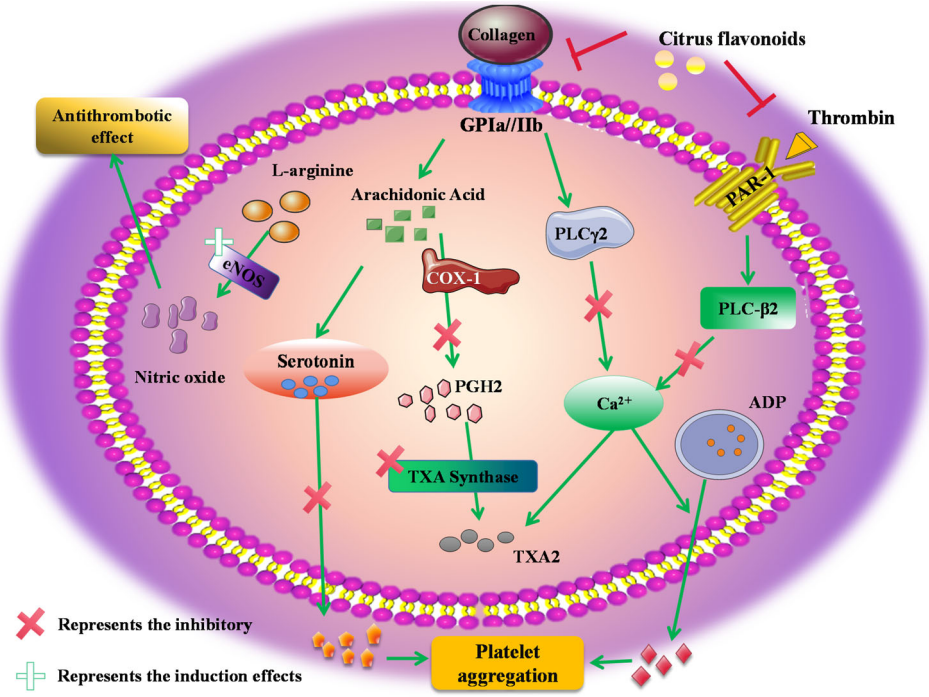


Figure 8. A schematic illustration of the reported targets that mediate anticoagulant effects of citrus flavonoids. Abbreviations: COX-1, cyclooxygenase-1; eNOS, endothelial nitric oxide synthase; TXA2, thromboxane A2; ADP, adenosine-diphosphate diphosphate; PLCγ2, phospholipase C-γ 2; PLC-β2, phospholipase C β 2; PAR-1, protease activated receptor 1; PGH2, prostaglandin H2.

might also be achieved by inhibiting the gene expression of thromboxane A_2 synthase and thromboxane B_2 synthase in vascular endothelial cells (Yamamoto et al. 2013). It can be seen that citrus flavonoids participate in the regulation of intracellular signaling pathways through different targets and play an anti-platelet aggregation role (Figure 8). But more research is still needed to determine the initial targets of these compounds, so as to understand the direct relationship between active substances and cell signaling molecules and pathways.

Regulation of intestinal flora

The intestinal flora is a requisite part of the human body's internal environment and lives in mutual benefit with the human body (Duda-Chodak 2012). The human body provides a unique living environment for the intestinal flora, while the main effects of the intestinal flora on human body are consumption, storage and redistribution of energy, regulation of innate and acquired immunity, and constitute the biological barrier of human intestine. The structure of intestinal flora will change with the age, dietary structure, immune status and surrounding environment of the host. The changes in the structure of intestinal flora are closely related to the occurrence of human diseases including heart failure, atherosclerosis, hypertension and other CVDs (Garcia-Rios et al. 2017). Intestinal microflora disorder may lead to atherosclerosis by promoting cholesterol accumulation and release of inflammatory factors, which can also give rise to chronic low-level inflammatory reactions in the intestine and even the whole body, resulting in excessive fat accumulation and obesity (Caesar, Fak, and Backhed 2010; Melania, Lorenza, and Franco 2010). Attention has been paid to the prevention and treatment of CVDs by targeting intestinal flora. Recent evidence suggests that citrus flavanones can modulate the composition and activity of the growing microbiome by inhibiting pathogenic bacteria and selectively stimulating beneficial bacteria. Studies have confirmed that citrus flavanone consumption might help maintain intestinal homeostasis and improve gastrointestinal health (Stevens et al. 2019; Mas-Capdevila et al. 2020). The effects of citrus flavonoids or citrus fruit foods on the intestinal or fecal microbiota mainly focus on their ability to inhibit pathogen growth, increase beneficial symbiotic bacteria (such as *Bifidobacterium* and *Lactobacillus* secies), and stimulate bacterial production of short-chain fatty acid (SCFA) (Ana et al. 2016).

Other mechanisms

Citrus flavonoids can reduce capillary fragility and permeability, improve microcirculation and blood rheology. It has been proved that rutin, hesperidin, d-catechin, gerberin, epimedium flavonoids, puerarin and other flavonoids can reduce the permeability of capillaries, and have the positive effects of preventing hypertension and atherosclerosis (Clark, Peter, and Taylor 2015). Yamamoto, Suzuki, and Hase (2008) conducted experiments on mice and found that hesperidin could decrease cardiac systolic blood pressure and perform a function in lowering blood pressure. Ohtsuki et al. (2003) had shown through experiments in mice that hesperidin could reduce the diameter of the vasculature,

improve the composition of serum cholesterol, and inhibit the hypertrophy of the vasculature.

Conclusion and future perspectives

Citrus flavonoids have attracted much attention due to their natural low toxicity and extensive pharmacological activity. They had great potential as natural cardiovascular protective agents, which provided theoretical basis for the development on clinical and food application of new drugs and functional foods that use citrus flavonoids as their main ingredients (Mulvihill and Huff 2012; Kou et al. 2013; Taher et al. 2020). Oral citrus flavonoids are metabolized in the GI, then, the metabolites are absorbed into the body and distributed to various organs through the circulatory system to play a role. The flavonoids in citrus can control the occurrence and development of CVDs through various mechanisms and different ways, including inhibiting oxidative stress, reducing inflammation, improving lipid metabolism, regulating apoptosis and autophagy, controlling platelet aggregation, affecting intestinal flora, and so on. However, there are still quite a few problems or controversies about the research on the role and mechanism of citrus flavonoids in the prevention and treatment of CVDs, thus further research is still needed. The following mechanisms need to be further explored.

The bioavailability of citrus flavonoids. The main flavonoids in citrus, such as hesperidin, hesperetin, naringenin, and PMFs, have poor water solubility, which limits their bioavailability. In view of the structure-activity relationship, measures including molecular modification, compounding of multiple active substances and targeted positioning can be considered to improve the bioavailability of citrus flavonoids.

The dose-effect relationship of citrus flavonoids in the body. The effects of citrus flavonoids in prevention and treatment of CVDs come from cell and animal experiments, and its effects in the human body are unclear. In order to avoid possible side effects caused by excessive intake, it is necessary that determine the safe and effective dose of flavonoids.

The new mechanism of citrus flavonoids in preventing and treating *CVDs*. For instance, the molecular mechanisms by which flavonoids exert lipid-lowering effects by regulating miRNAs have attracted the attention of scholars, which provides a new research perspective for illustrating the health effects of citrus flavonoids.

The specificity and selectivity of citrus flavonoids for diseases. Since citrus flavonoids have abundant targets and complex structures, lacking specificity and selectivity for diseases, it limits their development and application, which needs further research and discussion.

Author's contribution

Yudi Deng collected the references and wrote the majority of the manuscript. Yali Tu and Shenghui Lao was responsible for the drawing of the figures. Mengting Wu, Hantong Yin and Linqing Wang revised and improved the manuscript. Professor Wenzhen Liao reviewed and approved the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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