# Platelets and atherogenesis: Platelet anti-aggregation activity and endothelial protection from tomatoes (*Solanum lycopersicum* L.) (Review)

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**Abstract.** In recent years, it has been shown that platelets are not only involved in the arterial thrombotic process, but also that they play an active role in the inflammatory process of atherogenesis from the beginning. The interaction between platelets and endothelial cells occurs in two manners: activated platelets unite with intact endothelial cells, or platelets in resting adhere to activated endothelium. In this context, inhibition of the platelet function (adhesion/aggregation) could contribute to the prevention of atherothrombosis, the leading cause of cardiovascular morbidity. This can be achieved with antiplatelet agents. However, at the public health level, the level of primary prevention, a healthy diet has also been shown to exert beneficial effects. Among those elements of a healthy diet, the consumption of tomatoes (*Solanum lycopersicum* L.) stands out for its effect on platelet anti-aggregation activity and endothelial protection, which may be beneficial for cardiovascular health. This article briefly discusses the involvement of platelets in atherogenesis and the possible mechanisms of action provided by tomatoes for platelet anti-aggregation activity and endothelial protection.

**Contents**



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## 1. Introduction

According to the World Health Organization (WHO), cardiovascular disease (CVD) (i.e., acute myocardial infarction, cerebrovascular disease and peripheral arterial thrombosis) is the cause of approximately 30% of deaths worldwide (1), with a relative increase over time due to the aging of the population (2). The development and progression of CVD lies in the interactive processes of atherosclerotic lesions and thrombus formation, an interaction established primarily by platelet-endothelial binding (3).

The activation of vascular endothelium occurs early in the development of atherosclerosis (4), where the inflammatory component, present in all phases of atherosclerosis, is a vascular response to guard against cardiovascular risk factors (i.e., hypertension, diabetes, smoking and obesity) (5,6). This inflammatory process generates a microenvironment characterized by oxidative stress and cell damage (7). The process triggers a loss of endothelial function through a decrease in the bioavailability of nitric oxide (NO) and the physiological mechanisms of cardiovascular protection that are derived from it (8).

The role of platelets in arterial thrombosis is well known (9). When there is a damaged atheromatous plaque, platelets adhere, secrete their contents, and then attach to it (10). This activation causes a redistribution of anionic phospholipids, creating a negatively charged surface (11) which, in addition to the synthesis and expression of tissue factor (12), favors the consecutive formation of protein complexes in coagulation and fibrin and the consolidation of the thrombus (13).

Epidemiological studies have demonstrated the cardiovascular protective role of a healthy diet (14). In this context, the beneficial effects of fruits and vegetables (F&V) may be related to the bioactive compounds found therein (15), which

explains the increasing amount of attention in research on phytochemicals in the prevention of CVD (16). In addition to their nutritional value, tomatoes (*Solanum lycopersicum* L.), fresh or processed, have been found to provide a cardioprotective effect at both the endothelial and platelet levels (17).

This article discusses current knowledge of platelet-endothelial interaction during the development of atherosclerosis, and platelet anti-aggregation activity and the endothelial protective effects from tomatoes.

## 2. Role of platelets in atherogenesis

In the last decade, it has been shown that platelets are not only involved in the inflammatory complications of the atheromatous lesion (18) but are also involved in the initiation and progression of atherosclerotic plaque (19). Accordingly, platelets act as a bridge between the inflammatory processes characteristic of atherosclerosis and thrombosis (20). The interaction of platelets with endothelial cells (ECs) occurs in three forms: activated platelets join with ECs in the normal state; resting platelets adhere to activated ECs; or an interaction can occur between the two types of activated cells (21).

*Union of activated platelets to ECs in the normal state*. Endothelium in the normal state plays a fundamental role in regulating the hemostatic balance (22) through various mechanisms of antiplatelets, anticoagulants and fibrinolysis, which are regulated by the secretion of NO and prostacyclin (23).

*In vitro* studies have demonstrated platelet adhesion to ECs in the normal state (24). Platelet adhesion occurs because it is activated in circulation. By contrast, in other *in vivo* studies, the binding occurs under shear conditions (25). Once activated, platelets may adhere to ECs and promote local vascular inflammation through inflammatory mediators such as the secretion of chemokines (26), which disrupt normal functioning of the endothelium (27). For example, platelets store and express CD40L (inflammatory modulator) on their surface, releasing the protein into the environment once they are activated (28). CD40L also induces the expression and release of metalloproteinases, which degrade extracellular matrix proteins that are exposed to the circulation when damage occurs at the endothelial level (29). In addition to the release of sCD40, IL-1β, which promotes increased IL-6 and IL-8 and the expression of cell adhesion molecules such as E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the EC (30,31), is also released. This leads to the recruitment of leukocytes to the site from which the injury (i.e., damaged endothelium) originated (32). The leukocyte-platelet interaction causes a wide range of responses in the innate and adaptive immune systems (33), giving the platelet a new function as regulator of the immune system, which contributes to the pathogenesis of an inflammatory response (34,35).

Moreover, animal models have shown a high level of P-selectin expression and endothelial growth factor (VEGF) in atherosclerotic plaques with involvement in the progression of CVD (36). Platelet factor 4 (PF-4), CCL5 (RANTES) and platelet-derived growth factor (PDGF) molecules released from activated platelets cause chemotaxis of monocytes and other leukocytes on the EC, and promote the retention of low density lipoprotein (LDL) and oxidized LDL in the subendothelium (37). In patients with CVD, the presence of oxidized LDL promotes the release of neutrophil-peptide 78 (ENA-78), with an increase in chemoattractant capacity (38). It has been shown that RANTES and PF-4 have heterophile actions, and that the latter must undergo structural modifications in order to amplify its effects on monocytes (39). Moreover, the deposit of RANTES on the endothelium is promoted by platelet P-selectin (40). PDGF also stimulates smooth muscle cell proliferation, causing hyperplasia of the intima layer of the arterial wall, thus acting as an amplifier of the inflammatory response (41).

*Union of resting platelets with activated ECs*. Normally, ECs form neither a non-adherent nor a thrombogenic surface (42). Under physiological conditions this is able to exert important regulatory functions, such as maintaining the balance between procoagulant and anticoagulant factors, vascular tone regulation and control of vascular permeability (43,44). Under normal resting, platelets do not adhere to the endothelium (45). However, several factors can alter this balance, establishing what is known as endothelial dysfunction (46). A dysfunctional endothelium generates a proatherogenic environment characterized by inflammation, proliferation and a prothrombotic state favoring the development of atherosclerosis (47).

Changes in the endothelium can begin at a young age, with slow and progressive developments of atherosclerotic lesions occurring as an individual ages (48). The first evidence of the process is the decrease in NO synthesis (49), causing a decrease in vasodilatory capacity and the establishment of a proinflammatory and prothrombotic state in the endothelium (50). Furthermore, this decrease in NO creates an oxidative environment that eventually oxidizes LDL from the plasma passing the site of endothelial injury, where the presence of lysophosphatidylcholine increases oxidative stress (51), adhesion of monocytes (52) and induces in the EC the expression of adhesion molecules (ICAM-1, VCAM-1, P-selectin, among others) through two signaling pathways: i) lysophosphatidylcholine/nuclear factor κB (NF-κB) and ii) lysophosphatidylcholine/receivers coupled G protein 4/adenosine monophosphate (cAMP)/protein kinase A/cAMP response element binding protein (53,54). Endothelial P-selectin allows platelets to roll on the activated endothelium (55), permitting adhesion, activation and platelet aggregation in disturbed areas of the endothelium. This process represents one of the main events in the initiation and development of atherosclerosis (56).

Furthermore, it has been demonstrated that ADAM-15 expressed in activated ECs interacts with the GPIIb/IIIa complex and induces platelet activation (57). ADAM-15 also operates as a metalloproteinase, degrading endothelial matrix proteins (58). The two functions of this molecule could facilitate the instability and rupture of the fibrous cap of atherosclerotic lesions, causing the acceleration of the formation of a platelet thrombus (59).

## 3. Tomatoes: platelet anti-aggregation activity and endothelial protection

The report 'Diet, Nutrition and the Prevention of Chronic Diseases', published by the WHO in 2003, outlined the scientific evidence that has been associated with a decreased risk

of CVD. According to the report, individuals who consume at least 400 g of F&V daily were found to have a reduced risk of CVD (60). By consuming 5 servings of F&V daily there is a 17% reduction in the risk of CVD (61). Furthermore, the effects of consuming F&V have been replicated worldwide and are independent of ethnicity or geographical location (62).

Presently, in addition to their recognized high value in vitamins, minerals and dietary fiber, consuming F&V is associated with phytochemical content (63,64), with specific actions on target functions (65). These effects, in terms of primary prevention, could modify cardiovascular risk without any of the side effects normally associated with the majority of antiplatelet drugs (66).

The antioxidant properties of F&V are well known (67-69). However, their antithrombotic effects on platelet-endothelial interaction are less known. Preliminary studies have demonstrated the platelet anti-aggregation activity of fruits (red grapes, strawberries, kiwis and pineapples) and vegetables (garlic, onions, green onions, melons and tomatoes) (70,71). Among those beneficial elements mentioned above, the consumption of tomatoes, the fruit of a dicotyledonous plant belonging to the Solanaceae family, is emphasized (72). The possible mechanisms of action of bioactive compounds from tomatoes that have platelet anti-aggregation activity and endothelial protective effects are subsequently discussed (73,74).

*Platelet anti-aggregation activity of bioactive compounds from tomatoes*. It has been observed that the tomato has platelet anti-aggregation activity *in vitro* and *in vivo* by inhibiting platelet aggregation induced by ADP and collagen (75-80). This finding has also been confirmed by our research group (71). The various platelet anti-aggregation activity levels observed in different varieties of tomatoes can be explained by the existence of one or more bioactive compounds or different concentrations of the same compound (77). The platelet anti-aggregation activity of aqueous and methanol extracts of tomatoes ('cluster' type) *in vitro* were similar. Both types of extract showed inhibition of platelet aggregation (30-40%) at 1 mg/ml induced by ADP. When collagen was used as agonist, inhibition was lower, whereas the use of arachidonic acid and peptide receptor activator of thrombin showed no inhibitory effect (71).

The experimental results obtained by Fuentes *et al* indicate that aqueous and methanol extracts resuspended in 0.9% saline exhibit a pH of 4.5; when resuspended in more acidic (pH 2.0) and basic (pH 10.0) suspensions, they maintained their inhibitory activity of maximum platelet aggregation (78). As we know that carotenoids are unstable at pH extremes, this finding may exclude the possibility that these antioxidant compounds exhibit platelet anti-aggregation activity (82-84). In addition, the platelet anti-aggregation activity is inversely related to tomato ripening and the increase in the concentration of lycopene (77).

In the study by Fuentes *et al*, aqueous and methanol extracts under various temperatures (22, 60 and 100˚C) maintained their platelet anti-aggregation activity, indicating that the active compounds with platelet anti-aggregation activity present in the two extracts were not affected by heat treatment (78).

These results allow us to identify the tomato as a functional food, with one or more bioactive compounds with acid-base and thermal stability to exert its cardioprotective activity. This characteristic will benefit future efforts to protect the molecular structure and corresponding platelet anti-aggregation activity of tomato extracts during processing, storage, transport, management and molecular action. This will prove useful in the search for alternative antithrombotic therapy, a field in which most of the drugs used have a high instability in the environment of action (85).

The mechanism of action by which the tomato inhibits platelet aggregation has yet to be elucidated (86). It has been suggested that adenosine and other nucleosides may be responsible for this inhibition, possibly via a mechanism independent of cAMP generation (79) and thromboxanes (cyclooxygenase pathway) (75).

However, there are a wide range of bioactive compounds in tomatoes with platelet anti-aggregation activity, some of which have known bioavailability (flavonoid derivatives), and others whose bioavailability has yet to be identified (87). It is also necessary to determine those anatomical sites of the tomato (skin, pulp, seeds, etc.) in which compounds with platelet anti-aggregation activity are found. Based on the results of our research, the bioactive compounds of tomatoes have thermal and acid-base stability, are devoid of lycopene and have low molecular weight (<1000 Da). Moreover, we hypothesize that bioactive compounds exercise their antiplatelet activity via three platelet receptors: GPVI (collagen agonist) and P2Y1 and P2Y12 (ADP agonist) (71). Further studies of the possible mechanisms of action found in the search strategies of alternative pathways of platelet aggregation inhibition possessed by naturally occurring compounds are required. These studies will allow us to identify their therapeutic range of application (Fig. 1).

*Study of intraplatelet signaling pathways*. Studies of platelet aggregation with bioactive compounds (88) using ADP or collagen as agonist and subsequent platelet lysate for the Western blot test should examine: i) expression of phospholipase Cβ and ii) expression of phospholipase Cγ2. The first is the signaling pathway involved in the possible interaction of bioactive compounds with ADP receptor (P2Y1) (89). It is also necessary to study the proteins that are related to this signaling pathway. These proteins include total Akt and phospho-Akt (serine 473/ threonine 308) (90). The latter, expression of phospholipase Cγ2, is involved in the interaction of bioactive compounds with the collagen receptor (GPVI) (91). Future studies should examine the bioactive compounds of this signaling pathway: total Erk, phospho-Erk 1/2 (threonine 202/ tyrosine 204, threonine 185/ tyrosine 187), total- JNK, phospho-JNK (threonine 182/tyrosine 185) and total p38 MAPK and phospho-p38 MAPK (threonine 182/tyrosine 182) (92). This approach reveals active and inactive forms of these proteins after they have been treated with bioactive compounds, as well as a potential target of action in the corresponding signaling pathway.

*Analysis of cytosolic calcium*. If there is a decrease in cytosolic calcium levels when platelet aggregation is inhibited, it signifies that the bioactive compounds are exerting their action through GPVI or P2Y1 receptors (93).

*Cytosolic cAMP analysis*. An increase in cytosolic calcium level may indicate that the bioactive compounds are inhibiting platelet aggregation through the platelet receptor P2Y12 (94).

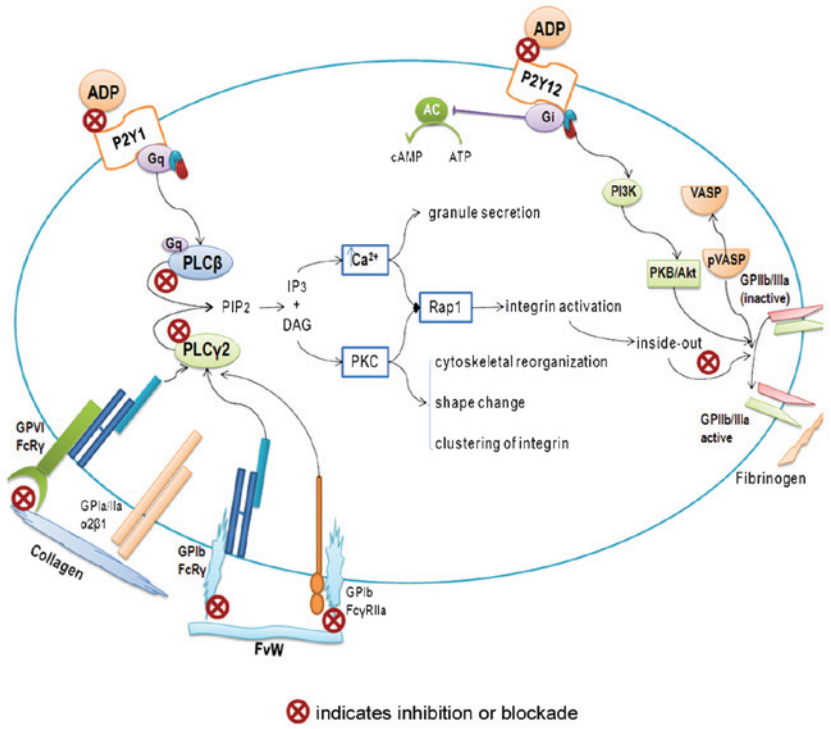


Figure 1. Platelet anti-aggregation activity. Schematic diagram showing possible mechanisms of action of bioactive compounds from the tomato in inhibiting platelet aggregation. ADP, adenosine 5'-diphosphate; AC, adenilate cyclase; ATP, adenosine 5'-triphosphate; cAMP, adenosine 3'5'cyclic monophosphate; DAG, dyacil glycerole; FvW, von Willebrand Factor; GP, glycoprotein; Gq and Gi, G protein-coupled receptors; IP3, inositol 1,4,5-trisphosphate; PLCβ, phospholipase Cβ; PLCy2, phospholipase Cy2; PIP2, phosphatidylinositol 4, 5-bisphosphate; PKC, protein kinase C; PI3K, phosphoinositide 3-kinase; PKB/Akt, protein kinase B; P2Y1 and P2Y12, ADP receptors; Rap1, ras-related protein 1; VASP, vasodilator-stimulated phosphoprotein; pVASP, phosphorylated-vasodilator-stimulated phosphoprotein.

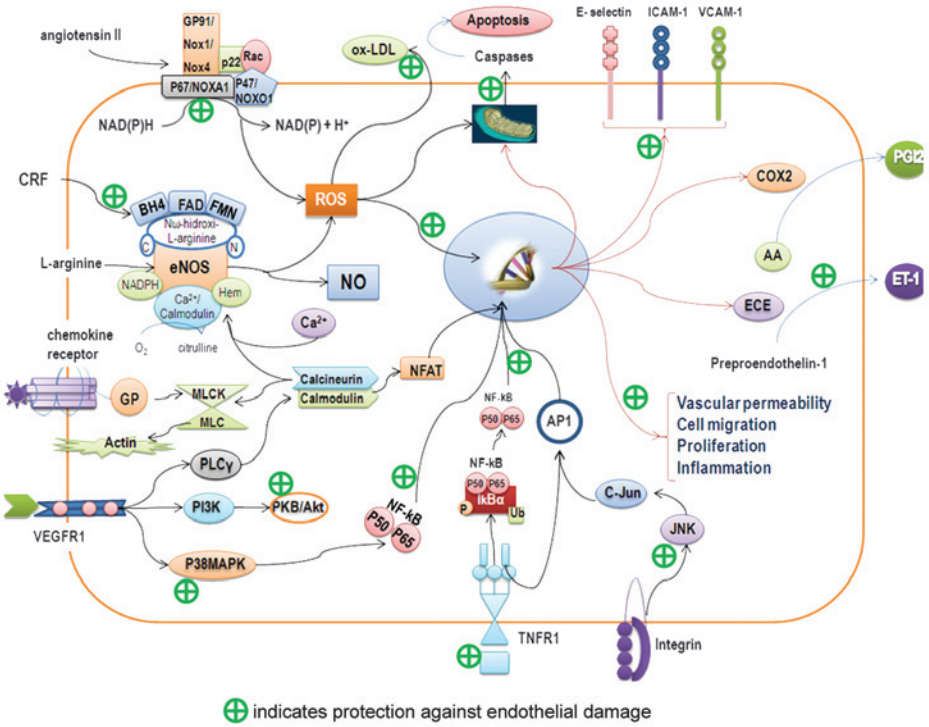


Figure 2. Endothelial protective mechanism. Schematic diagram showing possible mechanisms of action of bioactive compounds from the tomato in protecting endothelium. AA, arachidonic acid; AP-1, activator protein-1; BH4, tetrahydrobiopterin; COX2, cyclooxygenase 2; CRF, cardiovascular risk factor; ECE, endothelin-converting enzyme; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; ICAM-1, intercellular adhesion molecule 1; IkBα, inhibitor of I-κ-B-α; JNK, c-Jun N-terminal kinase; ox-LDL, oxidized low-density lipoprotein; MLCK/MLC, myosin light chain kinase-myosin light chain; NADPH, nicotinamide adenine dinucleotide phosphate; NFAT, nuclear factor of activated T-cells; NFkB, nuclear factor κB; NO, nitric oxide; GP, G protein; PGI2, prostacyclin I2; PI3K, phosphoinositide 3-kinase; PLCy, phospholipase Cy; PKB/Akt, protein kinase B; p38MAPK, p38 mitogen-activated protein kinase; ROS, reactive oxygen species; TNFR1, tumor necrosis factor receptor-1; VCAM-1, vascular cell adhesion molecule-1; VEGFR1, vascular endothelial growth factor receptor-1.

This receptor modulates the inhibition of GPIIb/IIIa through vasodilator-stimulated phosphoprotein (VASP) (95).

*Study of platelet adhesion*. After incubating platelets with bioactive compounds, they were deposited in fibrinogen or VWF matrices (96,97). A reduced matrix adhesion of fibrinogen (fibrinogen binding capacity) indicates that the bioactive compounds block GPIIb/IIIa-fibrinogen interaction via the amino acid sequence RGD (arginine-glycine-aspartic acid), or inhibit a signaling pathway involved in the expression of GPIIb/IIIa. Thus, complete blockage of the GPIIb/IIIa platelet receptor inhibits aggregation from forming a platelet thrombus (98). Bioactive compounds may affect the interaction of platelet (GPIb/IX/V) with endothelium (vWF) (24).

*Protective activity from tomatoes on the endothelial function of bioactive compounds*. It has been observed that aqueous and methanol extracts of tomatoes exhibit antioxidant activity *in vitro* (67,99). Carotenoids (β-carotene, lycopene, zeaxanthin, lutein and canxantina) and vitamins C and E, when they are found in tomatoes, possess endothelium-protective activity. These molecules have three main methods of action (Fig. 2): i) they cause antioxidant activity by protecting LDL and increasing resistance to oxidation (100); ii) in humans, it has been reported that supplementation with lycopene reduces oxidative damage to DNA and other markers of oxidative stress (101); and iii) lycopene has been found to inhibit the expression of adhesion molecules in ECs (102).

Cystine-knot miniproteins from tomato with low concentrations and low toxicity have active anti-angiogenic effects through their inhibition of Erk phosphorylation and do not affect the normal viability and proliferation of ECs (103). They have a similar function to carotenoids, which prevent the phosphorylation of Akt, p38 MAPK and JNK and are sensitive to reactive oxygen species (ROS) (104,105).

Lycopene is considered to be a chemopreventive agent (106), as it maintains the integrity of the vascular barrier, inhibits the expression of cell adhesion molecules and leukocytes, and inhibits EC migration by blocking the expression of NF-κB, CD14 and TLR4, and TNF-α production (107). In addition to decreased levels of malondialdehyde, programmed cell death prevents apoptosis by attenuating the expression of p53 and caspase-3 in ECs treated with $H_2O_2$ (108).

Newly discovered mechanisms include: the reduction of endothelial injury, control of lipid metabolism during the synthesis of cholesterol and oxysterol toxic activity and reduction of the inflammatory response through changes in cytokine production (109,110). While it is known that the consumption of tomato products is associated with a significant increase in plasma levels of lycopene, this has no substantial effect on endothelial function (111). Studies have also found that there are other potent bioactive compounds in tomatoes, such as flavonoids, whose nanomolar concentrations protect the cofactor tetrahydrobiopterin from peroxynitrite radicals and maintain the action of endothelial nitric oxide synthase (eNOS) (112). The inhibition of arginase enzyme and of NADPH oxidase combined with $O_2$, which causes a positive NO balance in the EC (113) and prevents apoptosis through p53, has also been shown to inhibit the synthesis of endothelian-1 (ET-1) (114). Its immediate anti-apoptotic function is to block the JNK and p38 MAPK signaling pathways, and its resistance to LDL oxidation through ROS takes place through action on the JAK2/STAT3 pathways (115).

To further understand the endothelial protective effects of bioactive compounds, such as those mentioned above, or those of other bioactive compounds that may be present in the tomato, future studies should focus on the three most important properties of the endothelium: i) markers of vascular tone control, i.e., concentrations of asymmetric dimethylarginine (ADMA), NO, eNOS and ET-1; ii) markers of the regulation of hemostasis, i.e., concentrations of prostacyclin, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA); and iii) markers of the immune system, i.e., the presence of sICAM-1, sE-selectin, sVCAM-1, IL-6, TNF-α and CRP high-sensitivity (hsCRP).

## 4. Conclusion

The initiation and development of CVD is marked by platelet-endothelial interaction. This interaction promotes the expression of adhesion molecules on the endothelium and the recruitment of inflammatory cells, and stimulates the activation of circulating platelets. In the prevention of CVD, the consumption of F&V is crucial. At the level of primary prevention, tomato consumption promotes cardiovascular health through its role in platelet anti-aggregation activity and its endothelium-protective effects. Platelet anti-aggregation activity is regulated by one or more bioactive compounds that act on ADP and collagen receptors. Further research is required in the identification of mechanisms of action of bioactive compounds. In the endothelium, carotenoids and polyphenols act mainly on eNOS and NAPDH-oxidase in order to control the levels of NO and to ensure a reduction in the inflammatory response.

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