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REVIEW

Impact of dietary polyphenols on human platelet function – A critical review of controlled dietary intervention studies

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Cardiovascular disease is a chronic disease influenced by many factors, with activated blood platelets being one of them. Platelets play a central role in the formation of plaques within blood vessels, contributing to early inflammatory events. Consumption of diets rich in plant-based products protects against the development of cardiovascular disease. Polyphenols, which are secondary plant metabolites found in a wide range of foodstuffs and beverages, may be partially responsible for these effects. Their protective properties include inhibitory effects on platelet function *in vitro* and *in vivo*. However, the bioavailability of many polyphenols is poor and it is unclear whether sufficient quantities can be obtained by dietary means to exert protective effects. Consequently, this review summarizes 25 well-controlled human intervention studies examining the effect of polyphenol-rich diets on platelet function. These studies report a huge variety of research methods, study designs, and study subjects, resulting in controversial assertions. One consistent finding is that cocoa-related products, however, have platelet-inhibiting effects when consumed in moderate amounts. To assess whether other classes of dietary polyphenols, or their metabolites, also beneficially affect platelet function requires more well-controlled intervention studies as well as the adoption of more uniform methods to assess platelet aggregation and activation.

Keywords:
Cardiovascular disease / Critical review / Dietary polyphenols / Human intervention studies / Platelet function

1 Introduction

Cardiovascular disease (CVD) is a widespread human epidemic in industrialized countries [1]. In the US alone it accounts annually for 36% of all deaths, which is more than the combined mortalities from cancer, chronic lower respiratory diseases, accidents, and diabetes mellitus. The estimated cost of CVD to the US economy in 2007 was 432 billion US dollars [2]. Similarly in Europe the annual 4.3 million deaths (equals 48% of all deaths) and associated health care ramifications from CVD cost the EU economy 192 billion Euros a year [3]. Moreover, the observed decline in CVD since the 1980s has slowed due to the increasing incidence of major risk factors like obesity and diabetes mellitus [2, 3].

CVD has a multi-factorial etiology and it has been recognized for a long time that platelet function is related to the risk of developing atherosclerosis [1], the principal cause of heart attack and stroke. Activated blood platelets play a central role as risk factors, comparable to hypertension or

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Abbreviations: ADP, adenosine diphosphate; **CAD,** coronary artery disease; **CVD,** cardiovascular disease; **LTA,** light transmission aggregometry; **PFA-100,** Platelet Function Analyzer-100; **PRP,** platelet-rich plasma; **vWF,** von Willebrand factor

diabetes [4], in this chronic inflammatory condition as they contribute to plaque formation within blood vessels in the early stages of atherogenesis. During this process, platelets become activated upon binding to collagen and von Willebrand factor (vWF) multimers, which are secreted in response to inflammatory stimuli from damaged endothelial cells [5]. Collagen and vWF bind to receptors (glycoprotein VI and integrin $\alpha_2\beta_1$, and the receptor complex glycoprotein Ib/IX/V, respectively) on the platelet surface [6]. The activated platelets then secrete a range of adhesion molecules, such as P-selectin and CD40 ligand, and bind fibrinogen from plasma. Additionally they synthesize and secrete agonists such as adenosine diphosphate (ADP) and thromboxane A_2, which also induce platelet aggregation and thus amplify and maintain the initial platelet response [6]. Activated platelets further intensify atherogenesis by contributing to recruitment and binding of leukocytes [5].

Despite extensive research on the pathways involved in platelet activation, understanding their functional role in the development of atherosclerosis and CVD in humans remains problematic [1, 5, 7]. This is in part due to the lack of a specific clinical test to evaluate the complex network of signaling pathways involved in platelet function and atherosclerosis [8]. Furthermore, mechanistic studies using animal models are often difficult to interpret as non-human platelets not only differ in morphology and numbers from human ones, but also vary in their responses to agonists and in the expression of platelet surface receptors [9–12].

Consumption of diets rich in plant-based products protects against the development of CVD. Such effects have been ascribed in part to non-nutritive but potentially bioactive secondary metabolites in fruits, vegetables, herbs, spices, teas, and wines. For example, simple phenolic compounds (hydroxybenzoic acid and hydroxycinnamic acid derivatives) (Fig. 1A) and polyphenolic flavonoids (Fig. 1B) are ubiquitous in plant-based foods (Table 1). Many have numerous potential anti-atherogenic properties including the modification of lipid profiles, vasodilatory effects and the ability to prevent the oxidation of low-density lipoproteins [13]. Some dietary phenolic compounds also affect platelet aggregation and function *in vitro* and *in vivo* after consumption of supplements [14–17]. However, whether such effects can be achieved from diet alone will depend on the amount of the phenolic compounds in foods and their subsequent bioavailability. Consequently, the aim of this review is to critically evaluate controlled human intervention trials in order to assess whether intake of polyphenol-rich diets or extracts impacts on platelet function. The main food sources, sub-classes of polyphenols, and nutritional relevance of polyphenol-rich diets are discussed.

2 Materials and methods

We searched PubMed, Scopus and Ovid MEDLINE® for human intervention studies where polyphenol-rich diets or supplements were provided and their influence on platelet activation and function was examined. The last search was performed on April 7, 2009. The used search term is included as Supporting Information.

2.1 Exclusion criteria

With the search term cited in Supporting Information a total of 2576 English-written references were obtained (Fig. 2). The search was then restricted to articles that had been published from 1980 onward, resulting in 2362 publications. As only original research articles were used for this review, all reviews, notes, and conference papers were excluded and the number of references was decreased further to 2091. We excluded all studies that were not performed within humans or human platelets and obtained 1153 articles that still matched our criteria. The next step was to exclude all trials examining compounds and supplements that were not derived from a diet or that did not have any dietary background, resulting in 136 remaining publications. Studies that did not discuss measurements related to platelet activation and function were also excluded. Of the remaining 115 references, 70 abstracts were excluded, as they did not describe an intervention trial. Finally, we excluded all studies that did not use valid controls, ending up with 25 controlled human intervention trials (Fig. 2).

2.2 Data evaluation

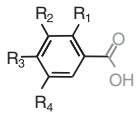
A meta-analytical approach was not possible due to a paucity of randomized controlled trials (rather than non-randomized trials) and a lack of uniformity in pre- and post-treatment outcome measures between studies. Consequently observational assessment was made of the 25 studies which survived the original systematically applied exclusion criteria (Table 2).

3 Results

Controlled intervention studies were categorized based on class of polyphenol (phenolic acids or flavonoids) and their relevant food sources: chocolate and cocoa products, grape seed extract, quercetin-rich diets/supplements, a soy protein supplement, black tea, wine and Armagnac, berries, purple grape juice, and sea buckthorn juice. Table 2 summarizes an overview of all studies including design, intervention compounds and food matrices, doses, parameters of the study populations, duration of intervention, platelet function tests, and outcomes. This summary of 25 intervention studies reveals a wide range between studies in polyphenol intake (18.6 mg to 2.20 g/day), study population (healthy, increased risk factors, and/or clinical disease), study size

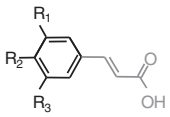
A Phenolic acids

1 Hydroxybenzoic acids



Hydroxybenzoic acid derivatives	R_1	R_2	R_3	R_4
Salicylic acid	OH	H	H	H
***p*-Hydroxybenzoic acid**	**H**	**H**	**OH**	**H**
Protocatechuic acid	H	OH	OH	H
Gentisic acid	OH	H	H	OH
Gallic acid	H	OH	OH	OH
Vanillic acid	H	MeO	OH	H
Syringic acid	H	MeO	OH	MeO

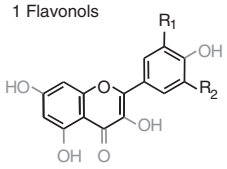
2 Hydroxycinnamic acids



Hydroxycinnamic acid derivatives	R_1	R_2	R_3
p-Coumaricd acid	H	OH	H
Caffeic acid	**OH**	**OH**	**H**
Ferulic acid	MeO	OH	H
Sinapic acid	MeO	OH	MeO

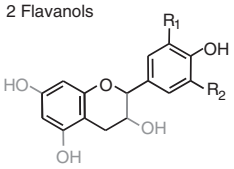
B Flavonoids

1 Flavonols



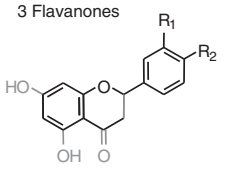
Flavonols	R_1	R_2
Kaempferol	H	H
Quercetin	**OH**	**H**
Myricetin	OH	OH

2 Flavanols



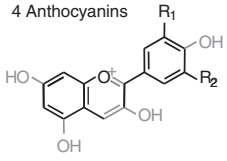
Flavanols	R_1	R_2
(+)-Catechein/ (-)-Epicatechin	OH	H
Gallocatechin/ Epigallocatechin	OH	OH

3 Flavanones



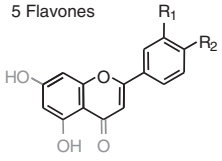
Flavanones	R_1	R_2
Naringenin	H	OH
Hesperetin	**OH**	**MeO**
Eriodictyol	OH	OH

4 Anthocyanins



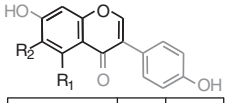
Anthocyanins	R_1	R_2
Pelargonidin	H	H
Peonidin	**MeO**	**H**
Malvidin	MeO	MeO
Cyanidin	OH	H
Petunidin	MeO	OH
Delphinidin	OH	OH

5 Flavones



Flavones	R_1	R_2
Chrysin	H	H
Apigenin	**H**	**OH**
Luteolin	OH	OH

6 Isoflavones



Isoflavones	R_1	R_2
Daidzein	H	H
Genistein	**OH**	**H**
Glycitein	H	MeO

Figure 1. Chemical structures of the two classes of phenolic acids (A), and of the various classes of flavonoids (B), and their respective compounds.

(between 6 and 1535 subjects), and length of supplementation (acute intake for 1 day to chronic intake for over 90 days).

3.1 Cocoa studies and flavanols

Dietary flavanols, mainly found in cocoa and grape seed extract, are the compounds most frequently tested in the studies included in this review. Ten studies [18–27] focused on the *in vivo* effects of food sources containing (–)-epicatechin and (+)-catechin, as well as procyanidin oligomers and polymers. Of these studies, five measured platelet function using the platelet function analyzer (PFA-100) [18, 24–27], allowing a straightforward comparison of the results obtained. This device tests shear stress-induced platelet aggregation by measuring the time taken by aggregating platelets to occlude a collagen-epinephrine or a collagen-ADP coated membrane (closure time). Data from these five studies show evidence of a dose-response relationship up to 900 mg flavanol intake (maximum daily dose tested), although linearity is not evident from the data available. We calculated that acute intake of flavanols led to approximately 3–11% inhibition of collagen-epinephrine-induced closure time *per* 100 mg flavanols consumed, 2–6 h after ingestion, and also to approximately 11% inhibition of collagen-ADP-induced closure time (Table 2). Conversely, chronic intake of flavanols led to inhibition of collagen-ADP-induced

Table 1. Main dietary sources of phenolic acids and flavonoids

Class of polyphenols	Main dietary sources	References
Phenolic acids		
Hydroxybenzoic acid derivatives	Berries (blackberries, blackcurrants, raspberries, redcurrants, strawberries, white currants), black and green tea, condiments/herbs	[52, 56]
Hydroxycinnamic acid derivatives	Blueberries, coffee, kiwi fruits, cherries, plums, aubergines, apples, pears, chicory, cider, spinach, cereal brans, broccoli, kale	[52, 57]
Flavonoids		
Flavonols	Broccoli, curly kale, yellow onions, leek, capers, parsley, lovage, beans, cherry tomatoes, blueberries, blackcurrants, black and green tea, apples, apricots	[29, 52, 58]
Flavanols and proanthocyanidins	Black and green tea, cocoa, dark chocolate, cinnamon, red wine, cider, sorghum, beans, nuts, chokeberries, apricots, plums, peaches, cherries, grapes, cranberries, blueberries, blackberries, blackcurrants, apples	[28, 29, 31, 52, 59, 60]
Flavones	Parsley, celery, sweet pepper	[29, 52, 58]
Isoflavones	Soy (soy flour, soy milk, soy bean, tofu, miso, tempeh), black beans, red clover	[52, 61–63]
Flavanones	Citrus fruits and their juices (orange, grapefruit, lemon, lime, mandarin)	[29, 52, 64]

closure time of 3–11% *per* 100 mg flavanols consumed daily, but did not affect the collagen-epinephrine-induced closure time (Fig. 3). Such effects may be nutritionally relevant as 100 mg flavanols can be obtained from 11 g dark chocolate with a cocoa content 70% w/w [28, 29], from 52 g milk chocolate [28–31], or from 50 to 100 mL cocoa drink containing 8% w/v pure cocoa [27–29]. Five other studies also found a significant inhibition of platelet aggregation and activation upon acute or chronic intake of dietary flavanols from cocoa [19–23] using alternative methodologies, namely the Impact Cone and Plate(let) Analyzer, light transmission aggregometry (LTA) in platelet-rich plasma (PRP), or whole blood platelet aggregation by impedance or platelet counting.

3.2 Flavonols and isoflavones

Four studies examined the effects of quercetin-rich foods and supplements on platelet function in healthy subjects [32–35]. Only one of these [34] observed a significant inhibition (21–65%) of platelet aggregation upon acute intake of 150 mg of quercetin-4′-*O*-β-D-glucoside, which is equivalent to the consumption of 400–700 g of raw yellow onions [29, 30]. Only one study examined the effects of isoflavones on platelet aggregation [36]. In this study consumption of a soy protein isolate beverage powder rich in genistein, daidzein, and glycitein did not affect LTA in PRP of healthy volunteers.

3.3 Polyphenol-rich beverages – studies using black tea, coffee, and alcoholic beverages

We found seven controlled studies testing black tea [37–39], coffee [40], Armagnac [41], or wines [42, 43] for their potential effects on platelet aggregation. Consumption of one liter of black tea rich in gallic acid, flavanols, tannins, theaflavins, and thearubigins *per* day inhibited platelet activation by 4–10% [39]. In this study platelet activation was measured by the very sensitive technique of flow cytometry scanning for circulating leukocyte–platelet aggregates [44]. Two other studies on tea consumption did not find significant effects on platelet function [37, 38]. Acute intake of a strong cup of coffee, mainly containing chlorogenic acid as a phenolic compound, also had significant anti-aggregatory effects [40].

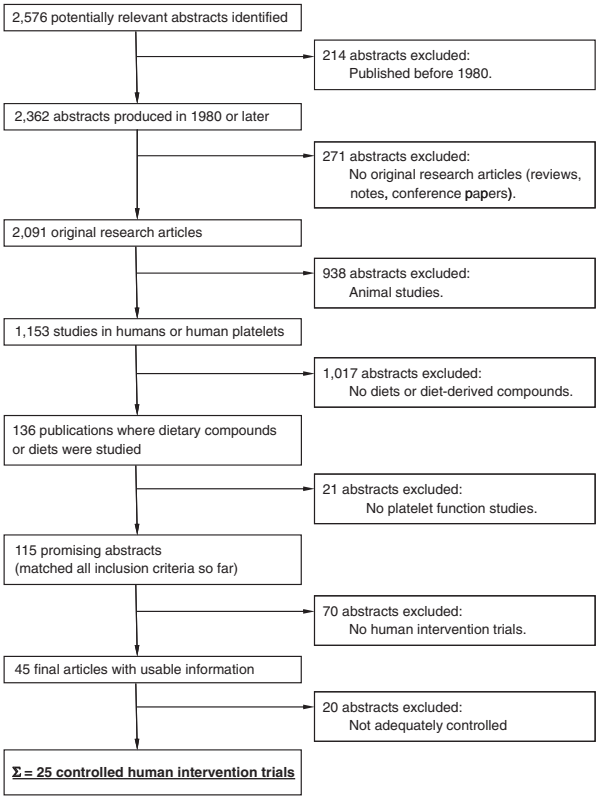


Figure 2. Organization of the literature search.

Table 2. Overview of controlled human intervention studies examining the effect of polyphenol-rich diets or supplementations on platelet aggregation and function, categorized according to the groups of dietary polyphenols

Study design	Compound and Food matrix	Dose *per* day (compound) / Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a)] Control	*P*	Intervention	*P*	Reference
Flavonoids: Flavanols ((−)-epicatechin, (+)-catechin, procyanidin oligomers)										
Controlled, randomized, double blind	Flavanols	446.4 mg	32 mildly hypercholesterolemic postmenopausal women 57.7 ± 2.2 years	8-wk chronic intake	**PFA-100 – closure time**[b)]					[27]
					Collagen-ADP induced	–	NS	13	NS	
	Cocoa beverage	240 mL			Collagen-epinephrine induced	–	NS	–	NS	
					Soluble P-selectin[b)]	3	NS	14	NS	
					Soluble E-selectin[b)]	5	NS	−7	NS	
					Soluble intercellular adhesion molecule 1 (ICAM-1)[b)]	−2	NS	0	NS	
					Soluble vascular cell adhesion molecule 1 (VCAM-1)[b)]	9	NS	−12	0.01	
Controlled, randomized, no baseline values	Flavonoids	~82 mg[c)]	1535 healthy males and females with increased risk for CVD 43 ± 13 years	1-day casual intake	**PFA-100 – closure time**					[18]
					Collagen-epinephrine induced	–	NS	–	0.01	
	Chocolate/cocoa	5.9 g cocoa			**Urinary 11-dehydro thromboxane B_2**	–	NS	–	0.03	
					Plasma fibrinogen	–	NS	–	NS	
Controlled	Flavanols (total epicatechin and procyanidins)	897.0 mg	30 healthy females males and 24–50 years	1-day acute intake	**PFA-100 – closure time**[b)]					[25]
					Collagen-ADP induced					
					After 2 h	1	0.00	16	NS	
					After 6 h	6	0.00	13	NS	
	Cocoa beverage	300 mL			Collagen-epinephrine induced					
					After 2 h	9	NS	8	0.00	
					After 6 h	10	NS	31	0.00	
					Platelet surface P-selectin[b)]					
					Without stimulation	10	NS	−56	0.05	
					Agonist 1:					
					20 μmol/L ADP	−3	NS	−19	0.01	
					100 μmol/L ADP	7	NS	−25	0.03	
					Agonist 2:					
					20 μmol/L epinephrine	37	NS	−11	NS	
					Activated glycoprotein IIb-IIIa[b)]					
					Without stimulation	−10	NS	−67	0.04	
					Agonist 1:					
					20 μmol/L ADP	−3	NS	−34	0.00	
					100 μmol/L ADP	0	NS	−25	NS	
					Agonist 2:					
					20 μmol/L epinephrine	42	NS	−66	0.01	
					Platelet microparticles[b)]	50	0.01	−68	0.00	
Controlled, randomized, double blind, no baseline values	Flavanols:		23 healthy male smokers 23 ± 2 years	1-day acute intake	**PFA-100 – closure time**					[24]
	Monomers	70.0 mg			Collagen-ADP induced					
	Dimmers and trimers	60.0 mg			After 1 h	4	NS	14	0.01	
	Total flavanols	130.0 mg			After 2 h	6	NS	13	0.03	
					After 6 h	0	NS	16	0.00	
	Flavanol-rich grape seed extract	400 mg			Collagen-epinephrine induced					
					After 1 h	9	NS	8	NS	
					After 2 h	9	NS	15	0.03	
					After 6 h	6	NS	10	NS	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
Controlled, randomized, double blind, no baseline values	Flavanols:		17 healthy, postmenopausal women 56±1.5 years	8-wk chronic intake	**PFA-100 – closure time**					[26]
	Monomers	70.0 mg			Collagen-ADP induced					
	Dimmers and trimers	60.0 mg			1 h after intake on 1st day	3	NS	8	NS	
	Total flavanols	130.0 mg			2 h after intake on 1st day	1	NS	24	0.01	
		Data taken from (23)			6 h after intake on 1st day	6	NS	14	0.05	
					on 56th day of trial	–	NS	–	0.05	
	Grape seed extract	400 mg			Collagen-epinephrine induced	–	NS	–	NS	
Controlled, randomized, double blind	Catechin	10.8 mg	22 male and female heart transplant recipients 52±15 years	1-day acute intake	**Shear stress-dependent platelet function by Impact Cone and Plate(let) Analyzer**[b] (% of surface covered by platelets)	−7	NS	−22	0.04	[19]
	Epicatechin	36.0 mg								
	Total polyphenols	624.0 mg								
	Dark chocolate	40 g			**Shear stress-dependent platelet function by Impact Cone and Plate(let) Analyzer**[b] (size of platelet aggregates in arbitrary units)	1	NS	−3	NS	
Controlled, randomized	Catechin	10.8 mg	20 healthy male smokers Age range not given	1-day acute intake	**Shear stress-dependent platelet function**[b] (% of surface covered by platelets)	–	NS	−36	0.03	[21]
	Epicatechin	36.0 mg								
	Total polyphenols	624.0 mg								
	Dark chocolate	40 g								
Controlled, randomized, single blind few data	Flavonoids	~192–279 mg[c]	30 healthy males and females 20–58 years	1-day acute intake	**LTA in PRP**[b]					[22]
	Milk chocolate (20% cocoa)	100 g			Agonist 1:					
					2.0 μmol/L ADP	–	NS	–	NS	
					1.0 μmol/L ADP	–	NS	–	NS	
					0.5 μmol/L ADP	–	NS	–	NS	
					Agonist 2:					
					2.0 mg/L collagen→aggregation	–	NS	–	NS	
					→slope	–	NS	–	NS	
					1.0 mg/L collagen→aggregation	–	NS	–	NS	
					→slope	–	NS	–	NS	
					0.5 mg/L collagen→aggregation	−7	NS	−82	NS	
					→slope	−5	NS	−48	NS	
	Flavonoids	~379–1045 mg			**LTA in PRP**[b]					
	Dark chocolate (75% cocoa)	100 g			Agonist 1:					
					2.0 μmol/L ADP	–	NS	–	NS	
					1.0 μmol/L ADP	–	NS	–	NS	
					0.5 μmol/L ADP	–	NS	–	NS	
					Agonist 2:					
					2.0 mg/L collagen→aggregation	–	NS	–	NS	
					→slope	–	NS	–	NS	
					1.0 mg/L collagen→aggregation	–	NS	−12	0.03	
					→slope	–	NS	−24	0.04	
					0.5 mg/L collagen→aggregation	−7	NS	−93	0.03	
					→slope	−5	NS	−48	0.01	
Controlled, randomized, double blind	Flavanols and procyanidins	234 mg	32 healthy males and females 44±7 years	4-wk chronic intake	**Platelet aggregation in whole blood**[b]					[23]
	Cocoa tablets	6 tablets			Agonist 1:					
					1.0 mmol/L arachidonic acid	–	NS	–	NS	
					Agonist 2:					
					8.0 μmol/L ADP	0	NS	−29	0.05	
					Agonist 3:					
					2.0 mg/L collagen	−8	NS	−17	0.05	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) / Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
		(≡50 g dark chocolate)			**ATP release of platelets**[b]					
					Agonist 2:					
					8.0 μmol/L ADP	−14	NS	−4	NS	
					Agonist 3:					
					2.0 mg/L collagen	−20	NS	−11	0.01	
					Platelet surface P-selectin[b]					
					Agonist 2:					
					3.0 μmol/L ADP	−3	NS	−8	0.01	
					10.0 μmol/LADP	−3	NS	−3	NS	
					Mean platelet count[b]	5	NS	−3	NS	
					Mean platelet volume[b]	1	NS	−1	NS	
Controlled, randomized, double blind	Flavanols: (−)-epicatechin, (+)-catechin, procyanidin oligomers up to decamers	380.0 mg	12 healthy males and females 23–50 years	1-day acute intake	**Platelet aggregation in whole blood**[d]					[20]
					Agonist:					
					0.125 mg/L collagen	−14 to −23	NS	−12 to −20	NS	
					0.250 mg/L collagen	2 to −15	NS	−15 to −17	NS	
					0.500 mg/L collagen	−2 to −5	NS	−1 to −3	NS	
					Platelet–monocyte conjugates[d]					
	Cocoa beverage	300 mL			Agonist:					
					0.125 mg/L collagen	−4 to −8	NS	0 to −14	NS	
					0.250 mg/L collagen	34 to 20	NS	36 to 14	0.02	
					0.500 mg/L collagen	15 to 4	NS	24 to 16	NS	
					Platelet–neutrophil conjugates[d]					
					Agonist:					
					0.125 mg/L collagen	−6 to −20	NS	−7 to −12	NS	
					0.250 mg/L collagen	17 to −13	NS	−6 to −12	NS	
					0.500 mg/L collagen	25 to 2	NS	8 to −5	NS	
					Platelet activation[d]					
					P-selectin on monocytes[d]					
					Agonist:					
					0.125 mg/L collagen	0 to −17	NS	−13 to −15	NS	
					0.250 mg/L collagen	10 to −6	NS	−6 to −8	NS	
					0.500 mg/L collagen	−10 to −19	0.02	−9 to −10	NS	
					P-selectin on neutrophils[d]					
					Agonist:					
					0.125 mg/L collagen	−8 to −22	NS	−9 to −16	0.04	
					0.250 mg/L collagen	11 to −15	NS	−11 to −19	0.03	
					0.500 mg/L collagen	−8 to −21	0.01	−5 to −15	0.04	
	Flavanols: (−)-epicatechin, (+)-catechin, procyanidin oligomers up to decamers	680.0 mg			**Platelet aggregation in whole blood**[d]					
					Agonist:					
					0.125 mg/L collagen	−14 to −23	NS	−25 to −30	0.03	
					0.250 mg/L collagen	2 to −15	NS	−12 to −14	NS	
					0.500 mg/L collagen	−2 to −5	NS	−3 to −7	NS	
					Platelet–monocyte conjugates[d]					
	Cocoa beverage	300 mL			Agonist:					
					0.125 mg/L collagen	−4 to −8	NS	−22 to −39	0.04	
					0.250 mg/L collagen	34 to 20	NS	0 to −11	NS	
					0.500 mg/L collagen	15 to 4	NS	36 to 17	0.05	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) / Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a)] Control	*P*	Intervention	*P*	Reference
					Platelet–neutrophil conjugates[d)]					
					Agonist:					
					0.125 mg/L collagen	−6 to −20	NS	−19 to −28	0.03	
					0.250 mg/L collagen	17 to −13	NS	−11 to −24	0.03	
					0.500 mg/L collagen	25 to 2	NS	2 to −6	NS	
					Platelet activation[d)]					
					P-selectin on monocytes[d)]					
					Agonist:					
					0.125 mg/L collagen	0 to −17	NS	−33 to −37	0.01	
					0.250 mg/L collagen	10 to −6	NS	−20 to −28	0.02	
					0.500 mg/L collagen	−10 to −19	0.02	−7 to −15	0.01	
					P-selectin on neutrophils[d)]					
					Agonist:					
					0.125 mg/L collagen	−8 to −22	NS	−31 to −36	0.01	
					0.250 mg/L collagen	11 to −15	NS	−22 to −31	0.02	
					0.500 mg/L collagen	−8 to −21	0.01	−15 to −20	0.02	
	Flavanols: (−)-epicatechin, (+)-catechin, procyanidin oligomers up to decamers	980.0 mg			**Platelet aggregation in whole blood**[d)]					
					Agonist:					
					0.125 mg/L collagen	−14 to −23	NS	−11 to −20	0.01	
					0.250 mg/L collagen	2 to −15	NS	1 to −12	NS	
					0.500 mg/L collagen	−2 to −5	NS	0 to −1	NS	
					Platelet–monocyte conjugates[d)]					
	Cocoa beverage	300 mL			Agonist:					
					0.125 mg/L collagen	−4 to −8	NS	−16 to −41	0.00	
					0.250 mg/L collagen	34 to 20	NS	34 to 14	0.05	
					0.500 mg/L collagen	15 to 4	NS	26 to 6	0.02	
					Platelet–neutrophil conjugates[d)]					
					Agonist:					
					0.125 mg/L collagen	−6 to −20	NS	−19 to −21	0.01	
					0.250 mg/L collagen	17 to −13	NS	4 to −4	NS	
					0.500 mg/L collagen	25 to 2	NS	15 to −3	NS	
					Platelet activation[d)]					
					P-selectin on monocytes[d)]					
					Agonist:					
					0.125 mg/L collagen	0 to −17	NS	−23 to −31	0.03	
					0.250 mg/L collagen	10 to −6	NS	−1 to −7	NS	
					0.500 mg/L collagen	−10 to −19	0.02	−3 to −15	NS	
					P-selectin on neutrophils[d)]					
					Agonist:					
					0.125 mg/L collagen	−8 to −22	NS	−16 to −23	0.01	
					0.250 mg/L collagen	11 to −15	NS	−7 to −9	NS	
					0.500 mg/L collagen	−8 to −21	0.01	−1 to −9	NS	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) / Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
Flavonoids: quercetin (= flavonol)										
Controlled, randomized, double blind	Quercetin anhydride Other mixed bioflavonoids Rutin Total flavonoids Supplementing capsules	1000.0 mg 1000.0 mg 200.0 mg 2200.0 mg 4	27 healthy males and females 42 ± 3 years	4-wk chronic intake	**LTA in PRP**[b] Agonist: 10 mg/L collagen **Thromboxane B2 production**[b]	−9 112	NS NS	−11 79	NS NS	[32]
Controlled, randomized, no baseline values	Quercetin Bouillon powder, boiling water + cooked yellow onions Apigenin Bouillon powder, boiling water + dried parsley	114.0 mg 6.6 g 393.4 mL 220 g 84.0 mg 6.6 g 393.4 mL 4.9 g	18 healthy males and females 25 ± 8 years	6-wk chronic intake	**Maximum whole blood aggregation** Agonist: 2.0 mg/L collagen **LTA in PRP** Agonist 1: 2.0 mg/L collagen Agonist 2: 1.5 μmol/L ADP 3.0 μmol/L ADP **Plasma concentration of fibrinogen** **Thromboxane B_2 production in PRP** **Platelet number**	– – – – – –	NS NS NS NS NS NS	– – – – – –	NS NS NS NS NS NS	[35]
Controlled, randomized, double blind, no baseline values	Quercetin Quercetin-rich onion soup	69.0 mg 600 mL	6 healthy males and females (pilot study) 34 ± 7 years	1-day acute intake	**LTA in washed platelets after 180 min** Agonist: 0.5 mg/L collagen 1.0 mg/L collagen 2.0 mg/L collagen 3.0 mg/L collagen **Syk tyrosine phosphorylation** After 60 min After 180 min **Phospholipase C gamma 2 (PLCγ2) tyrosine phosphorylation** After 60 min After 180 min	 – – – – – – – –	 NS NS NS NS NS NS NS NS	 −38 −10 −3 −2 −28 −60 −43 −23	 NS NS NS NS 0.00 NS NS NS	[33]
Controlled, randomized, no baseline values	Quercetin Quercetin-4′-*O*-β-D-glucoside supplement in 5% v/v ethanol	150.0 mg Data not given	6 healthy males and females (pilot study) Age not given	1-day acute intake	**LTA in washed platelets** Agonist: 0.5 mg/L collagen After 30 min After 120 min 1.0 mg/L collagen After 30 min After 120 min 2.0 mg/L collagen After 30 min After 120 min 3.0 mg/L collagen, after 120 min 4.0 mg/L collagen, after 120 min 5.0 mg/L collagen, after 120 min	 – – – – – – – – –	 NS NS NS NS NS NS NS NS NS	 −26 −65 −2 −36 −14 −21 −13 −15 −13	 0.00 0.00 NS 0.00 NS 0.00 NS NS NS	[34]

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
					Syk tyrosine phosphorylation					
					After 30 min	–	NS	−27	0.05	
					After 120 min	–	NS	−45	0.05	
					PLCγ2 tyrosine phosphorylation					
					After 30 min	–	NS	4	NS	
					After 120 min	–	NS	−42	0.05	
					Whole platelet protein tyrosine phosphorylation					
					After 30 min	–	NS	−12	0.00	
					After 120 min	–	NS	−60	0.00	
	Quercetin	300.0 mg			**LTA in washed platelets**					
					Agonist:					
	Quercetin-4′-*O*-β-D-glucoside supplement in 5% v/v ethanol	Data not given			0.5 mg/L collagen					
					After 30 min	–	NS	−44	0.00	
					After 120 min	–	NS	−53	0.00	
					1.0 mg/L collagen, after 120 min	–	NS	−27	NS	
					2.0 mg/L collagen, after 120 min	–	NS	−24	NS	
					3.0 mg/L collagen, after 120 min	–	NS	−11	NS	
					4.0 mg/L collagen, after 120 min	–	NS	−9	NS	
					5.0 mg/L collagen, after 120 min	–	NS	−14	NS	
					Syk tyrosine phosphorylation					
					After 30 min	–	NS	−24	0.05	
					After 120 min	–	NS	−40	0.05	
					PLCγ2 tyrosine phosphorylation					
					After 30 min	–	NS	3	NS	
					After 120 min	–	NS	−37	0.05	
					Whole platelet protein tyrosine phosphorylation					
					After 30 min	–	NS	−9	0.00	
					After 120 min	–	NS	−62	0.00	
Flavonoids: isoflavones					**LTA in PRP**					[36]
Controlled, randomized, no baseline values	Genistein Daidzein Glycitein Total isoflavones	80.3 mg 35.6 mg 15.1 mg 131.0 mg	20 healthy males 25 ± 1 years	4-wk chronic intake	Agonist 1:					
					1.5 mg/L collagen	–	NS	–	NS	
					3.0 mg/L collagen	–	NS	–	NS	
					10.0 mg/L collagen	–	NS	–	NS	
	Soy protein isolate beverage powder	60 g			Agonist 2:					
					0.6 μmol/L 9,11-dideoxy-11α-9α-epoxy-methanoprosta-glandin $F_{2\alpha}$(U46619)	–	NS	–	NS	
					1.0 μmol/L U46619	–	NS	–	NS	
Polyphenols: gallic acid, flavonols, and ellagitannins					**Platelet activation**[b]					[39]
Controlled, randomized, double blind	Flavonols Gallic acid Total polyphenols Black tea (tea extract)	67.2 mg 11.3 mg 78.5 mg 1000 mL (4 × 250 mL)	75 healthy males 18–55 years	6-wk chronic intake	Monocyte–platelet aggregates	9	NS	1	0.03	
					Neutrophil–platelet aggregates	4	NS	−4	0.02	
					Leukocyte–platelet aggregates	3	NS	−4	0.03	
					Soluble P-selectin[b]	−3	NS	2	NS	
					Platelet count[b]	6	NS	8	NS	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
Controlled, randomized	Polyphenols	1.465–2.257 g	22 healthy males and females	12-wk chronic intake	**LTA in PRP**[b] Agonist 1:					[38]
	Black tea	1250 mL	59 ± 2 years		2.0 μmol/L ADP	36	NS	45	NS	
					4.0 μmol/L ADP	5	NS	5	NS	
					8.0 μmol/L ADP	−1	NS	5	NS	
					Agonist 2:					
					0.2 mg/L collagen	5	NS	2	NS	
					0.6 mg/L collagen	1	NS	2	NS	
					2.0 mg/L collagen	0	NS	−1	NS	
					Fibrinogen[b]	3	NS	4	NS	
					Soluble P-selectin[b]	10	NS	−7	0.01	
					Soluble E-selectin[b]	4	NS	−1	NS	
					Soluble ICAM-1[b]	−1	NS	3	NS	
					Soluble VCAM-1[b]	3	NS	5	NS	
Controlled, randomized	Gallic acid	16.2 mg	49 male and female patients with CAD, taking 325 mg/d aspirin	1-day acute intake	**LTA in PRP** Agonist 1:					[37]
	Epigallocatechin	9.0 mg								
	Epigallocatechin gallate	17.6 mg			1.0 μmol/L ADP	–	NS	–	NS	
					2.5 μmol/L ADP	–	NS	–	NS	
					5.0 μmol/L ADP	–	NS	–	NS	
	Epicatechin	6.3 mg	55 ± 9 years		10.0 μmol/LADP	–	NS	–	NS	
	Epicatechin gallate	27.0 mg			Agonist 2:					
	Total catechins	59.9 mg			5.0 μmol/L Thombin receptor-activating peptide (TRAP)	–	NS	–	NS	
	Total theoflavins	27.0 mg			10.0 μmol/L TRAP	–	NS	–	NS	
	Total polyphenols	733.5 mg			20.0 μmol/L TRAP	–	NS	–	NS	
	Total flavonoids	477.0 mg			50.0 μmol/L TRAP	–	NS	–	NS	
	Black tea (fresh tea leaves)	450 mL								
	Gallic acid	62.1 mg		4-wk chronic intake	**LTA in PRP** Agonist 1:					
	Epigallocatechin	20.7 mg								
	Epigallocatechin gallate	43.2 mg			1.0 μmol/L ADP	–	NS	–	NS	
	Epicatechin	19.8 mg			2.5 μmol/L ADP	–	NS	–	NS	
					5.0 μmol/L ADP	–	NS	–	NS	
					10.0 μmol/LADP	–	NS	–	NS	
	Epicatechin gallate	32.4 mg			Agonist 2:					
	Total theoflavins	116.1 mg			5.0 μmol/L TRAP	–	NS	–	NS	
	Total catechins	22.5 mg			10.0 μmol/L TRAP	–	NS	–	NS	
	Total flavonoids	1350.0 mg			20.0 μmol/L TRAP	–	NS	–	NS	
	Total polyphenols	873.0 mg			50.0 μmol/L TRAP	–	NS	–	NS	
	Black tea (freeze-dried tea leaves)	900 mL								
Controlled[2]	Polyphenols	70.8–352.6 mg	10 healthy males and females	1-day acute intake	**LTA** in **PRP** Agonist 1:					[40]
	Caffeine	180 mg			2.0 μmol/L ADP after 60 min	29	NS	−15	NS	
					Agonist 2:					
	Coffee	200 ml (6% w/v)	24–35 years		3.0 mg/L collagen					
					After 30 min	1	NS	−2	0.05	
					After 60 min	0	NS	−1	NS	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) / Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
					Agonist 3:					
					0.5 μmol/L arachidonic acid					
					After 30 min	−15	NS	−20	NS	
					After 60 min	0	NS	−33	0.05	
					Platelet Thromboxane B_2 formation					
					Agonist:					
					3.0 mg/L collagen					
					After 30 min	–	NS	−29	NS	
					After 60 min	–	NS	−34	0.05	
Controlled, randomized, single (investigator) -blind	Polyphenols (mostly ellagitannins) Armagnac (40% alcohol)	18.6 mg 30 mL 12 g alcohol	20 healthy subjects 20–54 years Gender not given	2-wk chronic intake (then 2 wk follow up)	**LTA in PRP**[b]					[41]
					Agonist 1:					
					2.0 mmol/L ADP					
					After 1 wk	−11	NS	−29	0.05	
					After 2 wk	−11	NS	−31	0.05	
					After 3 wk (follow up)	−0	NS	−10	0.05	
					After 4 wk (follow up)	15	NS	−3	0.05	
					Agonist 2:					
					2.0 mg/L collagen					
					After 1 wk	−9	NS	−14	NS	
					After 2 wk	−11	NS	−21	NS	
					After 3 wk (follow up)	−5	NS	−11	NS	
					After 4 wk (follow up)	9	NS	−5	NS	
					Agonist 3:					
					0.3 U/mL thrombin					
					After 1 wk	−13	NS	−23	0.05	
					After 2 wk	−10	NS	−23	0.05	
					After 3 wk (follow up)	−5	NS	−8	0.05	
					After 4 wk (follow up)	4	NS	−6	0.05	
					Bleeding time[b]	18	NS	1	NS	
Controlled	Polyphenols Tannins (procyanidins) White wine	~13.8–21.1 mg[c] ~7.55 mg[c] 500 mL	16 healthy males 35 ± 5 years	90-day chronic intake	**LTA in PRP**[b]					[43]
					Agonist 1:					
					2.0 μmol/L ADP	22	0.05	−6	NS	
					Agonist 2:					
					500 mg/L arachidonic acid	6	NS	−10	NS	
					Agonist 3:					
					750 mg/L adrenalin	29	0.05	−3	NS	
					Cyclooxygenase activity[b]	8	NS	−7	NS	
					Thromboxane A_2 synthesis[b]	4	NS	−7	NS	
	Polyphenols Tannins (procyanidins) Red wine	~185–536 mg[c] ~105–279 mg[c] 500 mL			**LTA in PRP**[b]					
					Agonist 1:					
					2.0 μmol/L ADP	22	0.05	−16	0.05	
					Agonist 2:					
					500 mg/L arachidonic acid	6	NS	6	NS	
					Agonist 3:					
					750 mg/L adrenalin	29	0.05	−16	NS	
					Cyclooxygenase activity[b]	8	NS	−8	NS	
					Thromboxane A2 synthesis[b]	4	NS	−33	NS	

Table 2. Continued

Study design	Compound and Food matrix	Dose *per* day (compound) / Amount *per* day (food matrix)	Study population	Duration of study	Tests	% Change compared with baseline[a] Control	*P*	Intervention	*P*	Reference
Controlled	Polyphenols	2.0 mmol gallic acid equivalents	40 healthy males and females	1-day acute intake	**Platelet surface P-selectin after 6 h**[b]					[42]
					Without stimulation	36	NS	−19	NS	
	Dealcoholized red wine	300 mL	21–49 years		Agonist 1:					
					20 μmol/L ADP	−5	NS	4	NS	
					100 μmol/LADP	−2	NS	10	NS	
					Agonist 2:					
					20 μmol/L epinephrine	−6	NS	15	NS	
					Activated glycoprotein IIb-IIIa after 6 h[b]					
					Without stimulation	57	NS	50	NS	
					Agonist 1:					
					20 μmol/L ADP	−4	NS	−7	NS	
					100 μmol/LADP	−3	NS	9	0.03	
					Agonist 2:					
					20 μmol/L epinephrine	−3	NS	14	NS	
Polyphenols: phenolic acids and flavonoids										
Controlled, randomized, single (investigator) -blind	Polyphenols	837.0 mg	71 healthy males and females with increased risk for CVD 58 ± 6 years	8-wk chronic intake	**Platelet function (PFA-100) – closure time**[b]					[46]
					Collagen-ADP induced	−1	NS	11	0.02	
	Different berries (bilberries, lingonberry nectar, or blackcurrant and strawberry purée, chokeberry and raspberry juice)	~160 g (100 and 50 g or 100 g and 70 mL)			Collagen-epinephrine induced	3	NS	−2	NS	
					Fibrinogen[b]	−4	NS	4	NS	
					Prothrombin fragment 1 + 2[b]	20	NS	18	NS	
					Glycoprotein V[b]	−7	NS	−1	NS	
					Soluble ICAM-1[b]	−1	NS	0	NS	
					Soluble P-selectin[b]	4	NS	9	NS	
					CD40 ligand[b]	−18	NS	15	NS	
Controlled, randomized, double blind, no baseline values	Polyphenols	~3.84–4.04 mg/kg body weight[c]	20 male and female patients with stable CAD on standard medication 63 ± 9 years	2-wk chronic intake	**LTA in washed platelets**					[47]
	Purple grape juice	7 mL/kg body weight			Agonist:					
					5 μmol/L ADP	–	NS	–	NS	
					Plasma Thromboxane B_2	–	NS	–	NS	
					Soluble P-selectin	–	NS	–	NS	
					Soluble CD40 ligand	–	NS	–	0.03	
					Platelet derived nitric oxide (NO) release	–	NS	–	NS	
					Platelet superoxide release	–	NS	–	0.02	
Controlled, double blind, no baseline values	Flavonoids	354.6 mg	20 healthy males	8-wk chronic intake	**Platelet aggregation in whole blood**					[48]
	Sea buckthorn juice	300 mL	36 ± 2 years		Agonist 1:					
					10 μmol/L ADP	−1	NS	−16	NS	
					Agonist 2:					
					1.5 mg/L collagen	−18	NS	−35	NS	
					5.0 mg/L collagen	−5	NS	−13	NS	
					Agonist 3:					
					0.75 mol/L arachidonic acid	−21	NS	−13	NS	
					Soluble ICAM-1	5	NS	−1	NS	

ICAM-1, intercellular adhesion molecule 1; NS, not significant; PLCγ2, phospholipase C gamma 2; TRAP, thrombin receptor-activating peptide; U46619, 9,11-dideoxy-11α-9α-epoxy-methanoprosta glandin F_2 (thromboxane analogue); VCAM-1, vascular cell adhesion molecule 1; −"number", significant decrease *versus* baseline; –, data not given.

a) Results show percentage changes compared with baseline within treatment groups.

b) Percentage change was calculated based on baseline and post-treatment values provided in the manuscript.

c) Data were estimations based on the literature and databases [28–31, 52, 56, 65–70].

d) Percentage change was calculated based on baseline and post-treatment values obtained by personal communication with the authors.

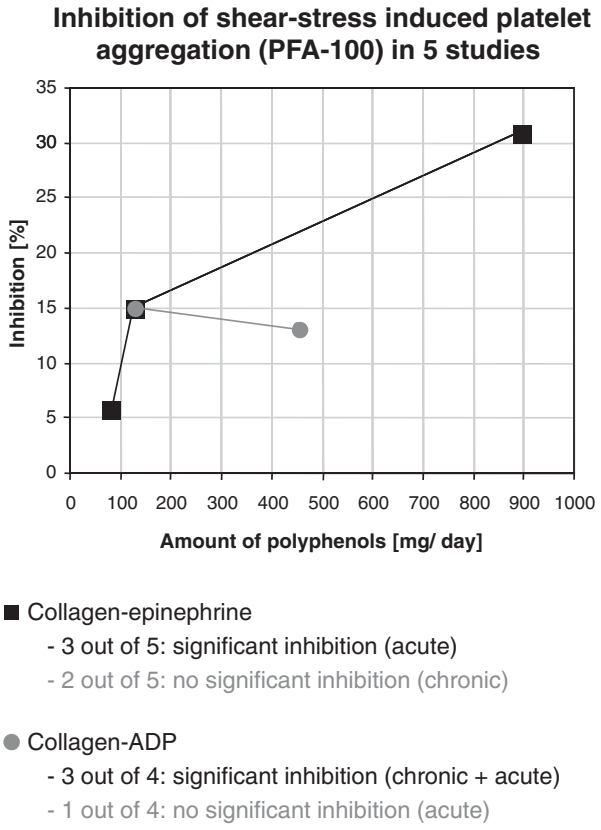


Figure 3. Overview of the inhibition of shear stress-induced platelet aggregation in healthy humans measured by the PFA-100 upon daily consumption of flavanols. The PFA-100 tests shear stress-induced platelet aggregation by measuring the time the aggregating platelets need to occlude a collagen-epinephrine-coated (black squares) or a collagen-ADP-coated (gray circles) membrane. The data were obtained from $n = 5$ studies where three were acute intake studies [18, 24, 25] and two were chronic intake studies [26, 27].

Furthermore, a daily intake of 30 mL of Armagnac, which is especially rich in ellagitannins, for 2 wk, inhibited platelet aggregation by 20–30% [41]. However, a study published by the same group 2 years later questions whether these effects are caused by the polyphenols present in Armagnac, as only polyphenol-free fractions of freeze-dried Armagnac extracts generated inhibition of platelet aggregation *ex vivo* [45]. Consumption of wine rich in proanthocyanidins, anthocyanins, and tannins has produced inconsistent effects on platelet function, showing inhibition of ADP-induced platelet aggregation [43], no effect on platelet aggregation [42], or even an increase in ADP-induced platelet aggregation by almost 10% [42].

3.4 Studies testing other phenolic-rich foods

Three studies assessed the effects of polyphenols from berries [46], purple grape juice [47], and sea buckthorn juice [48] on platelet function. Only the daily consumption of an average amount of 160 g fresh berries and berry juices for 8 wk caused a significant inhibition of collagen-ADP-induced platelet aggregation in subjects with higher risk for CVD [46].

4 Discussion

This review of controlled human intervention studies assessing the impact of dietary polyphenols on human platelet function revealed a plethora of research methods and study designs. Findings were markedly inconsistent. Chronic intake of polyphenols from berries, or juices derived from berries, may result in only a relatively low inhibition of collagen-ADP-induced platelet aggregation under shear stress. Due to the inconsistency in data generated in well-controlled studies, it is currently not possible to conclude whether polyphenols from black tea, coffee and alcoholic beverages have beneficial effects on platelet function when consumed in nutritionally relevant quantities. However, cocoa-related products consistently showed inhibition of platelet activation and aggregation when consumed in moderate amounts, either on an acute basis, or on a chronic basis.

4.1 Heterogeneity of experimental approaches

4.1.1 Assessment of platelet function

The complexity of platelet interactions with their environment results in a wide variety of experimental approaches for measuring platelet function, as illustrated by the studies included in this review (Table 2). Various tests and agonists have been used to assess the effect of a dietary intervention on platelet activation and function (Table 3). All established methods for platelet function monitoring have been reviewed and their advantages and disadvantages discussed [8, 49, 50]. Many older methods are unreliable and not platelet-specific (*e.g.* bleeding time) or very labor-intensive and therefore expensive, like LTA. In addition, LTA is, albeit considered a gold standard method for testing platelet function, relatively non-physiological as platelets are taken out of their natural environment (*i.e.* blood) and are not undergoing high-shear conditions. Modern equipment that measures platelet function is expensive and therefore not widely used, although the use of the PFA-100 for human intervention trials appears to be increasing (Table 2). The PFA-100 method uses whole blood, and platelets will become activated under high shear force, thereby adhering to a membrane and occluding an aperture. Such processes mimic the conditions in small arteries within the human body. Although this test appears to simulate *in vivo* conditions more accurately, the outcome is dependent on vWF and hematocrit values.

Table 3. Methods, platelet agonists and antibodies used for platelet function assessment in controlled human intervention studies, including the involved pathways/receptors

Test/measurement	Pathways/receptors involved	Description	References
Primary, platelet-specific measurements			
LTA in PRP			
→Agonists:		Standard method to examine agonist induced platelet aggregation by measuring decreasing turbidity of PRP and therefore increasing light transmission.	[71–73]
0.5 μmol/L–2.0 mmol/L ADP	$P2Y_1$, $P2Y_{12}$[a)]		
0.2–10.0 mg/L collagen	$\alpha_2\beta_1$, GPVI[b)]		
500 mg/L arachidonic acid	$COX \rightarrow TxA_2$[c)]		
5.0–50.0 μmol/L TRAP	PAR1 → thrombin[d)]		
0.3 U/L thrombin	PAR1 → thrombin[d)]		
750 mg/L adrenalin	α_{2A}-adrenergic receptor		
0.6–1.0 μmol/L 9,11-dideoxy-11α-9α-epoxy-methanoprostaglandin $F_2\alpha$ (U46619)	Thromboxane receptor		
LTA in washed platelets			
→Agonists:		Similar to aggregation in PRP but platelets are isolated, washed and resuspended in a modified Tyrodes–Hepes buffer	[72, 74, 75]
5.0 μmol/L ADP	$P2Y_1$, $P2Y_{12}$[a)]		
0.5–5 mg/L collagen	$\alpha_2\beta_1$, GPVI[b)]		
Platelet aggregation in whole blood – by impedance			
→ Agonists:		Measurement of increasing electrical impedance between electrodes upon agonist-induced platelet activation and accumulation on electrodes.	[72, 73]
8.0–10.0 μmol/L ADP	$P2Y_1$, $P2Y_{12}$[a)]		
1.5–5.0 mg/L collagen	$\alpha_2\beta_1$, GPVI[b)]		
1 mmol/L–0.75 mol/L arachidonic acid	$COX \rightarrow TxA_2$[c)]		
– by platelet counting			
→Agonist:		*Via* immunoplatelet counting *via* flow cytometry	[76]
0.125–0.5 mg/L collagen	$\alpha_2\beta_1$, GPVI[b)]		
PFA-100	GPIb-vWF[e)]	Simulates the *in vivo* hemostatic conditions during plug formation after injury to a small vessel wall. Measurement of the time needed for an agonist-coated membrane to occlude under high shear stress.	[72, 77, 78]
→Agonists:			
Collagen-ADP	$\alpha_2\beta_1$, GPVI[b)]-$P2Y_1$, $P2Y_{12}$[a)]		
Collagen-epinephrine	$\alpha_2\beta_1$, GPVI[b)] – α_{2A}-adrenergic receptor		
Shear stress-dependent platelet function (Impact Cone and Plate(let) Analyser)	Platelet adhesion/aggregation under shear stress – vWF	Arterial shear flow is mimicked by a cone rotating within a polystyrene well containing whole blood. Afterward the blood gets washed, stained, and platelet aggregates are evaluated by an image analyzer.	[5, 79]
Flow cytometry			
→Platelet surface P-selectin	GPIbα[f)]	Flow cytometric measurement of: Exposure of α-granule membrane proteins, antibody: anti-CD62P	[80,81]
– Agonists:			
3.0–100 μmol/L ADP			
20.0 μmol/L epinephrine			

Table 3. Continued

Test/measurement	Pathways/receptors involved	Description	References
none			
→Activated glycoprotein IIb/IIIa (PAC I) – Agonists: 20.0–100 μmol/L ADP 20.0 μmol/L epinephrine none	Binds multiple ligands leading to platelet aggregation: fibrinogen, fibronectin, vitronectin, thrombospondin-1 and vWF.	Activation-induced exposure of the fibrinogen binding site of the receptor glycoprotein IIb/IIIa, antibody: PAC1 or anti-fibrinogen.	[6, 82]
→Leukocyte–platelet aggregates	Adhesion initially mediated by P-selectin	Antibodies: anti-CD45 (leukocytes), anti-CD42a (platelets)	[81]
→Platelet-derived microparticles (PMP)	Agonist induced release[g].	Antibody: anti-CD42a	[83]
Secondary, not platelet-specific (additional) measurements			
Commercial immunoassays (mostly ELISA[a]):			
→ Soluble P-selectin	GPIbα[f]	Measured in platelet-poor plasma, quantified by ELISA	[8, 38, 40]
→ Soluble E-selectin	Expressed solely by endothelial cells.		
→ Soluble intercellular adhesion molecule 1 (ICAM-1)	Expressed by vascular endothelium, macrophages and lymphocytes		
→ Soluble vascular cell adhesion molecule 1 (VCAM-1)	Expressed by vascular endothelium		
→ Soluble CD40 ligand	Interacts with CD40 on endothelial cells[h].		[72]
→Thromboxane A_2 (TxA_2)→Thromboxane B_2 (= stable, biologically inactive metabolite of TxA_2) – Agonist: collagen	Thromboxane receptor	Maximal platelet aggregation in PRP is induced by an agonist. Thromboxane B_2 is isolated from the PRP and quantified by immunoassay.	[84]
→Glycoprotein V (platelet-specific)	Expressed on platelet surface[i]		[8, 85]
→Prothrombin fragments 1 and 2	Measurement for thrombin[d]		[72]
Fibrinogen:	Glycoprotein IIb/IIIa (integrin αIIbβ3)	Measured in platelet-poor plasma	[82]
→immunoassay		As a standard, coagulation of a known	[38]
→in coagulation analyser – Agonist: thrombin		fibrinogen concentration in different dilutions of plasma is induced by thrombin. The clotting times are measured and compared with the one of the sample. Thus fibrinogen content can be calculated.	[35]
COX activity	COX →TxA_2[c]	Measured by audioradiography (with radioactive labelled arachidonic acid)	[43, 72]
Platelet count (platelet-specific)		*Via* impedance analysis by a hematology analyser or *via* immunoplatelet counting *via* flow cytometry	[76]
Mean platelet volume (**MPV**, platelet specific)		By a hematology analyzer	[19]
Bleeding time	Time until bleeding from a small fresh wound stops[j]	Ivy' s method, very heterogeneous results	[86]
Nitric oxide (NO)	Antithrombotic, vasodilatory effects[k]	With the help of a nitric oxide-selective microelectrode measured during platelet aggregation in washed platelets	[87, 88]
Superoxide (ROS)	Released by activated platelets to recruit more platelets and leukocytes	Measured by lucigenin amplified chemiluminescence.	[89, 90]

Table 3. Continued

Test/measurement	Pathways/receptors involved	Description	References
Adenosine triphosphate (ATP) release	Released by activated platelets – receptor: $P2X_1$	The dense granule ATP secreted by activated platelets reacts with the firefly luciferin-luciferase. This results in luminescence, which is measured to quantify the ATP release.	[91]
Tyrosine phosphorylation: →Whole platelet protein →SYK (Spleen Tyrosine Kinase) → PLCγ2	Collagen-induced signalling pathway downstream of glycoprotein Ib/IX/V involves tyrosine kinases (Src family kinases, phosphatidylinositol-3 kinase) and thus protein tyrosine phosphorylation.	Quantified by Western blotting.	[33, 34, 85]

ATP, adenosine triphosphate; GPVI, glycoprotein VI; ICAM-1, intercellular adhesion molecule 1; PAR1, protease activated receptor 1; PLCγ2, phospholipase C gamma 2; PMP, platelet-derived microparticles; TRAP, thrombin receptor-activating peptide; U46619, 9,11-dideoxy-11α-9α-epoxy-methanoprostaglandin F_2 (thromboxane analogue); VCAM-1, vascular cell adhesion molecule 1.

a) $P2Y_1$ receptor, $P2Y_{12}$ receptor.

b) Integrin $\alpha_2\beta_1$, glycoprotein VI.

c) Cyclooxygenase (COX)→thromboxane A_2 generation.

d) Protease-activated receptor 1 after thrombin hydrolysis.

e) Glycoprotein Ib – vWF interaction causes initial adhesion to membrane within PFA-100 cartridge.

f) Glycoprotein Ibα (GPIbα), also called P-selectin, is rapidly expressed by activated platelets and activated endothelial cells.

g) Agonists (*e.g.* collagen, thrombin) induce changes in the levels of intracellular second messengers (*e.g.* Ca^{2+}), which leads to the release of platelet-derived microparticles.

h) Rapidly expressed by activated platelets, interacts with CD40 on endothelial cells and thus causes an inflammatory response.

i) Expressed on platelet surface, subunit of the glycoprotein Ib/IX/V complex (receptor for vWF, collagen, P-selectin, thrombin, *etc.*).

j) Ivy's method measures the bleeding time under a standard pressure (blood pressure cuff).

k) Inhibition of platelet adhesion to endothelium, platelet activation, and platelet aggregation by upregulation of cyclic guanosine monophosphate production.

Methods based on flow cytometry, like assessment of platelet activation markers, leukocyte–platelet aggregates or platelet-derived microparticles are also performed in whole blood. These methods appear very sensitive, especially the measurement of circulating monocyte–platelet aggregates as marker for *in vivo* platelet activation [44]. However, this method is performed under low shear conditions in solution, which is again not an accurate simulation of *in vivo* processes [8, 49, 50]. The use of different methods often results in outcomes, which are not comparable. For example, Lordkipanidze *et al.* measured platelet function in response to daily aspirin use in 201 patients with stable coronary artery disease (CAD) to examine aspirin resistance using six different methods: LTA in PRP induced by 1.6 mmol/L arachidonic acid or different concentrations (5, 10, and 20 μmol/L) of ADP, whole blood aggregometry induced by 1.6 mmol/L arachidonic acid, the PFA-100 with a collagen-epinephrine coated membrane, the VerifyNow Aspirin point-of-care system, and an enzyme immunoassay kit to measure urinary 11-dehydro-thromboxane B_2 (the stable metabolite of thromboxane A_2) concentrations. The authors reported variable responses between methods, and the correlation between the measurements was low [51]. In addition, different agonists activate different pathways of platelet function (Table 3), which introduces another layer of complexity when comparing outcomes of controlled human intervention trials. Ideally, a panel of agonists targeting different pathways of aggregation should be used, in order to obtain an all-round picture of nutritional or supplemental effects on platelet function. This may allow easy comparison between studies. However, this approach is rarely followed. As a result, the difficulty in comparing study outcomes obtained using different methods and agonists significantly decreases the impact of the overall body of research already carried out.

4.1.2 Chronic *versus* acute intake

Most of the studies discussed in this review (13 out of 25) examined the effects of chronic dietary intake of polyphenols on platelet function with interventions ranging between 2 and 13 wk. Nine studies examined acute effects of polyphenols on platelet function, *i.e.* within 30 min to 6 h after ingestion, whereas one study [37] examined the effects of both chronic and acute polyphenol intake. A large proportion of the chronic intake studies did not find a significant effect on platelet function [27, 32, 35–38, 47, 48]. This may reflect the use of blood samples obtained after an overnight fast. Most polyphenols (which can be considered xenobiotics) would have been rapidly metabolized and excreted soon after ingestion [52]. Intervention compounds and their metabolites may only circulate in the blood for a limited amount of time. Indeed, a review of 97 bioavailability studies of polyphenols revealed that catechins, gallic acid, and flavanones have very short half-lives within the human body [53]. Therefore, perhaps only acute effects of polyphenols on platelet function may be measurable, with platelet function returning to baseline levels once the polyphenols or their metabolites have been removed from the circulation. Nevertheless, since some chronic-intake studies taking blood samples after an overnight fast did find an improvement of platelet function [23, 39, 41, 43, 46], the platelet-inhibiting effects of some of these compounds or metabolites may have persisted. Such persistence could arise in several ways. For example, the compounds responsible for inhibition of platelet aggregation and activation could have an irreversible effect, so that daily consumption results in a cumulative effect as increasing numbers of the whole platelet pool are affected, until a plateau is reached (approximately one-tenth of the platelet pool turns over each day). Alternatively, daily acute suppression of platelet activation and aggregation could improve the inflammatory status and reduce baseline platelet activation status as a secondary benefit. The studies reviewed here suggest that both acute and chronic effects may result from intake of dietary polyphenols. However, as the mechanisms by which these effects are achieved are likely to be different, it is not possible to directly compare outcomes of acute and chronic studies, and this limits the conclusions that can be drawn. Design of intervention studies to measure both acute (day 1) and chronic effects is necessary to enable the true effects of these dietary components to be assessed.

4.1.3 Health status of the study population

The underlying health status of the volunteers often influences the outcome of the study, with more significant anti-platelet effects observed in volunteers having single or multiple risk factors for or suffering from disease. Seventeen of the studies discussed in this review were performed in healthy subjects [20, 22, 23, 25, 26, 32–36, 38–43, 48], whereas six studies were performed in healthy subjects with risk factors for CVD [18, 19, 21, 24, 27, 46], and two studies were performed in CAD patients taking routine medication [37, 47]. Significant improvements in platelet function were observed after supplementation in healthy subjects as well as in high-risk subjects but not in CAD patients and therefore the health status of the volunteers did not appear to determine the outcome. The effects of health status may be linked to baseline platelet function, which cannot be fully assessed for the studies in this review. Study populations with very different baseline platelet function might be expected to respond very differently to supplementation with any compounds that inhibit platelet aggregation and activation. This may be a source of variability in the studies included in this review, which could contribute to the sometimes conflicting nature of the outcomes.

4.2 Active compounds or metabolites?

In the face of a large body of *in vitro* anti-platelet efficacy data for many polyphenolic compounds [16], it is somewhat surprising that only the flavanols clearly mirror their *in vitro* efficacy *in vivo*. While it appears clear that many sources of variability in the studies reviewed prevent us from drawing clear conclusions about the efficacy of dietary polyphenols, it is also possible that the lack of coherence between studies is not the only reason for the *in vitro versus in vivo* discrepancy. For example, the concentrations of phenolic compounds that inhibit platelet function *in vitro* on a detectable level are often not physiological [16]. Furthermore, bioavailability and metabolism may explain the divergent effects of *in vitro* and *in vivo* results. It is often not known whether the polyphenols or their metabolites influence platelet function. The bioavailability of most polyphenols is very low. Plasma concentrations of total metabolites after an oral intake of 50 mg aglycone equivalents range between 0 μmol/L for poorly absorbed polyphenols, such as proanthocyanidins and anthocyanins, and 4 μmol/L for well-absorbed polyphenols, *e.g.* quercetin glucosides [53]. Furthermore, it is questionable whether individual dietary polyphenols and their metabolites or the total amount of polyphenols in the diet cause changes in platelet function [15]. More information about the bioavailability of polyphenols and their metabolites obtained from different food matrices is required in order to better assess the effects of individual compounds and compound mixtures on pathways involved in platelet function *in vitro* [54].

In conclusion, it is currently difficult to deduce from a relatively small number of well designed and controlled human intervention studies if and how polyphenols improve platelet function. This is mainly due to heterogeneity in assessment methods. However, a relatively large body of evidence suggests that moderate consumption of especially dark chocolate significantly inhibits platelet activation and aggregation. The physiological relevance of the beneficial effects of flavanols acquired from cocoa consumption on platelets is even comparable to standard doses of aspirin: consumption of approximately 100 g dark chocolate with 70% cocoa solids could result in similar effects to 81 mg of aspirin in an acute setting [55]. The anti-platelet effect of flavanols can be obtained in both healthy subjects as well as people at risk for CVD, and appears to be largest upon acute consumption. To assess whether other classes of dietary polyphenols, or their metabolites, also beneficially affect platelet function, more well-controlled intervention studies as well as the adoption of more uniform methods to assess platelet aggregation and activation are needed.

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