

Review Article

Platelet Responses in Cardiovascular Disease: Sex-Related Differences in Nutritional and Pharmacological Interventions

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Cardiovascular diseases (CVD) represent one of the biggest causes of death globally, and their prevalence, aetiology, and outcome are related to genetic, metabolic, and environmental factors, among which sex- and age-dependent differences may play a key role. Among CVD risk factors, platelet hyperactivity deserves particular mention, as it is involved in the pathophysiology of main cardiovascular events (including stroke, myocardial infarction, and peripheral vascular injury) and is closely related to sex/age differences. Several determinants (*e.g.*, hormonal status and traditional cardiovascular risk factors), together with platelet-related factors (*e.g.*, plasma membrane composition, receptor signaling, and platelet-derived microparticles) can elucidate sex-related disparity in platelet functionality and CVD onset and outcome, especially in relation to efficacy of current primary and secondary interventional strategies. Here, we examined the state of the art concerning sex differences in platelet biology and their relationship with specific cardiovascular events and responses to common antiplatelet therapies. Moreover, as healthy nutrition is widely recognized to play a key role in CVD, we also focused our attention on specific dietary components (especially polyunsaturated fatty acids and flavonoids) and patterns (such as Mediterranean diet), which also emerged to impact platelet functions in a sex-dependent manner. These results highlight that full understanding of gender-related differences will be useful for designing personalized strategies, in order to prevent and/or treat platelet-mediated vascular damage.

1. Introduction

Noncommunicable diseases (mostly, cardiovascular disease (CVD), cancer, diabetes, and chronic respiratory disease) are the leading cause of mortality worldwide: with 41 million deaths, they were responsible for 71% of all global deaths in 2016 [1]. Among them, CVD remains the biggest cause of death globally in the last 20 years: according to the last World Health Organization Report, ischemic heart disease and stroke collectively accounted for 15.2 million deaths in 2016 [1].

Genetic, metabolic, and environmental aspects interact together, leading to metabolic and/or physiological changes (overweight and obesity, rise in blood pressure, and increase in blood glucose and cholesterol levels), which represent key CVD risk factors. Some of the risk behaviours (tobacco use, physical inactivity, unhealthy diet, and alcohol abuse) can be deeply modified, in order to lower CVD prevalence.

Remarkably, CVD prevalence, aetiology, and outcome are also strictly related to differences in sex (based on biological characteristics, such as gene expression, hormones, anatomy, and immune system) and gender (based on social and structural determinants). Usually, life expectancy is greater for women than men, but CVD, including myocardial infarction, stroke, cardiomyopathy, and hypertensive heart disease, displays sex- and age-related incidence: indeed, it accounts for 40% of all deaths in men and up to 49% of all deaths in women over the age of 65 years [2]. This finding may be due to increased prevalence of CVD risk factors in women with respect to their male counterparts [3, 4]. In this context, it should also be recalled that CVD prevalence, outcome, prevention, and treatment in women are often underestimated, due to underrepresentation of women in CVD clinical trials [5, 6].

Among biological risk factors, platelets are emerging as new players, since increased platelet aggregation is a major

determinant for heart attacks, stroke, and thrombosis: indeed, activated platelets are major components of thrombi occluding arteries and play a role in plaque formation within blood vessels during atherogenesis [7]. As a consequence, either antiplatelet therapy or other interventional strategies (such as those to promote consumption of foods rich in antioxidant and phytochemical compounds, fiber, and mono- and polyunsaturated fatty acids (PUFA)) are becoming increasingly relevant for preventing and treating vascular events in high-risk patients [8–16]. Accidentally, also in this context, a sex and gender disparity can be identified, in terms of aggregation response capacity and susceptibility to platelet aggregation inhibitors [17–19].

Based on this background, in this review, we will examine the state of the art concerning the main differences in platelet function between men and women, in order to establish their relationship with specific cardiovascular events and with responses to primary and secondary interventional strategies.

2. CVD Risk, Platelet Function, and Gender

Platelet count and mean platelet volume, usually linked to markers of platelet activation, are significantly associated with increased risk (as well as with outcome and mortality) of stroke, myocardial infarction, and coronary artery disease [20–22]. As a consequence, platelet number and size are commonly used tools for diagnosing and monitoring thromboembolic disorders [23, 24]. Activated platelets are also main players in atherogenesis, as they secrete proinflammatory chemokines that promote expression of adhesion molecules in atherosclerotic endothelial cells [25]. In atherosclerosis animal models, platelet/endothelium interaction precedes the onset of atherosclerotic lesions and inhibition of platelet adhesion decreases endothelium dysfunction and leucocyte recruitment in the atherosclerotic plaque [25]. Accordingly, currently used antiplatelet therapy has been proven to be effective in reducing thrombotic events (associated with a marked risk reduction of atherothrombotic events in high-risk settings, including patients with acute coronary syndromes), not only by inhibiting platelet activation but also by downregulating endothelial dysfunction and inflammation [26].

In 1999, Miller and collaborators highlighted that a gender-specific release of vasoactive factors from platelets could be found. In particular, they found that secretion of cyclooxygenase 1 (COX-1) metabolites of the ω -6 polyunsaturated fatty acid (PUFA) arachidonic acid (20:4 ω -6, AA) (*i.e.*, thromboxane A₂ (TxA₂) and prostacyclin (PGI₂)), as well as secretion of serotonin from platelets, was higher in male with respect to female pigs, but platelets from ovariectomized females had the highest concentration of all vasoactive compounds, with respect to male counterparts. This pioneering study highlighted, therefore, that sex differences in platelet activity might explain differences in response to injury in the coronary circulation, usually observed in males and females [27]. Since then, several studies have been undertaken to unravel sex-related dissimilarities and to understand how these differences can affect platelet biology and CVD onset.

It is well established that traditional CVD risk factors (including obesity, dyslipidaemia, inflammation, diabetes, hypertension, and smoking) are greater in women than in men and that CVD risks are more age-dependent in females than in males [28, 29]. Nonetheless, prevalence of CVD is greater in men than in women [30, 31]; this sex disparity persists until women reach menopause, when CVD incidence rapidly rises, until it overtakes that of men [32–35]. Accordingly, a study enrolling 59 men (58.6 ± 9.9 years) and 75 postmenopausal women (61.4 ± 10.6 years) with angina and nonobstructive coronary artery disease (ANOCA) found significant differences between the two groups in relation to lipid profile and thrombogenicity [36]. Indeed, females display higher levels of total cholesterol, total LDL-C, HDL-C and their subtypes and IDLs, as well as elevated thrombin-induced platelet-fibrin clot strength, clotting index, and fibrinogen activity. As no differences were seen on inflammatory markers (including OxLDL, OxLDL/ β 2GPI, and urinary 11-dehydrothromboxane B₂), adverse cardiovascular events more frequently observed in females may be ascribed to the basal prothrombotic phenotype occurring in women with ANOCA [36–38].

The cardioprotective effects exerted by female hormonal levels produced during menstrual cycle (and lost after menopause) may explain the observed differences between sexes. Although the present review does not focus on molecular and cellular mechanisms of sex hormones, some details about their action (especially concerning platelets) need to be highlighted. Human platelets from both sexes express receptors for 17 β -oestradiol (ER α and ER β) and ER-regulatory proteins, as well as androgen and progesterone receptors [39–42]. However, available literature data regarding interactions among sex, steroid hormones, and platelet functions are controversial. Indeed, either positive or negative and even no effects on platelet aggregation, in response to different agonists, have been reported in relation to sex [27, 43–47]. Additionally, a proaggregatory effect of 17 β -oestradiol has been reported in both healthy male and female platelets [48]; on the contrary, Coleman et al.'s group [44] has recently reported that female platelets have both increased aggregation and activation potential, and 17 β -oestradiol pretreatment feminizes male platelets leading to similar platelet behaviour in response to platelet-activating factor (PAF). Accordingly, as emerged by a large, population based, case-control study on risk factors for venous thrombosis (MEGA study), women taking oral contraceptives have a significant thromboembolic risk, with a positive association with estrogen dose used [49]. As recently reviewed, results concerning the impact of menopausal hormonal therapy on platelet activation in women are also largely contradictory, much likely due to heterogeneity in experimental settings among studies [44, 50]. This rather complex puzzle is further complicated by the evidence that testosterone can enhance TxA₂ receptor density and platelet aggregability [51, 52].

Beside hormonal changes, other several factors related to platelet biology can account for the prothrombotic state; some of them, highlighted by several experimental and clinical evidence, will be described in the next paragraphs.

2.1. Platelet Fatty Acid Composition of Plasma Membrane.

The main feature distinguishing male and female platelets is the fatty acid composition of plasma membrane, especially concerning ω -6 (AA) and ω -3 (eicosapentaenoic (20:5 ω -3, EPA) and docosahexaenoic (22:6 ω -3, DHA) acids) PUFAs. A study enrolling healthy 40 men and 34 women (age 20-50 years) of Caucasian origin showed that (albeit same daily intake of these lipids, as well as ω -6/ ω -3 PUFA ratio) women show greater incorporation of DHA and EPA into phosphatidylcholine and phosphatidylethanolamine, compared to men, who, on the contrary, show higher levels of AA and other ω -6 PUFAs [53]. Conversely, a controlled, randomized, double-blind study reported no significant sex differences in EPA and DHA incorporation into platelet membrane after 12-month supplementation with oily fish [54], although the too wide age range of subjects enrolled (*i.e.*, 20-90) might represent an important confounding factor not to be overlooked.

Higher ω -3 PUFA proportion in female phospholipids might be related to sex-dependent differences in conversion of the essential α -linolenic acid (18:3 ω -3, ALA) to EPA and DHA. Humans, indeed, can endogenously synthesize EPA and DHA from ALA through a series of desaturations and elongations, but young women show a better capacity to produce long-chain PUFAs which is dependent on estrogen effects [55]. In particular, in healthy young females, about 21% of dietary ALA is converted to EPA, and 9% is converted to DHA [56], whereas only 8% of dietary ALA is converted to EPA and 0-4% is converted to DHA in healthy young men [57]. As a consequence, women have greater circulating plasma DHA concentrations, thus increasing DHA supply for incorporation into platelet membranes [55, 58, 59]. Replacement of AA with EPA and/or DHA in female circulating platelets alters the phospholipid bilayer, thus modifying the activity of membrane-associated molecules (*i.e.*, phosphatidylserine, GPIIb/IIIa exposure, and P-selectin) [60, 61]. It also reduces generation of proaggregatory and proinflammatory eicosanoids, such as TxA₂ (through competitive inhibition of COX-1) and 12-hydro(pero)xyeicosatetraenoic (12-H(P)ETE) acids (by competition for 12-lipoxygenase (12-LOX)) [62-64]. Consequently, the differential incorporation in membrane phospholipids leads to a different degree of platelet aggregation and vessel occlusion, thus contributing to the protective effects of ω -3 PUFAs on cardiovascular risk [64].

2.2. Platelets Receptors and Platelet-Derived Microparticles.

Platelet receptors and downstream signaling cascades are affected by sex (and age), depending on the receptor engaged. A study carried out on healthy donors (53 men and 56 women; age range: 19-82 years in men and 21-70 years in women), indeed, showed sex- and aging-dependent decrease of platelet glycoprotein (GP) Ib/von Willebrand factor (vWF) interaction, with age-related changes more profound in women than in men [65]. Conversely, Sestito and coworkers [47] reported no changes in relation to sex (and age) in 62 healthy subjects (11 men and 17 women < 55 years and 22 men and 12 women > 55 years) in platelet response to collagen and ADP, although platelets from older men had

higher tendency towards aggregability with respect to younger ones. Based on these findings, it should be considered that different experimental settings and sample stratification may explain different platelet behaviours.

Although no gender difference in total number of GPIIb-IIIa (fibrinogen receptor) was expressed on platelet surface, nonetheless, women show higher receptor reactivity: in response to ADP and thrombin receptor activating peptide (TRAP), indeed, the amount of fibrinogen/GPIIb-IIIa complexes was significantly greater in healthy women (especially in fertile subjects) than in men [66]. Sex-specific difference in GPIIb/IIIa activity also seems to emerge from the CRUSADE study, where female patients with non-ST-segment elevation acute coronary syndromes (NSTE ACS) and treated with GPIIb/IIIa antagonists (eptifibatide, tirofiban) showed more haemorrhagic events than males [67]. Although female gender is recognized as a risk factor for bleeding, especially following medical or surgery [68-70], nonetheless several confounding factors can be identified in the study, in particular, (i) difference in mean age (women: 65 \pm 10 years, men: 60 \pm 12 years), (ii) presence of other risk factors in women (obesity, hypertension) and, importantly, (iii) excessive dose administration in women compared to men could have biased results. Finally, it has been showed that platelets from males generally respond stronger to activation of the α 2-adrenergic receptor by epinephrine and serotonin signaling pathways [71], while showing stronger TxA₂ receptor-related aggregation responses [51].

During activation, platelets release microparticles (pMPs), a heterogeneous population of membrane vesicles with distinct functional properties: based on their cargo of molecules and antigenic composition, indeed, these pMPs can modulate several biological functions, such as coagulation, inflammation, and transfer of bioactive molecules to other cells [72-74]. High prothrombotic activity has extensively been reported for circulating pMPs [75, 76], which can be considered specific candidate biomarkers for CVD diagnosis and prognosis in early and late disease processes. Indeed, plasma pMP levels are high in healthy subjects showing high coronary heart disease risk score [77], and their number and phenotype (*i.e.*, surface expression of P-selectin and phosphatidylserine) positively correlate with recurrent CVD events [78, 79].

According to their parental origin, a significant gender-specific difference has been found, with the amount of pMPs significantly greater in healthy women than in the corresponding counterparts [80]. A menstrual cycle-dependent difference in pMPs also exists: a case-control study enrolling 27 healthy women and 18 healthy men demonstrated increased pMP release in females, especially in the luteal phase [81]. This finding suggests, therefore, that higher circulating pMPs (together with other risk factors, including pregnancy, oral contraceptives, and hormone therapy) may contribute to higher risk of developing venous thromboembolism observed in women < 45 years [49, 82, 83].

2.3. Platelet and CVD Risk in Metabolic Syndrome.

Metabolic syndrome is a cluster of cardiometabolic risk factors, including central obesity, hypertension, impaired glucose

metabolism, and dyslipidaemia [84, 85]. Several meta-analyses have shown that CVD risk in metabolic syndrome is higher in females than in males and sex differences in adiposity and insulin resistance may partly account for this increased risk [86, 87]. A Korean cross-sectional study, carried on 3827 participants (2169 men and 1658 women), showed a positive correlation for platelet count and an inverse correlation for mean platelet volume in women with metabolic syndrome, but such trend was not observed in men [88]. Moreover, a recent prospective longitudinal, observational, cohort study (the Framingham Heart Study) evaluated protein biomarker profiles in 3289 men and 3895 women (mean age 49 years), in order to identify key biological pathways differing between sexes [89]. Of 71 biomarkers analyzed, 86% were significantly different in the two groups; in particular, women showed upregulation of pathways involved in inflammation, immune response, and adiposity, while platelet homeostasis and fibrosis pathways were enriched in men [89]. According to available literature data, these sex differences in circulating CVD biomarkers were attenuated in postmenopausal women, confirming that CVD risk dramatically increases following menopause [89]. Given these gender-related divergences, physicians are encouraged to take care of sex-specific risks in primary cardiovascular prevention and for design of personalized therapeutic strategies [90].

3. Intervention Studies

Several interventional strategies have been established to reduce incidence of clinical events related to coronary heart, cerebrovascular, and peripheral vascular diseases. World guidelines are drawn up for primary (for lowering risk in people without clinical symptoms) and secondary (for people with clinically manifest CVD) prevention, and concern both lifestyle changes and drug use. Herein, we will focus on dietary habits and antiplatelet therapy since these are the interventions where gender differences are most evident.

3.1. Nutritional Strategies. Until few years ago, in studies concerning correlations between nutrition and CVD, the traditional research orientation was to identify harmful foods (e.g., unprocessed red and processed meats, sugar-sweetened beverages) and nutrients (such as *trans*- and saturated fats, cholesterol, and sodium), whose consumption is now strictly prohibited [15, 91]. As it is recently emerging, instead, the importance of the so-called “positive food/nutrients,” whose diet reduction or absence plays an equally crucial role in increasing cardiovascular risk [15, 91]. Coherently, diets based on foods particularly rich in antioxidants, phytochemicals, fiber, vitamins, monounsaturated fatty acids, and PUFAs, such as Mediterranean diet (MD) and vegetarian diet, are recognized worldwide as protective against CVD and its risk factors [8–15]. Also, in this context, it has been recognized a sex-related difference in individual responses to specific dietary habits (e.g., adherence to MD), with men displaying more favorable specific cardiometabolic changes, with respect to premenopausal women [92–95].

Some food patterns and bioactive components appear particularly interesting, as, besides their beneficial cardiovascular effects, they also act on platelets with a sex-dependent impact.

3.1.1. Mediterranean Diet. MD, a typical eating pattern of the Mediterranean basin, is characterized by wide consumption of fruits, vegetables, cereals, legumes, fish, olive oil as main fat source, and moderate red wine intake. Due to the consumption of these food items, subjects adhering to MD assume significant amounts of main nutrients of a healthy diet [96].

Effects of MD adherence on platelets have been investigated in the Moli-sani population-based cohort study that enrolled 14586 Italian healthy men and women [97]. Food intake was determined by food frequency questionnaire, while adherence to the MD was analyzed by using the MD Score (MDS) that evaluates intake of specific MD items and the Italian Mediterranean Index (IMI), a score particularly related to typical products consumed in Italy. What emerged from this study is that (i) greater adherence to MD was significantly associated with reduction in platelet count; (ii) subjects with greater adherence had reduced odds of being in the highest platelet count group; and (iii) hypercholesterolemia and increase in C-reactive protein (marker of inflammation) were prevalent in high-platelet count individuals. However, the most intriguing finding was that, although the mean platelet count of all individuals was within normal-range values, nonetheless, it directly correlates with predicted CVD risks in men, but not in women [97]. In line with this finding, a previous Moli-sani population-based cohort study demonstrated that specific healthy MD foods, particularly rich in antioxidant and phytochemicals, protected men much more than women against hypertension and inflammation [96]. This finding, therefore, suggests that healthy dietetic habits represent a valid strategy for primary CVD prevention that, however, requires particular attention to sex-related responses.

3.1.2. ω -3 PUFA. Beyond different efficiency of ω -3 PUFA biosynthesis in men and women, the rate of conversion is however low to satisfy physiological needs [55–57]; therefore, nutritional guidelines recommend to take EPA and DHA from fish (particularly, cold-water fatty fish, such as salmon, mackerel, tuna, herring, and sardine fish) and other seafood. Sometimes, also EPA and DHA supplementation, in the form of oily fish or fish oil (often fish liver oil) or krill oil capsules, is advised, although content of EPA and DHA varies in each of these preparations [98].

Several population studies have shown that dietary fish intake (as part of a healthy eating pattern) is inversely associated with stroke incidence and mortality, and therefore, EPA and DHA are counted among those nutrients that benefit cardiovascular health [99, 100]. As emerged from epidemiological studies, among mechanisms underlying the EPA and/or DHA cardiovascular protective role, reduction of platelet activation deserves special mention. The Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) study, where over 8000 high-risk

patients were enrolled and followed for five years, demonstrated that supplementation with EPA-ethyl ester (providing more EPA per gram of oil, with respect to other supplements) significantly reduced major CVD events by 25% [101]. The beneficial effect much likely derives from the ability of EPA to compete with AA as a substrate for platelet COX-1, thus counterbalancing production of proaggregatory thromboxane A₂ [102].

The main finding emerging from different studies is that ω -3 PUFAs counterbalance CVD risk factors in a gender-specific manner, both in primary and secondary prevention [103]. A meta-analysis indicated that dietary intake of fish and ω -3 PUFAs was correlated with lower incidence and mortality of stroke, especially in women and those with body mass index (BMI) < 24 kg/m² [104]. Phang and collaborators [105] have shown that 1.0 μ M EPA, DHA, and docosapentaenoic acid (DPA) reduced aggregation of platelets isolated from healthy subjects. When data from male and female populations were combined together, according to already published literature [106–108], all tested ω -3 PUFAs reduced platelet aggregation, but with significant differences in terms of efficaciousness: EPA resulted to be the most effective showing a significantly higher percentage inhibition with respect to both DHA and DPA. When data were separated by sex, the same pattern of inhibition of platelet aggregation by ω -3 PUFAs was observed only in the male group, while differences were lost in females. The most pronounced antiaggregatory effect of EPA observed only in men might, therefore, suggest a positive interaction between sex hormones and ω -3 PUFAs in modulating platelet activation cascades [105]. The same group [109] confirmed these findings also *in vivo*: in a blinded placebo-controlled intervention trial (enrolling 15 male and 15 female participants), the effects of a single acute oral dose of EPA- (providing 1 g EPA; EPA/DHA ratio = 5 : 1) or DHA- (providing 1 g DHA; EPA/DHA ratio = 1 : 5) rich oils on the aggregation response were investigated. Again, a gender-specific response was seen: DHA, but not EPA, significantly reduced platelet aggregation in women, whereas EPA, but not DHA, exerted an inhibitory action only in men. Accordingly, an inverse relationship between testosterone levels and platelet aggregation following EPA supplementation was observed. What emerges is, therefore, that males may benefit more from EPA supplementation and this may partly be explained considering that, among ω -3 PUFAs, EPA is more efficiently incorporated into male platelets [59]. Moreover, the finding that females are more responsive to DHA seems to be coherent with the evidence that, independently of dietary intake, females have higher circulating DHA concentrations, compared to males [59]. In the same population, authors also observed a gender-dependent response in the procoagulant activity of circulating pMPs. In male subjects, the single dose of EPA-rich oil inhibits pMP activity (-22%), in parallel with inhibition of platelet aggregation; on the contrary, DHA-rich oil reduces platelet aggregation, independently of pMP activity, in female subjects [110], thus pointing out to pMPs as one of the potential mechanistic pathways whereby ω -3 PUFAs might differentially modulate platelet activity and, therefore, yield cardiovascular benefits.

However, the study has some limitations, including differences in baseline characteristics (females were older and of postmenopausal age, while males had greater BMI and high testosterone levels) and in platelet-related parameters (longer lag time in males; higher platelet count and baseline platelet aggregation in females).

Thus, although available evidence highlights gender-specific effects of ω -3 PUFAs on platelet function, further work is needed to establish exact mechanisms underlying the interactions between sex hormones and this class of nutrients and future well-powered studies should be assessed to justify dietary recommendations for distinctive ω -3 PUFAs in men and women.

Although there are several data on beneficial effects of ω -3 PUFAs (taken from fish or supplements) in high-risk CVD patients, nonetheless, their therapeutic value, up to now, is not clear, as results are not conclusive and sometimes controversial [101]. Moreover, several confounding, often perplexing, factors should be considered, such as (i) differences in taking PUFAs from fish (which also is a source of other important nutrients, like selenium, iodine, zinc, calcium, and proteins), fortified foods (e.g., enriched margarine), or supplements; (ii) harmful effects related to high-PUFA intake, especially through supplements, *i.e.*, high concentrations of toxic compounds (namely, mercury, dioxins, and polychlorinated biphenyls) in fish oils; (iii) other negative events dependent on ω -3 PUFAs themselves, such as prolonged bleeding time, increased lipid peroxidation, and abrogation of normal immune responses.

Although dietary modifications may help in preventing pathological conditions, all these elements point out that we are far from a solid, scientific-based knowledge for development of individualized PUFA supplementation regimens to prevent and manage CVD, and further studies are required to better define precise dietary indications.

3.1.3. Flavonoids. Flavonoids are a large family of over 5,000 hydroxylated polyphenolic compounds, which encompass six major subclasses of dietary significance, named anthocyanidins, flavan-3-ols (also referred as flavanols), flavonols, flavanones, flavones, and isoflavones. These phytochemicals are abundantly found in fruits, vegetables, and cocoa, as well as in beverages, such as tea and wine. Several factors may affect flavonoid content in food, among which are agricultural practices, environmental conditions, ripening, storage, and food processing; consequently, reported value contents in plants should be considered approximate [54, 111].

Flavonoids are often present as glycosides (such as isoflavones and anthocyanins), and this chemical feature, together with other factors (including some other chemical characteristics, interactions with other components of food matrix, composition of colonic microbiota, and gut and liver metabolism), influences their metabolic fate and bioavailability [54, 111]. For example, anthocyanins and galloylated catechins are poorly absorbed, while isoflavones seem to be the most bioavailable flavonoids [111].

Beyond these evidences, flavonoids have received particular attention for potential health benefits of fruit- and vegetable-rich diets, especially in relation to the cardiovascular

system [111]. Most (but not all) epidemiological studies, indeed, greatly suggest that high intake of dietary flavonoids (approximately 200 mg/day of total flavonoids) is inversely related to CVD risk and mortality [112–117]. Nonetheless, it must not be overlooked that some of their beneficial effects may also be attributed to other bioactive constituents, (including other polyphenols, vitamins, and minerals), synergizing with flavonoids.

If initially flavonoids were believed to mainly act as antioxidants, nowadays, it is well established that they positively impact cardiovascular health by exerting other biological activities, such as (i) induction of vascular endothelium relaxation, (ii) inhibition of endothelial dysfunction, (iii) stimulation of nitric oxide release, (iv) inhibition of platelet aggregation, and (vi) downregulation of proinflammatory mediators [116, 118]. There is, indeed, the consistent view that these compounds directly act on various signaling pathways, among them, those related to P2Y₁/P2Y₁₂ (ADP receptors), GPVI (collagen receptor), protease-activated receptor 1 (PAR1; thrombin receptor), and COX-1 signaling, through which flavonoids (especially, those extracted from cocoa, tea, pigmented rice, chokeberry, and oat) mitigate platelet adhesion, degranulation, and aggregation [119].

In this context, interventional studies have shown that flavanols, which include catechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate monomers; their dimers (theaflavins, thearubigins); and polymers (proanthocyanidins), appear the most efficacious in attenuating platelet hyperactivation. Most of the studies are focused on two of principal dietary sources of flavanols, *i.e.*, cocoa-based products and green tea [120]. Just an example, a double-blind randomized placebo-controlled trial, enrolling twenty patients with congestive heart failure, evaluated acute and chronic effects of commercially available flavanol-rich chocolate on platelet and endothelial functions and compared it with a chocolate-free cocoa liquor, as control. The authors reported that, shortly after ingestion, only flavanol-rich cocoa led to peripheral vasodilatation, endothelial function improvement, and reduction in platelet activation [121]. The exact contribution of flavanols in the beneficial effect of cocoa has further been assessed by Ostertag and coworkers [122], who evaluated potential sex differences in platelet responses. In their blinded randomized, controlled trial, the researchers compared flavanol-enriched dark chocolate (containing 907.4 ± 22.75 mg of flavan-3-ols) with both standard dark (containing 382.3 ± 45.20 mg of flavanols) and white (with no flavanols) chocolates, in relation to effects on platelets derived from healthy men and women. By pooling data from male and female groups, they found that acute consumption of the two types of dark chocolates reduced ADP- and thrombin-dependent activation and aggregation of platelets and increased the collagen/epinephrine-induced *ex vivo* bleeding time; these effects were time-dependent and more evident with flavanol-enriched dark chocolate. According to gender-related differences in platelet signaling cascades [51, 66, 71], ADP-triggered pathways were significantly inhibited in men, while thrombin-dependent signaling was exclusively attenuated in women,

after consumption of flavanol-enriched dark chocolate. Analysis of plasma or urine concentrations of flavanols and their metabolites revealed gender-related absorption or metabolism of flavanols that might partially explain the different efficacy by which these phytochemicals can affect platelet functions [122]. However, it should be underlined that also white chocolate improved platelet profile in males [122], thus indicating the presence of other compounds, not yet identified, capable of exerting antiplatelet effects in a sex-dependent manner.

Besides flavanols, isoflavones (such as daidzein and genistein) deserve to be mentioned. These flavonoids, mainly found in soybeans and soy foods, show both estrogenic and antiestrogenic effects; therefore, they are also classified as phytoestrogens [111]. Accordingly, the effects derived from their intake (*via* foods and supplements) are object of extensive investigations, especially in the hormone-sensitive cancer research field. Moreover, it is well known that their assumption ameliorates some symptoms of menopause, such as hot flashes [123]. This evidence, together with the finding that isoflavones ameliorate lipid profile and endothelial function in a gender-specific manner, strengthens the idea that these phytoestrogens are also beneficial for cardiovascular health [124–126], especially for menopausal women, missing estrogen-dependent protection. For example, three prospective cohort studies have found positive correlation between higher intake of isoflavones and tofu (but not soy drinks) and moderately lower risk of developing coronary heart disease in both men and women; nonetheless, in women, the favorable association of tofu was more pronounced in young subjects or postmenopausal women without hormone use [127].

The capability of isoflavones to inhibit *in vitro* platelet activation induced by collagen or AA, through a mechanism dependent on their ability to act as TxA₂ receptor antagonists, seems noteworthy [128]. A double-blind, randomized study has clearly underlined that supplementation with soy-derived isoflavones reduced the risk of thrombogenesis, by decreasing platelet TxA₂ signaling [129]. Twenty-nine healthy postmenopausal women (aged 45–60 years) were randomly assigned to two groups, receiving either 100 mg/day soy isoflavone extract or placebo, for 3 months; what emerged is that supplementation had no significant effect on common CVD risk factors (lipid profile, blood pressure, and anthropometric measures), while significantly decreasing TxA₂ receptor density (from 181.9 ± 30.9 to 115.2 ± 16.2 fmol/10⁸ platelets) [129]. Conversely, a previous study evaluating the chronic effect of soy protein supplements (that are rich in isoflavones) in healthy young males showed that, although soy supplementation critically increased plasma concentration of isoflavones, nonetheless, such increase was not sufficient either to significantly inhibit *ex vivo* platelet aggregation or to ameliorate lipid profile [130].

In conclusion, although the impact of diet and gender on platelets is suggestive (Table 1), dietary manipulation of platelet function is still far from being realized, since gaps in our knowledge (especially concerning sex differences on bioavailability, metabolism, and activity of food components) persist and more research is required.

TABLE 1: Nutritional studies aimed at investigating gender-related differences in platelet responses.

Dietary factors	Experimental protocol	Main findings	Refs
Mediterranean diet (MD)	Population-based cohort study: 6975 males and 7611 females (mean age: 54.2 ± 11.5 yrs) adhering to MD and subdivided into 3 groups according to their PLT count: high-, medium-, and low-PLT count groups (2.5%, 95.6%, and 1.9% of the population, respectively).	In both sexes: PLT count was inversely associated with both MDS and IMI scores. Subjects with very high MD adherence had lower odds of having high-PLT count compared with individuals with poor adherence (OR 50.50; 95% CI: 0.31-0.80 and OR 5 0.73; 95% CI: 0.52-1.02 for MDS and IMI, respectively). In males: the mean PLT count increased with increasing of predicted CVD risk (low CVD risk: 236.5 ± 54.7, medium CVD risk: 239.6 ± 57.1, and high CVD risk: 247.1 ± 58.7; <i>P</i> for trend > 0.027 in multivariable analysis of variance). In females: no differences in PLT count within the predicted CVD risk.	[97]
ω-3 PUFA	Ex vivo study: PLT isolated from healthy 20 males (33.5 ± 2.1 yrs) and 22 females (35.7 ± 2.5 yrs) preincubated with 1 μM EPA, DHA, or DPA for 6 min at 37°C, before stimulation with 5 μg/mL collagen.	In both sexes: DHA and DPA equally reduced PLT aggregation (36.4% and 33.5% in men and women, respectively). EPA was the most efficacious PUFA (51.7%, <i>P</i> < 0.01 vs. DPA and <i>P</i> < 0.004 vs. DHA). In males: DHA (25.3%) and DPA (21.7%) were less effective, with respect to EPA (48.9%, <i>P</i> < 0.002 and <i>P</i> < 0.001, respectively). In females: DHA (46.5%), DPA (44.2%), and EPA (54.3%) equally reduced PLT aggregation.	[105]
	Blinded placebo-controlled trial: healthy 15 males (40.1 ± 2.1 yrs old) and 15 females (47.4 ± 1.9 yrs), alternatively receiving a single dose of 2 × 1 g capsules containing either (i) placebo or (ii) EPA-rich oil (providing 1 g EPA with EPA/DHA ratio = 5 : 1) or (iii) DHA-rich oil (providing 1 g DHA with EPA/DHA ratio = 1 : 5). Fasting blood samples collected for PLT aggregation assay at 0, 2, 5, and 24 hrs after supplementation.	In both sexes: EPA- and DHA-rich oils reduced PLT aggregation. EPA was the most effective at 2 (-3.6%, <i>P</i> < 0.001), 5 (-8.8%, <i>P</i> < 0.001), and 24 (-13.3%, <i>P</i> < 0.006) hrs postsupplementation. DHA was ineffective at 2 and 5 hrs, but equally effective (-11.9%, <i>P</i> < 0.016) as EPA at 24 hrs. In males: only EPA reduced PLT aggregation at 2 (-11%, <i>P</i> < 0.001), 5 (-10.6%, <i>P</i> < 0.003), and 24 (-20.5%, <i>P</i> < 0.008) hrs. In females: only DHA reduced PLT aggregation at 24 hrs (-13.7%, <i>P</i> < 0.05).	[109]
	Blinded placebo-controlled trial: healthy 15 males (40.1 ± 2.1 yrs) and 15 women (47.4 ± 1.9 yrs), alternatively receiving a single dose of 2 × 1 g capsules containing either (i) placebo or (ii) EPA-rich oil (providing 1 g EPA with	In both sexes: neither oil affected pMP numbers, and only EPA-rich oil produced a decrease in pMP activity (-19.4%, <i>P</i> = 0.003). In males: EPA, but not DHA, increased the mean lag time (60 vs. 79 sec, +29.5%) and reduced ADP-induced PLT	[110]

TABLE 1: Continued.

Dietary factors	Experimental protocol	Main findings	Refs
	<p>EPA/DHA ratio = 5 : 1) or (iii) DHA-rich oil (providing 1 g DHA with EPA/DHA ratio = 1 : 5). Fasting blood samples collected at 0 and 24 hrs after supplementation for PLT aggregation assay and measurement of pMP number and procoagulant activity.</p> <p>Double-blind, randomized, controlled intervention trial: 79 men and 95 women aged 20–80 yrs receiving six 0.75 g capsules/day providing a total of 1.5 g EPA and 1.77 g DHA (i.e., 3.27 g EPA plus DHA), as TAG, equivalent to the amount in one portion of oily fish and six 0.75 g placebo capsules (high oleic sunflower oil), over 12 months. Fasting blood samples collected at 0 and 12 months after supplementation for lipid composition of platelet membrane.</p>	<p>aggregation (-20.5%, $P = 0.008$) and pMP activity (-22%, $P = 0.008$). Inverse relationship between PLT aggregation activity and testosterone levels ($r = -0.443$, $P = 0.04$). In females: DHA, but not EPA, was effective in reducing PLT aggregation (-13.7%), without affecting pMP number and activity. In both sexes: no differences in basal content of EPA and DHA. Equal dose-dependent increases of EPA and DHA in platelet membrane between male and females after 12-month supplementation.</p> <p>In males: EPA increased in PLT membrane, but without statistical significance.</p>	[131]
Flavanols	<p>Blinded randomized, controlled acute trial: healthy 26 women (23–62 yrs; mean: 38 ± 2.4 yrs) and 16 males (25–65 yrs; mean: 46 ± 3.4 yrs), who acutely ingested 60 g of (i) flavanol-enriched dark chocolate (FDC; 907.4 ± 22.75 mg of flavan-3-ols), (ii) standard dark chocolate (SDC; 382.3 ± 45.20 mg of flavan-3-ols), and (iii) white chocolate (WC; not detectable). Fasting blood collected at 0, 2, and 6 hrs after supplementation for PLT activity assays.</p>	<p>Ex vivo bleeding time In both sexes: <i>ex vivo</i> bleeding time increased 6 hrs after consumption of FDC and SDC, but not of WC ($P = 0.011$), in both sexes. In females: <i>ex vivo</i> bleeding time increased 6 hrs after the consumption of FDC and SDC, but not with WC ($P = 0.016$). In males: <i>ex vivo</i> bleeding time increased 6 hrs after the consumption of FDC and WC ($P = 0.042$).</p> <p>PLT aggregation In both sexes: ADP-induced platelet aggregation reduced at 2 hrs, but not 6 hours, after consumption of FDC and SDC. In males: ADP-induced PLT aggregation was reduced at 2 and 6 hrs after consumption of FDC and SDC ($P = 0.008$ and $P = 0.020$ vs. women). In females: TRAP-induced PLT aggregation was reduced at 2 hrs, but not 6 hours, after consumption of FDC ($P = 0.010$, P value for interaction between treatment and gender: $P = 0.213$).</p> <p>PLT activation In both sexes: TRAP-induced fibrinogen binding decreased at 2 and 6 hrs after consumption of FDC and WC (respectively, $P = 0.014$ and $P = 0.021$ vs. SDC).</p>	[122]

TABLE 1: Continued.

Dietary factors	Experimental protocol	Main findings	Refs
		In males: ADP-triggered P-selectin exposure decreased at 2 hrs, but not 6 hrs, after consumption of FDC and WB, but not with SDC ($P = 0.002$). In females: TRAP-induced fibrinogen binding was decreased at 2 hrs, but not 6 hours, after consumption of FDC ($P = 0.041$, P value for interaction between treatment and gender: $P = 0.304$).	
Isoflavones	Double-blind, randomized, placebo-controlled study: 29 postmenopausal women (45–60 yrs), who randomly received two daily capsules of a soybean isoflavone extract (23.4 ± 3.4 mg daidzein and 24.1 ± 4.6 mg genistein per capsule) or placebo for 12 weeks. Blood collected at 0 and 12 weeks after supplementation for PLT TxA2 receptor binding assay. Double-blind, randomized, placebo-controlled study: healthy 10 men (25.8 ± 1.2 yrs) receiving 60 mg of soy proteins in the form of beverage powder and providing 131 mg of total isoflavones (80.3 mg genistein, 35.6 mg daidzein, and 15.1 mg glycitein) and 10 men (23.9 ± 0.9 yrs), receiving 60 mg of calcium caseinate powder (control), for 28 days. Blood was collected at 0, 28, and 56 days after supplementation for quantification of isoflavone content in plasma and PLT aggregation.	In females: PLT TxA2 receptor density decreased in isoflavone-treated subjects from 181.9 ± 30.9 to 115.2 ± 16.2 fmol/ 10^8 PLT ($P < 0.02$ vs. the placebo group). Decrease in TxA2 receptor density inversely correlated with serum concentrations of isoflavones. In males: plasma isoflavone content increased after 28 day in the supplementation group ($P < 0.05$ vs. basal values) and returned to baseline after 56 days (washout period). PLT aggregation was not affected by soy protein supplementation.	[129] [130]

CI: confidence interval; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid; IMI: Italian Mediterranean Index; MD: Mediterranean diet; MDS: Mediterranean Diet Score; OR: odds ratio; PLT: platelet; pMP: platelet microparticles; TAG: triglycerides; TRAP: thrombin receptor activating peptide; TxA2: thromboxane A2.

4. Antiplatelet Therapy

Current antiplatelet therapies mainly target enzymes (such as COX-1), receptors (such as thromboxane or ADP receptors), and glycoproteins (such as GPIIb or GPVI) [132, 133]. Among antiplatelet drugs, the most widely used is aspirin that irreversibly inhibits COX-1, thus preventing conversion of AA into TxA2; nonetheless, it does not act on TxA2-independent signaling pathways and, moreover, long-term usage leads to increased risk of bleeding events [134]. To overcome these limitations, other drugs have been developed,

such as the P2Y₁₂ receptor inhibitors clopidogrel, prasugrel and ticagrelor. The first one is the most commonly used, but it shows a delayed therapeutic onset and may cause coagulation dysfunction; prasugrel inhibits platelet aggregation more rapidly than clopidogrel; the last antiplatelet drug exerts CVD protective effects without increasing overall bleeding and, being a P2Y₁₂ receptor reversible inhibitor, loses pharmacological activity upon body clearance [135, 136].

Although women are less represented in cardiovascular clinical trials, nonetheless, numerous investigations have

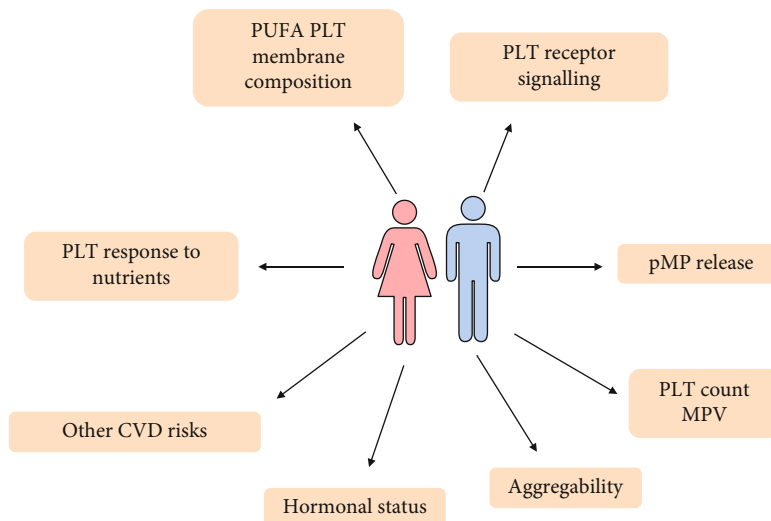


FIGURE 1: Schematic representation of the main sex-based differences in platelets in relation to cardiovascular disease. See text for details. CVD: cardiovascular disease; MPV: mean platelet volume; PLT: platelet; pMPs: platelet microparticles; PUFA: polyunsaturated fatty acids.

pointed out that some of the abovementioned female-related conditions (such as hormonal status and platelet biology) have to be taken into account in view of aspirin administration for primary prevention. Just an example, the Women's Health Study, evaluating the efficacy of aspirin in 39876 healthy women (≥ 45 years of age) monitored for 10 years, reported a significant prevention of ischemic stroke ($RR = 0.83$; $P < 0.04$); however, the drug also led to a parallel increment of gastrointestinal bleeding risk [137]. Other clinical trials and meta-analyses confirmed no significant benefit of aspirin treatment in women concerning cardiovascular events and CVD mortality, but a huge increase in risk of overall bleeding [134, 138]. Based on these findings, special attention should be paid when treating women with aspirin.

A meta-analysis of five randomized trials, involving 79,613 (of whom 23,533 are women) patients with cardiovascular heart disease, showed that clopidogrel (in addition to aspirin) significantly decreased cardiovascular events in both men and women. Although gender differences in the absolute benefit are not striking, during long-term antiplatelet therapy, risk of events was higher in women than in men and clopidogrel therapy seemed to be effective only in men. As documented by the *post hoc* subanalysis of the BleeMACS study, collecting data from fifteen centres in Europe (Germany, Greece, Italy, Netherlands, Poland, and Spain), Asia (China and Japan), North America (Canada), and South America (Brazil), the increased bleeding rates observed only in females were associated with prasugrel-/ticagrelor-based dual antiplatelet therapy [139]. Finally, the multicentre, Italian START ANTIPLATELET registry investigated the choice of antiplatelet treatment and its impact on one-year clinical outcome, in 625 males and 215 females presenting with acute coronary syndrome. In this study, what emerged is that dual antiplatelet therapy was more commonly prescribed in men and, when administered in both sexes, clopidogrel was the best choice for women, while prasugrel was preferentially used in men. However, gender-related differences in terms of therapy did not lead to different outcomes. Therefore,

P2Y₁₂ inhibitor choice in dual antiplatelet therapy is gender-specific (in order to counteract the increased bleeding risk usually observed in females), but it has a similar clinical outcome irrespective of sex [140].

High percentage of individuals usually experiences antiplatelet therapy resistance that impairs successful prevention of cardiovascular events, and some determinants of resistance to antiplatelet therapy are gender responsive [141–146]. A prospective study on 160 patients with stable coronary heart disease (118 men and 42 women, aged 65.2 ± 7.8 years), indeed, showed a sex-related response to long-term double antiplatelet therapy (75 mg/day aspirin and clopidogrel for three months): female gender was more predisposed to resistance to both aspirin and clopidogrel compared to men [19]. Two main factors may account for the worst responsiveness: women possess (i) greater aggregation capacity, maybe because of higher density of platelet receptors able to bind fibrinogen, and (ii) increased inflammatory status, as highlighted by higher concentrations of the proaggregatory C-reactive protein (CRP) and number of leukocytes and granulocytes.

5. Conclusions

Evidence to date has revealed sex-based differences in CVD prevention, diagnosis, and management. Among modifiable and nonmodifiable risk factors, platelet hyperactivity deserves particular mention, as activation and aggregation of platelets, as well as their interaction with endothelial cells and crosstalk with immune cells, play a major role in the pathophysiology of main cardiovascular events, including stroke, myocardial infarction, and peripheral vascular injury. Moreover, platelet biology is profoundly modulated by several elements, including sex hormones, nutrients, and inflammatory mediators (Figure 1). Consequently, men and women not only display a different platelet functionality but also distinctively respond to common antiplatelet drugs, as well as to specific dietetic habits. In conclusion, full

understanding of gender-related differences is the final goal in order to design tailored strategies for preventing and treating platelet-mediated vascular damage.

Abbreviations

12-H(P)ETE:	12-Hydro(pero)xyicosatetraenoic acid
12-LOX:	12-Lipoxygenase
ANOCA:	Angina and nonobstructive coronary artery disease
AA:	Arachidonic acid
BMI:	Body mass index
CRP:	C-reactive protein
CVD:	Cardiovascular disease
COX-1:	Cyclooxygenase 1
DHA:	Docosahexaenoic acid
DPA:	Docosapentaenoic acid
EPA:	Eicosapentaenoic acid
GP:	Glycoprotein
PAF:	Platelet-activating factor
pMP:	Platelet microparticle
PGI ₂ :	Prostacyclin
PUFA:	Polyunsaturated fatty acid
RR:	Relative ratio
TxA ₂ :	Thromboxane A ₂
TPA:	Thrombin receptor activating peptide
vWF:	von Willebrand factor.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contributions

M. Valeria Catani and Isabella Savini are equally senior authors.

References

- [1] World Health Organization WHO, *The top 10 causes of death*, <http://www.who.int/en/news-room/fact-sheets/detail/the-top-10-causes-of-death>.
- [2] N. M. Townsend, R. Luengo-Fernandez, J. Leal, A. Gray, P. Scarborough, and M. Rayner, "European cardiovascular disease statistics," *European Heart Network*, vol. 2012, pp. 1–125, 2012.
- [3] M. Mishali, S. Prizant-Passal, T. Avrech, and Y. Shoenfeld, "Association between dairy intake and the risk of contracting type 2 diabetes and cardiovascular diseases: a systematic review and meta-analysis with subgroup analysis of men versus women," *Nutrition Reviews*, vol. 77, no. 6, pp. 417–429, 2019.
- [4] I. Campesi, C. Carru, A. Zinellu et al., "Regular cigarette smoking influences the transsulfuration pathway, endothelial function, and inflammation biomarkers in a sex-gender specific manner in healthy young humans," *American Journal of Translational Research*, vol. 5, no. 5, pp. 497–509, 2013.
- [5] M. Woodward, "Cardiovascular disease and the female disadvantage," *International Journal of Environmental Research and Public Health*, vol. 16, no. 7, p. 1165, 2019.
- [6] C. Vitale, M. Fini, I. Spoletini, M. Lainscak, P. Seferovic, and G. M. Rosano, "Under-representation of elderly and women in clinical trials," *International Journal of Cardiology*, vol. 232, pp. 216–221, 2017.
- [7] M. D. Linden and D. E. Jackson, "Platelets: Pleiotropic roles in atherogenesis and atherothrombosis," *The International Journal of Biochemistry & Cell Biology*, vol. 42, no. 11, pp. 1762–1766, 2010.
- [8] R. Micha, M. L. Shulkin, J. L. Peñalvo et al., "Etiologic effects and optimal intakes of foods and nutrients for risk of cardiovascular diseases and diabetes: systematic reviews and meta-analyses from the nutrition and chronic diseases expert group (NutriCoDE)," *PLoS One*, vol. 12, no. 4, article e0175149, 2017.
- [9] B. Jakše, B. Jakše, S. Pinter et al., "Dietary intakes and cardiovascular health of healthy adults in short-, medium-, and long-term whole-food plant-based lifestyle program," *Nutrients*, vol. 12, no. 1, p. 55, 2020.
- [10] D. Kargin, L. Tomaino, and L. Serra-Majem, "Experimental outcomes of the Mediterranean diet: lessons learned from the Predimed randomized controlled trial," *Nutrients*, vol. 11, no. 12, p. 2991, 2019.
- [11] A. Sekikawa, C. Cui, D. Sugiyama, A. Fabio, W. S. Harris, and X. Zhang, "Effect of high-dose marine omega-3 fatty acids on atherosclerosis: a systematic review and meta-analysis of randomized clinical trials," *Nutrients*, vol. 11, no. 11, p. 2599, 2019.
- [12] A. Tuttolomondo, I. Simonetta, M. Daidone, A. Mogavero, A. Ortello, and A. Pinto, "Metabolic and vascular effect of the Mediterranean diet," *International Journal of Molecular Sciences*, vol. 20, no. 19, p. 4716, 2019.
- [13] V. Tosti, B. Bertozzi, and L. Fontana, "Health benefits of the Mediterranean diet: metabolic and molecular mechanisms," *The Journals of Gerontology: Series A*, vol. 73, no. 3, pp. 318–326, 2018.
- [14] L. Serra-Majem, B. Román-Viñas, A. Sanchez-Villegas, M. Guasch-Ferré, D. Corella, and C. La Vecchia, "Benefits of the Mediterranean diet: epidemiological and molecular aspects," *Molecular Aspects of Medicine*, vol. 67, pp. 1–55, 2019.
- [15] B. De Roos, X. Zhang, G. Rodriguez Gutierrez et al., "Anti-platelet effects of olive oil extract: in vitro functional and proteomic studies," *European Journal of Nutrition*, vol. 50, no. 7, pp. 553–562, 2011.
- [16] J. Milionis, M. S. Elisaf, and H. Dimit, "Platelet function and lipid-lowering interventions," *Platelets*, vol. 10, no. 6, pp. 357–367, 2009.
- [17] G. Berlin, M. Hammar, L. Tapper, and N. Tynngård, "Effects of age, gender and menstrual cycle on platelet function assessed by impedance aggregometry," *Platelets*, vol. 30, no. 4, pp. 473–479, 2018.
- [18] N. J. Breet, M. A. Sluman, M. A. J. P. J. van Berkel et al., "Effect of gender difference on platelet reactivity," *Netherlands Heart Journal*, vol. 19, no. 11, pp. 451–457, 2011.
- [19] M. Jastrzebska, Z. Marcinowska, S. Oledzki et al., "Variable gender-dependent platelet responses to combined antiplatelet therapy in patients with stable coronary-artery disease," *Journal of Physiology and Pharmacology*, vol. 69, pp. 595–605, 2018.

- [20] R. J. Butterworth, "The relationship between mean platelet volume, stroke subtype and clinical outcome," *Platelets*, vol. 9, no. 6, pp. 359–364, 2009.
- [21] J. Du, Q. Wang, B. He et al., "Association of mean platelet volume and platelet count with the development and prognosis of ischemic and hemorrhagic stroke," *International Journal of Laboratory Hematology*, vol. 38, no. 3, pp. 233–239, 2016.
- [22] K. Vasudeva and A. Munshi, "Genetics of platelet traits in ischaemic stroke: focus on mean platelet volume and platelet count," *International Journal of Neuroscience*, vol. 129, no. 5, pp. 511–522, 2019.
- [23] G. Slavka, T. Perkmann, H. Haslachner et al., "Mean platelet volume may represent a predictive parameter for overall vascular mortality and ischemic heart disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 5, pp. 1215–1218, 2011.
- [24] F. Mayda-Domaç, H. Mısırlı, and M. Yılmaz, "Prognostic role of mean platelet volume and platelet count in ischemic and hemorrhagic stroke," *Journal of Stroke and Cerebrovascular Diseases*, vol. 19, no. 1, pp. 66–72, 2010.
- [25] H. Lebas, K. Yahiaoui, R. Martos, and Y. Boulaftali, "Platelets are at the nexus of vascular diseases," *Frontiers in Cardiovascular Medicine*, vol. 6, 2019.
- [26] M. Hamilos, S. Petousis, and F. Parthenakis, "Interaction between platelets and endothelium: from pathophysiology to new therapeutic options," *Cardiovascular Diagnosis and Therapy*, vol. 8, no. 5, pp. 568–580, 2018.
- [27] V. M. Miller, D. A. Lewis, and D. A. Barber, "Gender differences and endothelium- and platelet-derived factors in the coronary circulation," *Clinical and Experimental Pharmacology and Physiology*, vol. 26, no. 2, pp. 132–136, 1999.
- [28] D. De Smedt, D. De Bacquer, J. De Sutter et al., "The gender gap in risk factor control: effects of age and education on the control of cardiovascular risk factors in male and female coronary patients. The EUROASPIRE IV study by the European Society of Cardiology," *International Journal of Cardiology*, vol. 209, pp. 284–290, 2016.
- [29] J. Dallongeville, D. De Bacquer, J. Heidrich et al., "Gender differences in the implementation of cardiovascular prevention measures after an acute coronary event," *Heart*, vol. 96, no. 21, pp. 1744–1749, 2010.
- [30] A. H. E. M. Maas and Y. E. A. Appelman, "Gender differences in coronary heart disease," *Netherlands Heart Journal*, vol. 18, no. 12, pp. 598–603, 2010.
- [31] R. Y. Khamis, T. Ammari, and G. W. Mikhail, "Gender differences in coronary heart disease," *Heart*, vol. 102, no. 14, pp. 1142–1149, 2016.
- [32] L. L. Yanes and J. F. Reckelhoff, "Postmenopausal hypertension," *American Journal of Hypertension*, vol. 24, no. 7, pp. 740–749, 2011.
- [33] W. B. Kannel, "Menopause and risk of cardiovascular disease," *Annals of Internal Medicine*, vol. 85, no. 4, pp. 447–452, 1976.
- [34] B. D. Pardhe, S. Ghimire, J. Shakya et al., "Elevated cardiovascular risks among postmenopausal women: a community based case control study from Nepal," *Biochemistry Research International*, vol. 2017, 5 pages, 2017.
- [35] D. Zhao, E. Guallar, P. Ouyang et al., "Endogenous sex hormones and incident cardiovascular disease in postmenopausal women," *Journal of the American College of Cardiology*, vol. 71, no. 22, pp. 2555–2566, 2018.
- [36] R. Chaudhary, A. Sukhi, R. Chaudhary et al., "Gender differences in thrombogenicity among patients with angina and non-obstructive coronary artery disease," *Journal of Thrombosis and Thrombolysis*, vol. 48, no. 3, pp. 373–381, 2019.
- [37] E. M. Buchholz, K. M. Strait, R. P. Dreyer et al., "Editor's choice—sex differences in young patients with acute myocardial infarction: a VIRGO study analysis," *European Heart Journal: Acute Cardiovascular Care*, vol. 6, no. 7, pp. 610–622, 2015.
- [38] A. Gupta, Y. Wang, J. A. Spertus et al., "Trends in acute myocardial infarction in young patients and differences by sex and race, 2001 to 2010," *Journal of the American College of Cardiology*, vol. 64, no. 4, pp. 337–345, 2014.
- [39] G. Khetawat, N. Faraday, M. L. Nealen et al., "Human megakaryocytes and platelets contain the estrogen receptor β and androgen receptor (AR): testosterone regulates AR expression," *Blood*, vol. 95, no. 7, pp. 2289–2296, 2000.
- [40] M. Jayachandran and V. M. Miller, "Human platelets contain estrogen receptor α , caveolin-1 and estrogen receptor associated proteins," *Platelets*, vol. 14, no. 2, pp. 75–81, 2009.
- [41] J.-F. Arnal, et al. F. Lenfant, R. Metivier et al., "Membrane and nuclear estrogen receptor alpha actions: from tissue specificity to medical implications," *Physiological Reviews*, vol. 97, no. 3, pp. 1045–1087, 2017.
- [42] X. Fan, X. Chen, C. Wang et al., "Drospirenone enhances GPIIb-IX-V-mediated platelet activation," *Journal of Thrombosis and Haemostasis*, vol. 13, no. 10, pp. 1918–1924, 2015.
- [43] M. Otahbachi, J. Simoni, G. Simoni et al., "Gender differences in platelet aggregation in healthy individuals," *Journal of Thrombosis and Thrombolysis*, vol. 30, no. 2, pp. 184–191, 2010.
- [44] J. R. Coleman, E. E. Moore, M. R. Kelher et al., "Female platelets have distinct functional activity compared with male platelets: implications in transfusion practice and treatment of trauma-induced coagulopathy," *Journal of Trauma and Acute Care Surgery*, vol. 87, no. 5, pp. 1052–1060, 2019.
- [45] C. Beyan, K. Kaptan, A. Ifran, S. Savaşçı, Y. Öztürk, and B. Ökmen, "Effect of sex difference on platelet aggregation using an optical method in healthy subjects," *Clinical and Laboratory Haematology*, vol. 28, no. 1, pp. 14–16, 2006.
- [46] M. Johnson, E. Ramey, and P. W. Ramwell, "Sex and age differences in human platelet aggregation," *Nature*, vol. 253, no. 5490, pp. 355–357, 1975.
- [47] A. Sestito, A. Sciahbasi, R. Landolfi, A. Maseri, G. A. Lanza, and F. Andreotti, "A simple assay for platelet-mediated hemostasis in flowing whole blood (PFA-100): reproducibility and effects of sex and age," *Cardiologia*, vol. 44, no. 7, pp. 661–665, 1999.
- [48] Y. Nakano, T. Oshima, H. Matsuura, G. Kajiyama, and M. Kambe, "Effect of 17β -estradiol on inhibition of platelet aggregation in vitro is mediated by an increase in NO synthesis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 18, no. 6, pp. 961–967, 1998.
- [49] A. van Hylckama Vlieg, F. M. Helmerhorst, J. P. Vandembroucke, C. J. M. Doggen, and F. R. Rosendaal, "The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study," *BMJ*, vol. 339, no. aug13 2, pp. b2921–b2921, 2009.
- [50] M. Dupuis, S. Severin, E. Noirrit-Esclassan, J. F. Arnal, B. Payrastre, and M. C. Valéra, "Effects of estrogens on

- platelets and megakaryocytes," *International Journal of Molecular Sciences*, vol. 20, no. 12, p. 3111, 2019.
- [51] A. A. L. Ajayi, R. Mathur, and P. V. Halushka, "Testosterone increases human platelet thromboxane A2 receptor density and aggregation responses," *Circulation*, vol. 91, no. 11, pp. 2742–2747, 1995.
- [52] A. A. L. Ajayi and P. V. Halushka, "Castration reduces platelet thromboxane A2 receptor density and aggregability," *QJM - Monthly Journal of the Association of Physicians*, vol. 98, no. 5, pp. 349–356, 2005.
- [53] J. Geppert, Y. Min, M. Neville, C. Lowy, and K. Ghebremeskel, "Gender-specific fatty acid profiles in platelet phosphatidyl-choline and -ethanolamine," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 82, no. 1, pp. 51–56, 2010.
- [54] J. A. Rothwell, M. Urpi-Sarda, M. Boto-Ordoñez et al., "Systematic analysis of the polyphenol metabolome using the Phenol-Explorer database," *Molecular Nutrition & Food Research*, vol. 60, no. 1, pp. 203–211, 2016.
- [55] E. J. Giltay, L. J. G. Gooren, A. W. F. T. Toorians, M. B. Katan, and P. L. Zock, "Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects," *American Journal of Clinical Nutrition*, vol. 80, no. 5, pp. 1167–1174, 2004.
- [56] G. C. Burdge and S. A. Wootton, "Conversion of α -linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women," *British Journal of Nutrition*, vol. 88, no. 4, pp. 411–420, 2002.
- [57] G. C. Burdge, A. E. Jones, and S. A. Wootton, "Eicosapentaenoic and docosapentaenoic acids are the principal products of α -linolenic acid metabolism in young men," *British Journal of Nutrition*, vol. 88, no. 4, pp. 355–363, 2002.
- [58] G. Burdge, "n-3 Fatty acid metabolism in women – Reply," *British Journal of Nutrition*, vol. 90, no. 5, pp. 994–995, 2003.
- [59] C. E. Childs, "Sex hormones and n-3 fatty acid metabolism," *Proceedings of the Nutrition Society*, vol. 79, no. 2, pp. 219–224, 2020.
- [60] M. K. Larson, G. W. Tormoen, L. J. Weaver et al., "Exogenous modification of platelet membranes with the omega-3 fatty acids EPA and DHA reduces platelet procoagulant activity and thrombus formation," *American Journal of Physiology - Cell Physiology*, vol. 304, no. 3, pp. C273–C279, 2013.
- [61] V. L. Serebruany, M. Miller, A. N. Pokov et al., "Early impact of prescription omega-3 fatty acids on platelet biomarkers in patients with coronary artery disease and hypertriglyceridemia," *Cardiology*, vol. 118, no. 3, pp. 187–194, 2011.
- [62] O. Adam, "Cardiovascular effects of n-6 fatty acids," *Klinische Wochenschrift*, vol. 68, pp. 16–22, 1990.
- [63] S. D. Kristensen, E. B. Schmidt, and J. Dyerberg, "Dietary supplementation with n-3 polyunsaturated fatty acids and human platelet function: a review with particular emphasis on implications for cardiovascular disease," *Journal of Internal Medicine*, vol. 225, no. S731, pp. 141–150, 1989.
- [64] R. Adili, M. Hawley, and M. Holinstat, "Regulation of platelet function and thrombosis by omega-3 and omega-6 polyunsaturated fatty acids," *Prostaglandins and Other Lipid Mediators*, vol. 139, pp. 10–18, 2018.
- [65] J. Cowman, E. Dunne, I. Oglesby et al., "Age-related changes in platelet function are more profound in women than in men," *Scientific Reports*, vol. 5, no. 1, 2015.
- [66] N. Faraday, P. J. Goldschmidt-Clermont, and P. F. Bray, "Gender differences in platelet GPIIb-IIIa activation," *Thrombosis and Haemostasis*, vol. 77, no. 4, pp. 748–754, 2018.
- [67] K. P. Alexander, A. Y. Chen, L. K. Newby et al., "Sex differences in major bleeding with glycoprotein IIb/IIIa inhibitors," *Circulation*, vol. 114, no. 13, pp. 1380–1387, 2006.
- [68] E. Y. Cheng and M. H. Kong, "Gender differences of thromboembolic events in atrial fibrillation," *American Journal of Cardiology*, vol. 117, no. 6, pp. 1021–1027, 2016.
- [69] J. R. O'Brien, "The bleeding time in normal and abnormal subjects," *Journal of Clinical Pathology*, vol. 4, no. 3, pp. 272–285, 1951.
- [70] S. Orsini, P. Noris, L. Bury et al., "Bleeding risk of surgery and its prevention in patients with inherited platelet disorders," *Haematologica*, vol. 102, no. 7, pp. 1192–1203, 2017.
- [71] A. L. Bailey, D. C. Scantlebury, and S. S. Smyth, "Thrombosis and antithrombotic therapy in women," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 3, pp. 284–288, 2009.
- [72] V. Gasperi, C. Vangapandu, I. Savini, G. Ventimiglia, G. Adorno, and M. V. Catani, "Polyunsaturated fatty acids modulate the delivery of platelet microvesicle-derived microRNAs into human breast cancer cell lines," *The Journal of Nutritional Biochemistry*, vol. 74, p. 108242, 2019.
- [73] M. Pathan, P. Fonseca, S. V. Chitti et al., "Vesiclepedia 2019: A compendium of RNA, proteins, lipids and metabolites in extracellular vesicles," *Nucleic Acids Research*, vol. 47, no. D1, pp. D516–D519, 2019.
- [74] M. V. Catani, I. Savini, V. Tullio, and V. Gasperi, "The 'Janus face' of platelets in cancer," *International Journal of Molecular Sciences*, vol. 21, no. 3, p. 788, 2020.
- [75] E. Sinauridze, D. Kireev, N. Popenko et al., "Platelet micro-particle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets," *Thrombosis and Haemostasis*, vol. 97, no. 3, pp. 425–434, 2017.
- [76] M. Zarà, G. F. Guidetti, M. Camera et al., "Biology and role of extracellular vesicles (EVs) in the pathogenesis of thrombosis," *International Journal of Molecular Sciences*, vol. 20, no. 11, p. 2840, 2019.
- [77] T. Ueba, S. Nomura, N. Inami et al., "Plasma level of platelet-derived microparticles is associated with coronary heart disease risk score in healthy men," *Journal of Atherosclerosis and Thrombosis*, vol. 17, no. 4, pp. 342–349, 2010.
- [78] J. Rosińska, W. Ambrosius, J. Maciejewska, R. Narożny, W. Kozubski, and M. Łukasik, "Association of platelet-derived microvesicles and their phenotypes with carotid atherosclerosis and recurrent vascular events in patients after ischemic stroke," *Thrombosis Research*, vol. 176, pp. 18–26, 2019.
- [79] L. Badimon, R. Suades, E. Fuentes, I. Palomo, and T. Padró, "Role of platelet-derived microvesicles as crosstalk mediators in atherothrombosis and future pharmacology targets: a link between inflammation, atherosclerosis, and thrombosis," *Frontiers in Pharmacology*, vol. 7, 2016.
- [80] C. M. Gustafson, A. J. Shepherd, V. M. Miller, and M. Jayachandran, "Age- and sex-specific differences in blood-borne microvesicles from apparently healthy humans," *Biology of Sex Differences*, vol. 6, no. 1, 2015.
- [81] B. Toth, K. Nikolajek, A. Rank et al., "Gender-specific and menstrual cycle dependent differences in circulating microparticles," *Platelets*, vol. 18, no. 7, pp. 515–521, 2009.

- [82] S. Nikol and K. Middendorf, "Thromboembolie- und Arterioskleroserisiko bei Frauen," *Zeitschrift für Kardiologie*, vol. 91, no. 7, pp. 523–535, 2002.
- [83] M. D. Silverstein, J. A. Heit, D. N. Mohr, T. M. Petterson, W. M. O'Fallon, and L. J. Melton, "Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study," *Archives of Internal Medicine*, vol. 158, no. 6, pp. 585–593, 1998.
- [84] M. B. Lanktree and R. A. Hegele, *Metabolic Syndrome. In Genomic and Precision Medicine*, Elsevier, 2017.
- [85] C. Barale and I. Russo, "Influence of cardiometabolic risk factors on platelet function," *International Journal of Molecular Sciences*, vol. 21, no. 2, p. 623, 2020.
- [86] S. Mottillo, K. B. Filion, J. Genest et al., "The metabolic syndrome and cardiovascular risk: a systematic review and meta-analysis," *Journal of the American College of Cardiology*, vol. 56, no. 14, pp. 1113–1132, 2010.
- [87] A. S. Gami, B. J. Witt, D. E. Howard et al., "Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies," *Journal of the American College of Cardiology*, vol. 49, no. 4, pp. 403–414, 2007.
- [88] B. J. Park, J. Y. Shim, H. R. Lee, D. H. Jung, J. H. Lee, and Y. J. Lee, "The relationship of platelet count, mean platelet volume with metabolic syndrome according to the criteria of the American Association of Clinical Endocrinologists: a focus on gender differences," *Platelets*, vol. 23, no. 1, pp. 45–50, 2011.
- [89] E. S. Lau, S. M. Paniagua, J. S. Guseh et al., "Sex differences in circulating biomarkers of cardiovascular disease," *Journal of the American College of Cardiology*, vol. 74, no. 12, pp. 1543–1553, 2019.
- [90] F. Santilli, D. D'Ardes, M. T. Guagnano, and G. Davi, "Metabolic syndrome: sex-related cardiovascular risk and therapeutic approach," *Current Medicinal Chemistry*, vol. 24, no. 24, pp. 2602–2627, 2017.
- [91] D. Mozaffarian, "Dietary and policy priorities for cardiovascular disease, diabetes, and obesity," *Circulation*, vol. 133, no. 2, pp. 187–225, 2016.
- [92] A. Bédard, M. Riverin, S. Dodin, L. Corneau, and S. Lemieux, "Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile," *British Journal of Nutrition*, vol. 108, no. 8, pp. 1428–1434, 2012.
- [93] A. Bédard, L. Corneau, B. Lamarche, S. Dodin, and S. Lemieux, "Sex differences in the impact of the Mediterranean diet on LDL particle size distribution and oxidation," *Nutrients*, vol. 7, no. 5, pp. 3705–3723, 2015.
- [94] A. Bédard, B. Lamarche, L. Corneau, S. Dodin, and S. Lemieux, "Sex differences in the impact of the Mediterranean diet on systemic inflammation," *Nutrition Journal*, vol. 14, no. 1, 2015.
- [95] R. Baetta, M. Pontremoli, A. Martinez Fernandez, C. M. Spickett, and C. Banfi, "Proteomics in cardiovascular diseases: unveiling sex and gender differences in the era of precision medicine," *Journal of Proteomics*, vol. 173, pp. 62–76, 2018.
- [96] G. Pounis, on behalf of the Moli-sani Project Investigators, S. Costanzo et al., "Consumption of healthy foods at different content of antioxidant vitamins and phytochemicals and metabolic risk factors for cardiovascular disease in men and women of the Moli-sani study," *European Journal of Clinical Nutrition*, vol. 67, no. 2, pp. 207–213, 2013.
- [97] M. Bonaccio, A. di Castelnuovo, A. de Curtis et al., "Adherence to the Mediterranean diet is associated with lower platelet and leukocyte counts: results from the Moli-sani study," *Blood*, vol. 123, no. 19, pp. 3037–3044, 2014.
- [98] D. B. Jump, C. M. Depner, and S. Tripathy, "Omega-3 fatty acid supplementation and cardiovascular disease," *Journal of Lipid Research*, vol. 53, no. 12, pp. 2525–2545, 2012.
- [99] N. G. Kumar, D. Contaifer, P. Madurantakam et al., "Dietary bioactive fatty acids as modulators of immune function: implications on human health," *Nutrients*, vol. 11, no. 12, p. 2974, 2019.
- [100] R. Preston Mason, "New insights into mechanisms of action for omega-3 fatty acids in atherothrombotic cardiovascular disease," *Current Atherosclerosis Reports*, vol. 21, no. 1, p. 2, 2019.
- [101] W. S. Harris, "Understanding why REDUCE-IT was positive – mechanistic overview of eicosapentaenoic acid," *Progress in Cardiovascular Diseases*, vol. 62, no. 5, pp. 401–405, 2019.
- [102] L. Dong, H. Zou, C. Yuan, Y. H. Hong, D. V. Kuklev, and W. L. Smith, "Different fatty acids compete with arachidonic acid for binding to the allosteric or catalytic subunits of cyclooxygenases to regulate prostanoid synthesis," *Journal of Biological Chemistry*, vol. 291, no. 8, pp. 4069–4078, 2016.
- [103] R. N. Thota, J. J. A. Ferguson, K. A. Abbott, C. B. Dias, and M. L. Garg, "Science behind the cardio-metabolic benefits of omega-3 polyunsaturated fatty acids: biochemical effects vs . clinical outcomes," *Food & Function*, vol. 9, no. 7, pp. 3576–3596, 2018.
- [104] S. C. Larsson, N. Orsini, and A. Wolk, "Long-chain omega-3 polyunsaturated fatty acids and risk of stroke: a meta-analysis," *European Journal of Epidemiology*, vol. 27, no. 12, pp. 895–901, 2012.
- [105] M. Phang, M. L. Garg, and A. J. Sinclair, "Inhibition of platelet aggregation by omega-3 polyunsaturated fatty acids is gender specific—redefining platelet response to fish oils," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 81, no. 1, pp. 35–40, 2009.
- [106] E. C. Leigh-Firbank, A. M. Minihane, D. S. Leake et al., "Eicosapentaenoic acid and docosahexaenoic acid from fish oils: differential associations with lipid responses," *British Journal of Nutrition*, vol. 87, no. 5, pp. 435–445, 2002.
- [107] Y. Nagakawa, H. Orimo, M. Harasawa, I. Morita, K. Yashiro, and S. Murota, "Effect of eicosapentaenoic acid on the platelet aggregation and composition of fatty acid in man: a double blind study," *Atherosclerosis*, vol. 47, no. 1, pp. 71–75, 1983.
- [108] J. A. Lovegrove, S. S. Lovegrove, S. V. M. Lesauvage et al., "Moderate fish-oil supplementation reverses low-platelet, long-chain n-3 polyunsaturated fatty acid status and reduces plasma triacylglycerol concentrations in British Indo-Asians," *The American Journal of Clinical Nutrition*, vol. 79, no. 6, pp. 974–982, 2004.
- [109] M. Phang, A. J. Sinclair, L. F. Lincz, and M. L. Garg, "Gender-specific inhibition of platelet aggregation following omega-3 fatty acid supplementation," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 22, no. 2, pp. 109–114, 2012.
- [110] M. Phang, L. Lincz, M. Seldon, and M. L. Garg, "Acute supplementation with eicosapentaenoic acid reduces platelet microparticle activity in healthy subjects," *The Journal of Nutritional Biochemistry*, vol. 23, no. 9, pp. 1128–1133, 2012.
- [111] D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges, and A. Crozier, "Dietary

- (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases,” *Antioxidants and Redox Signaling*, vol. 18, no. 14, pp. 1818–1892, 2013.
- [112] H. Speer, N. M. D’Cunha, M. Botek et al., “The effects of dietary polyphenols on circulating cardiovascular disease biomarkers and iron status: a systematic review,” *Nutrition and Metabolic Insights*, vol. 12, article 117863881988273, 2019.
- [113] P. Ditano-Vázquez, J. D. Torres-Peña, F. Galeano-Valle et al., “The fluid aspect of the Mediterranean diet in the prevention and management of cardiovascular disease and diabetes: the role of polyphenol content in moderate consumption of wine and olive oil,” *Nutrients*, vol. 11, no. 11, p. 2833, 2019.
- [114] K. Yamagata, M. Tagami, and Y. Yamori, “Dietary polyphenols regulate endothelial function and prevent cardiovascular disease,” *Nutrition*, vol. 31, no. 1, pp. 28–37, 2015.
- [115] A. Serino and G. Salazar, “Protective role of polyphenols against vascular inflammation, aging and cardiovascular disease,” *Nutrients*, vol. 11, no. 1, p. 53, 2019.
- [116] I. Campesi, M. Marino, M. Cipolletti, A. Romani, and F. Franconi, “Put “gender glasses” on the effects of phenolic compounds on cardiovascular function and diseases,” *European Journal of Nutrition*, vol. 57, no. 8, pp. 2677–2691, 2018.
- [117] X. M. Liu, Y. J. Liu, Y. Huang et al., “Dietary total flavonoids intake and risk of mortality from all causes and cardiovascular disease in the general population: a systematic review and meta-analysis of cohort studies,” *Molecular Nutrition and Food Research*, vol. 61, no. 6, 2017.
- [118] A. Rees, G. Dodd, and J. Spencer, “The effects of flavonoids on cardiovascular health: a review of human intervention trials and implications for cerebrovascular function,” *Nutrients*, vol. 10, no. 12, p. 1852, 2018.
- [119] B. Ed Nignpense, K. A. Chinkwo, C. L. Blanchard, and A. B. Santhakumar, “Polyphenols: modulators of platelet function and platelet microparticle generation?,” *International Journal of Molecular Sciences*, vol. 21, no. 1, p. 146, 2020.
- [120] G. Raman, M. Shams-White, E. E. Avendano, F. Chen, J. A. Novotny, and A. Cassidy, “Dietary intakes of flavan-3-ols and cardiovascular health: a field synopsis using evidence mapping of randomized trials and prospective cohort studies,” *Systematic Reviews*, vol. 7, no. 1, p. 100, 2018.
- [121] A. J. Flammer, I. Sudano, M. Wolfrum et al., “Cardiovascular effects of flavanol-rich chocolate in patients with heart failure,” *European Heart Journal*, vol. 33, no. 17, pp. 2172–2180, 2012.
- [122] L. M. Ostertag, P. A. Kroon, S. Wood et al., “Flavan-3-ol-enriched dark chocolate and white chocolate improve acute measures of platelet function in a gender-specific way—a randomized-controlled human intervention trial,” *Molecular Nutrition and Food Research*, vol. 57, no. 2, pp. 191–202, 2013.
- [123] T. Zhou, C. Meng, and P. He, “Soy isoflavones and their effects on xenobiotic metabolism,” *Current Drug Metabolism*, vol. 20, no. 1, pp. 46–53, 2019.
- [124] S. C. Ho, J. L. F. Woo, S. S. F. Leung, A. L. K. Sham, T. H. Lam, and E. D. Janus, “Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population,” *The Journal of Nutrition*, vol. 130, no. 10, pp. 2590–2593, 2000.
- [125] D. P. Beavers, K. M. Beavers, M. Miller, J. Stamey, and M. J. Messina, “Exposure to isoflavone-containing soy products and endothelial function: a Bayesian meta-analysis of randomized controlled trials,” *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 22, no. 3, pp. 182–191, 2012.
- [126] S. H. Li, X. X. Liu, Y. Y. Bai et al., “Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials,” *American Journal of Clinical Nutrition*, vol. 91, no. 2, pp. 480–486, 2010.
- [127] L. Ma, G. Liu, M. Ding et al., “Isoflavone intake and the risk of coronary heart disease in US men and women,” *Circulation*, vol. 141, no. 14, pp. 1127–1137, 2020.
- [128] J. A. Guerrero, M. L. Lozano, J. Castillo, O. Benavente-García, V. Vicente, and J. Rivera, “Flavonoids inhibit platelet function through binding to the thromboxane A2 receptor,” *Journal of Thrombosis and Haemostasis*, vol. 3, no. 2, pp. 369–376, 2005.
- [129] A. Garrido, M. P. De la Maza, S. Hirsch, and L. Valladares, “Soy isoflavones affect platelet thromboxane A2 receptor density but not plasma lipids in menopausal women,” *Maturitas*, vol. 54, no. 3, pp. 270–276, 2006.
- [130] M. H. Gooderham, H. Adlercreutz, S. T. Ojala, K. Wähälä, and B. J. Holub, “A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men,” *Journal of Nutrition*, vol. 126, no. 8, pp. 2000–2006, 1996.
- [131] C. G. Walker, L. M. Browning, A. P. Mander et al., “Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans,” *British Journal of Nutrition*, vol. 111, no. 4, pp. 679–689, 2014.
- [132] M. Holinstat and J. Yeung, “Newer agents in antiplatelet therapy: a review,” *Journal of Blood Medicine*, vol. 33, 2012.
- [133] M. Holinstat and A. Reheman, “Dual antiplatelet therapy for PCI: are we tailored to all?,” *Thrombosis Research*, vol. 135, no. 6, pp. 1045–1046, 2015.
- [134] Antithrombotic Trialists’ (ATT) Collaboration, C. Baigent, L. Blackwell et al., “Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials,” *The Lancet*, vol. 373, no. 9678, pp. 1849–1860, 2009.
- [135] D. Capodanno, K. Dharmashankar, and D. J. Angiolillo, “Mechanism of action and clinical development of ticagrelor, a novel platelet ADP P2Y12receptor antagonist,” *Expert Review of Cardiovascular Therapy*, vol. 8, no. 2, pp. 151–158, 2014.
- [136] A. Rana, E. Westein, B. Niego, and C. E. Hagemeyer, “Shear-dependent platelet aggregation: mechanisms and therapeutic opportunities,” *Frontiers in Cardiovascular Medicine*, vol. 6, 2019.
- [137] P. M. Ridker, N. R. Cook, I.-M. Lee et al., “A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women,” *Obstetrical & Gynecological Survey*, vol. 60, no. 8, pp. 519–521, 2005.
- [138] G. Renda, G. Patti, I. M. Lang et al., “Thrombotic and hemorrhagic burden in women: gender-related issues in the response to antithrombotic therapies,” *International Journal of Cardiology*, vol. 286, pp. 198–207, 2019.
- [139] K. Grodecki, Z. Huczek, P. Scisło et al., “Gender-related differences in post-discharge bleeding among patients with

- acute coronary syndrome on dual antiplatelet therapy: a Bleeding MACS sub-study,” *Thrombosis Research*, vol. 168, pp. 156–163, 2018.
- [140] P. Cirillo, L. Di Serafino, G. Patti et al., “Gender-related differences in antiplatelet therapy and impact on 1-year clinical outcome in patients presenting with ACS: the START ANTI-PLATELET Registry,” *Angiology*, vol. 70, no. 3, pp. 257–263, 2018.
- [141] A. Mărginean, C. Bănescu, A. Scridon, and M. Dobreanu, “Anti-platelet therapy resistance – concept, mechanisms and platelet function tests in intensive care facilities,” *The Journal of Critical Care Medicine*, vol. 2, no. 1, pp. 6–15, 2016.
- [142] F. Zaccardi, D. Pitocco, P. Willeit, and J. A. Laukkanen, “Efficacy and safety of P2Y₁₂ inhibitors according to diabetes, age, gender, body mass index and body weight: systematic review and meta-analyses of randomized clinical trials,” *Atherosclerosis*, vol. 240, no. 2, pp. 439–445, 2015.
- [143] J.-Y. Chiang, S.-H. Lee, Y.-C. Chen et al., “Metabolomic analysis of platelets of patients with aspirin non-response,” *Frontiers in Pharmacology*, vol. 10, 2019.
- [144] A. Gallo, S. Galliazzo, S. Grazioli, L. Guasti, W. Ageno, and A. Squizzato, “Epidemiology and secondary prevention of ischemic stroke in patients on antiplatelet drug: a retrospective cohort study,” *Journal of Thrombosis and Thrombolysis*, vol. 48, no. 2, pp. 336–344, 2019.
- [145] E. M. K. Warlo, H. Arnesen, and I. Seljeflot, “A brief review on resistance to P2Y₁₂ receptor antagonism in coronary artery disease,” *Thrombosis Journal*, vol. 17, no. 1, 2019.
- [146] B. C. Lu, X. J. Shi, L. Liang, N. Dong, and Z. Z. Liu, “Platelet surface CD62p and serum vitamin D levels are associated with clopidogrel resistance in Chinese patients with ischemic stroke,” *Journal of Stroke and Cerebrovascular Diseases*, vol. 28, no. 5, pp. 1323–1328, 2019.