Nutrition, Supplements, and Vitamins in Platelet Function and Bleeding

Francesco Violi, MD; Pasquale Pignatelli, MD; Stefania Basili, MD

Basic, experimental, and clinical studies have provided definite evidence on the key role played by platelets in the process of atherothrombosis. Interventional trials with aspirin (a platelet COX-1 inhibitor),\(^1\) thienopyridines such as ticlopidine or clopidogrel (a platelet P2Y\(_{12}\) receptor inhibitor),\(^2\) or the combination of the 2 drugs, i.e., aspirin plus clopidogrel, reduced clinical outcomes in patients with acute coronary syndromes.\(^3\) A meta-analysis of trials with antiplatelet drugs in patients with stable atherosclerosis such as those with stable angina, peripheral arterial disease, or cerebrovascular disease confirmed the clinical efficacy of this drug category.\(^4\)

Despite the success of interventional trials, the real world of atherothrombosis is complicated, with a high rate of morbidity and mortality. Several issues related to the antiplatelet treatment may account for cardiovascular relapses. Poor compliance with an antiplatelet regimen may play a relevant role because the risk of adverse clinical outcome is higher in patients who do not adhere to aspirin treatment.\(^5\) Concomitant multiple antiatherosclerotic treatments are an important cause of poor aspirin compliance and should be taken into account in the monitoring of patients’ adherence to antiplatelet treatment.\(^6\) Insufficient antiplatelet effect by the present armamentarium may be another relevant explanation for vascular relapses;\(^7\) thus, prasugrel, a P2Y\(_{12}\) receptor antagonist more potent than clopidogrel, improved vascular outcome in patients with acute coronary syndrome.\(^8\) Even if clinical investigation of more potent antiplatelet drugs represents an important future objective to improve the prevention of cardiovascular disease, a nonpharmacological approach to lower platelet function may be another intriguing perspective.

Observational and interventional studies have demonstrated that a significant reduction in cardiovascular events might be achieved by following particular diets or using specific nutrients.\(^9\) Relative to this, it is worthwhile to mention the significant reduction in cardiovascular events observed in subjects following the Mediterranean diet,\(^10\) a diet rich in fruits and vegetables,\(^11\) or the Eskimo diet.\(^12\) A potential explanation of such success is that these diets could reduce cardiovascular events by affecting platelet function. This review focuses on the strengths and weaknesses of clinical studies assessing whether diet per se or single components of “antiatherosclerotic diets” may represent a nonpharmacological antiplatelet approach potentially usable in the prevention of atherosclerotic progression and its clinical manifestations.

Nutrition, Cardiovascular Events, and Platelets

Since the classic study by Keys et al.,\(^13\) it has been evident that people living in the South of Europe who follow the Mediterranean diet had lower incidence of cardiovascular disease compared with people living in the North of Europe. The Mediterranean diet is characterized by a high intake of fruits, vegetables, legumes, and monounsaturated fatty acids (essentially olive oil) and a moderate intake of fish and wine. The relevance of the Mediterranean diet in preventing cardiovascular disease has been documented by a large prospective study performed in 22 043 adults in Greece.\(^10\) During a median follow-up of 44 months, people who closely followed the Mediterranean diet had lower probability (−25%) of dying compared with those who adhered poorly to the diet.

The relationship between adherence to the Mediterranean diet and a reduction in mortality has been supported by a recent meta-analysis including >1 million people.\(^14\) The beneficial effect of the Mediterranean diet has been observed in healthy subjects and in those with previous cardiovascular diseases and includes a reduction in myocardial infarction and stroke.\(^15,16\)

Many nutrients in the Mediterranean diet may account for the reduction in cardiovascular events. Fruits and vegetables are thought to play an important role among them. This is consistently supported by observational studies in either healthy subjects or patients with coronary heart disease. For instance, the Nurses’ Health Study and the Health Professionals’ Follow-Up Study demonstrated that the consumption of fruits and vegetables is associated with reduced risk of coronary heart disease.\(^17\) The Indian Heart Study randomized 505 survivors of myocardial infarction to usual care or a diet rich in fruits, vegetables, nuts, and grains. After 1 year of follow-up, there were fewer cardiac events and a lower overall mortality in patients on the interventional diet.\(^18\)
Nutrition based on high fish intake has also proved to account for such a beneficial effect, a reduction in platelet function when given to healthy subjects or patients at risk of cardiovascular disease. On the contrary, many studies have analyzed whether constituents of the above-mentioned diets may affect platelet function when given to healthy subjects or patients at risk of cardiovascular disease. We review below the clinical trials assessing whether nutrients or supplements containing n-3, vitamins, or antioxidant molecules had some effect on platelet function and discuss whether they may be a tool to inhibit platelet function in clinical practice.

**n-3 and Platelet Function**

The Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico trial is one of the largest randomized controlled trials testing the effect of n-3 supplementation on cardiovascular events. In thus open-label, multicenter trial, 11,324 patients surviving a recent myocardial infarction were randomly assigned to ≥850 mg n-3 polyunsaturated fatty acids (EPA+DHA) or no supplement. After 3.5 years of follow-up, there was a 10% reduction in the combination of vascular death and nonfatal myocardial infarction. Subgroup analysis revealed the most favorable effect was for cardiovascular death (~17%). This finding was confirmed by a meta-analysis of the effect of fish oil on vascular outcomes among patients given fish oil. Among the mechanisms potentially accounting for such a beneficial effect, a reduction in platelet function has been hypothesized.

We found 21 studies analyzing the effect of fish oil or the n-3 fatty acids EPA and DHA on platelet function. We

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**Table 1. n-3 and Platelet Function**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Follow-Up</th>
<th>PA/Platelet Function/Platelet Survival</th>
<th>Platelet TxB2</th>
<th>Serum TxB2</th>
<th>Platelet Survival</th>
<th>Urinary TxB2</th>
<th>sCD40L</th>
<th>sPs</th>
<th>Platelet-Monocyte Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mori et al23</td>
<td>120 HC and AH</td>
<td>EPA + DHA=3.65 g/d vs P (randomized study)</td>
<td>12 wk</td>
<td>↓ (−7%) Collagen- and (−9%) PAF-induced PA after omega 3-fatty acids</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Agren et al23</td>
<td>55 HS</td>
<td>Control EPA vs DHA (1.68 g DHA/d)</td>
<td>15 wk</td>
<td>↓ Collagen (50 μg/mL)-induced PA in the fish diet (−36%) and the fish oil (−68%) groups</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Pirch et al23</td>
<td>26 HC</td>
<td>EPA (216 mg)/DHA (140 mg) + omega-3 fatty acids (390 mg)/LA (3480 mg/d) vs P (randomized, double-blind study)</td>
<td>6 wk</td>
<td>↑ (3%) Platelet survival (11% in-ozone-radiolabeled platelets) and ↓ (−6.7%) MDA formation in fish oil group</td>
<td>NE</td>
<td>↓ (−5.8%)</td>
<td>In fish oil group</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Woodman et al23</td>
<td>59 Hyperensive, T2DM</td>
<td>EPA (4 g/d) vs DHA (4 g/d) vs olive oil (P) (randomized, double-blind trial)</td>
<td>6 wk</td>
<td>↓ (−16.9%) Collagen (1 μg/mL)-induced PA with DHA</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Aarsetøy et al23</td>
<td>300 post-AMI (on day 4 to 6)</td>
<td>4 g/d n-3 fatty acids or corn oil (randomized study)</td>
<td>1 y</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Larson et al24</td>
<td>10 HS</td>
<td>P-OM3=4g (pilot study)</td>
<td>30 d</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Din et al24</td>
<td>14 HS</td>
<td>EPA + DHA=1g/d vs P (controlled study)</td>
<td>4 wk</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
</tbody>
</table>

PA indicates platelet aggregation; Tx, thromboxane; sCD40L, soluble CD40 ligand; sPs, soluble P selectin; HC, hypercholesterolemia; LA, linoleic acid; P, placebo; NE, not evaluated; HS, healthy subjects; AH, arterial hypertension; P-OM3, omega-3 fatty acids product; T2DM, type 2 diabetes mellitus; PUFA, polyunsaturated fatty acids; AMI, acute myocardial infarction; MDA, malondialdehyde; and ↔, no significant changes or differences.
excluded 14 studies because they were not controlled. Among the 7 controlled studies, 4 were done on healthy subjects and 3 on patients at risk of atherosclerosis (Table 1). The study population was treated with a quite different dosage of n-3; thus, the daily amount could range from as low as 1 g/d to as high as 4 g/d. Among these studies, 2 showed no effect of n-3 on platelet aggregation; conversely, 5 showed inhibition of platelet function or prolongation of platelet survival. It is of note that such an inhibitory effect was not dose related because it was observed with 1 or 4 g/d. Apparently, n-3 supplementation has no side effect because none of these studies reported adverse reaction during the follow-up.

We found 8 studies that specifically investigated the effect of α-linolenic acid on platelet function. One compared the antiplatelet effect of α-linolenic acid versus EPA + DHA. As in the case of fish oil, the studies analyzed quite different dosages of α-linolenic acid, with daily dosage ranging from as low as 0.86 g/d to as high as 5.9 g/d. The study population included healthy subjects or patients at risk of atherosclerosis. Globally considered, the studies did not consistently show an inhibitory effect of α-linolenic acid on platelet function. As in the case of fish oil, no side effects were recorded.

Together, these studies seem to suggest a different effect of fish- and plant-derived n-3 on platelet function. Thus,
Olive Oil, Oleic Acid, and Platelet Function

Because olive oil is a key component of the Mediterranean diet, several studies have been done to assess whether olive oil or its principal component, oleic acid, is capable of affecting platelet function.\textsuperscript{49–59} As far as olive oil is concerned, a daily dosage of 30 to 45 g/d has been assessed in 3 studies.\textsuperscript{49–51} Globally considered, the trials do not support an antiplatelet effect of either olive oil or oleic acid in healthy patients or those at risk of atherosclerosis. However, most studies were not controlled against placebo and included a small number of patients. Therefore, a larger sample size and more adequate study design are needed to further investigate whether olive oil or oleic acid has some effect on platelet function.

Wine, Polyphenols, and Platelet Function

Large epidemiological studies suggest the existence of an inverse relationship between moderate consumption of wine and cardiovascular risk. In one of the largest reports on this argument, Grønbaek et al.\textsuperscript{60} showed that moderate daily drinkers (3 to 5 glasses daily) had a lower risk of cardiovascular death compared with nondrinkers. This finding was confirmed by a meta-analysis that included 13 studies involving 209,418 people; thus, a 32% risk reduction of vascular disease was observed in people drinking 150 mL wine daily.\textsuperscript{61} Even if this finding may be biased by the coexistence of socioeconomic confounders potentially explaining the reduction of cardiovascular risk independently from wine intake,\textsuperscript{62} several hypotheses, including the inhibition of platelet function, have been postulated to explain such an inverse association.\textsuperscript{63} We found cross-sectional and interventional studies comparing platelet function in wine consumers versus abstainers, nonconsumers, or subjects given red or white wine over the short or long term.\textsuperscript{64–72}

Globally considered, the studies inconsistently showed that short- or long-term wine intake is associated with an inhibition of platelet function in humans.\textsuperscript{64–70} Negative results were also reported with dealkoholized wine,\textsuperscript{71,72} which is in contrast to an animal study showing that dealkoholized wine intake is associated with platelet inhibition.\textsuperscript{73} The fact that experimental studies suggested a role for the nonalcoholic components of wine in inhibiting platelet function\textsuperscript{74} raised the hypothesis that polyphenols, which are abundantly present in wine, possess an antiplatelet property. This also generated the hypothesis that the inhibition of platelet function could

with few exceptions, an inhibitory effect of fish oil or EPA + DHA would emerge in human studies; this is in agreement with animal studies that consistently showed not only an inhibitory effect of platelet aggregation\textsuperscript{43–45} but also an antithrombotic effect of fish oil supplementation.\textsuperscript{46,47} Such changes have been attributed to a reduced decrease of thromboxane A\textsubscript{2} and an increase of the nonaggregating thromboxane A\textsubscript{3}.\textsuperscript{48}

### Table 3. Vitamins and Bleeding

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Design</th>
<th>Intervention</th>
<th>Follow-Up, y</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATBC Trial\textsuperscript{110}</td>
<td>29 133 Male smokers</td>
<td>Randomized, double-blind, placebo-controlled, primary-prevention trial</td>
<td>α-Tocopherol 50 mg/d, β-Carotene 20 mg/d, α-Tocopherol + β-carotene Placebo</td>
<td>5–8</td>
<td>↑ Fatal hemorrhagic stroke (mortality rate per 10 000 person-y=7.8) in α-tocopherol vs non-α-tocopherol group</td>
</tr>
<tr>
<td>ATBC Trial\textsuperscript{111}</td>
<td>28 519 Male smokers free of stroke at baseline</td>
<td>Randomized, double-blind, placebo-controlled, primary-prevention trial</td>
<td>α-Tocopherol 50 mg/d, β-Carotene 20 mg/d, α-Tocopherol + β-carotene Placebo</td>
<td>6 (median)</td>
<td>↑ Subarachnoid hemorrhage in α-tocopherol vs non-α-tocopherol group (RR=1.50; 95% CI, 0.97–2.32; ( P =0.07 ))</td>
</tr>
<tr>
<td>Physicians’ Health Study II\textsuperscript{112}</td>
<td>14 641 US male physicians</td>
<td>Randomized, double-blind, placebo-controlled, factorial trial</td>
<td>Vitamin E 400 IU every other day, Vitamin C 500 mg/d Placebo</td>
<td>8 (mean)</td>
<td>↑ Hemorrhagic stroke in active vitamin E group vs placebo vitamin E group (HR, 1.74; 95% CI, 1.04–2.91; ( P =0.04 ))</td>
</tr>
<tr>
<td>Women’s Health Study\textsuperscript{113}</td>
<td>39 876 Apparently healthy US women</td>
<td>Randomized, double-blind, placebo-controlled, 2×2 factorial trial</td>
<td>Vitamin E 600 IU every other day Placebo</td>
<td>10.1 (mean)</td>
<td>↔ No increase in hemorrhagic stroke associated with vitamin E use</td>
</tr>
<tr>
<td>Women’s Antioxidant Cardiovascular Study\textsuperscript{114}</td>
<td>8171 Female health professionals at increased risk</td>
<td>Randomized, double-blind, placebo-controlled trial</td>
<td>Vitamin C 500 mg/d Placebo</td>
<td>9.4 (mean)</td>
<td>↔ No significant increase in hemorrhagic stroke associated with vitamin E or β-carotene use</td>
</tr>
<tr>
<td>HOPE\textsuperscript{115}</td>
<td>9541 At high risk for cardiovascular events</td>
<td>Double-blind, randomized trial with a 2×2 factorial design</td>
<td>Vitamin E from natural sources 400 IU daily Placebo</td>
<td>4.5 (mean)</td>
<td>↔ No increase in hemorrhagic stroke associated with vitamin E use</td>
</tr>
</tbody>
</table>

ATBC indicates Alpha-Tocopherol, Beta Carotene Cancer; RR, relative risk; CI, confidence interval; HR, hazard ratio; and HOPE, Heart Outcomes Prevention Evaluation.
Several sources of polyphenols have been investigated to test their antiplatelet effect in human. Grape juice, berries, pomegranate, apples, garlic, onion, tea, cocoa, tomato, and garlic have been tested to assess whether polyphenol-rich nutrients may affect platelet function differently because of their polyphenol content. Additionally, because an experimental study provided evidence of an antithrombotic effect mediated by half-maximal effective concentration; and blood concentration.

Table 4. Vitamin E and Platelet Function in Subjects With Atherosclerotic Risk Factors

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Intervention/Design</th>
<th>Follow-Up</th>
<th>PA</th>
<th>TxB2</th>
<th>sCD40L</th>
<th>sPs</th>
<th>Platelet NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gisinger et al122</td>
<td>T1DM</td>
<td>DL-α-tocopherol acetate (400 mg/d) vs placebo (double-blind, crossover study)</td>
<td>4 wk</td>
<td>NE</td>
<td>↓ (−67%) Collagen (0.15 μg) platelet TxB2 in DL-α-tocopherol acetate group</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Jain et al123</td>
<td>T1DM</td>
<td>DL-α-tocopherol (100 IU/d) vs placebo (double-blind, controlled study)</td>
<td>3 mo</td>
<td>NE</td>
<td>↓ (−51%) Serum TxB2 in DL-α-tocopherol group</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Davi et al124</td>
<td>T2DM</td>
<td>L-α-tocopherol acetate (600 mg/d) (open study)</td>
<td>2 wk</td>
<td>NE</td>
<td>↓ (−43%) Urinary TxB2</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Clarke et al125</td>
<td>T2DM</td>
<td>α-Tocopherol (500 mg) vs γ-tocopherol-rich compound daily (randomized, controlled study)</td>
<td>6 wk</td>
<td>NE</td>
<td>↔ Serum and Urinary TxB2</td>
<td>↔</td>
<td>↔</td>
<td>NE</td>
</tr>
<tr>
<td>Vignini et al126</td>
<td>T2DM</td>
<td>Vitamin E 500 IU/d (open study)</td>
<td>10 wk</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>↑ At the 5th (−20%) and 10th (−50%) wk</td>
</tr>
<tr>
<td>Patrignani et al127</td>
<td>Moderate cigarette smokers</td>
<td>Vitamin E 300, 600, or 1200 mg/d vs placebo (randomized, double-blind study)</td>
<td>3 wk</td>
<td>NE</td>
<td>↔ Serum TxB2 and urinary TxB2</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Williams et al128</td>
<td>HC</td>
<td>Vitamin E 400 IU/d vs placebo (nonrandomized, controlled study)</td>
<td>6 wk</td>
<td>↑ (132%) EC50 thrombin-induced PA with vitamin E</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
</tbody>
</table>

HS indicates healthy subjects; HC, hypercholesterolemic patients; T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; sCD40L, soluble CD40 ligand; PA, platelet aggregation; sPs, soluble P-selectin; NE, not evaluated; Platelet NO, platelet nitric oxide production; urinary TxB2, 11-dehydro-TxB2 excretion; EC50, half-maximal effective concentration; and ↔, no significant changes or differences.

Contribute to the reduction in cardiovascular events observed after high polyphenol intake.75

Several sources of polyphenols have been investigated to test their antiplatelet effect in human. Grape juice, berries, pomegranate, apples, garlic, onion, tea, cocoa, tomato, and garlic have been tested to assess whether they possess antiplatelet activity.76–100 Data obtained with grape juice and cocoa (Table 2) seem to show an antiplatelet effect more consistently than other sources of polyphenols. Thus, inhibition of platelet function was seen in all the studies of grape juice and in all but 1 study86 with chocolate. These different findings may be related to the concentrations of polyphenols in the source of polyphenols investigated. This is likely to represent a crucial issue because the concentration of polyphenols in human blood seems to influence platelet function.99 Accordingly, an inhibitory effect was observed with blood concentration >5 μmol/L,99,103 which is consistent with in vitro study showing that 5 to 10 μmol/L polyphenols inhibits platelet recruitment via increase of nitric oxide production.101,102 On the basis of these considerations, pharmacokinetic and pharmacodynamic studies should be done to assess whether polyphenol-rich nutrients may affect platelet function differently because of their polyphenol content. Additionally, because an experimental study provided evidence of an antithrombotic effect mediated by prolongation of primary hemostasis,103 it is crucial to evaluate whether polyphenol supplementation increases the risk of bleeding.

Vitamins

Antioxidant vitamins, particularly vitamin E, have been given in primary and secondary interventional trials to see whether they can prevent cardiovascular events. The scientific background of these trials stems from the oxidative hypothesis of atherosclerosis suggesting that oxidative stress has a pivotal role in the initiation and progression of atherosclerosis.104 Vitamin E is a chain-breaking antioxidant that prevents the propagation of free radical reaction.105 A meta-analysis of these trials demonstrated that vitamin E supplementation does not favorably influence vascular outcome in patients with a low or high risk of vascular accidents.106 The reason for this negative finding may merely depend on the fact that vitamin E supplementation has no impact on the progression of atherosclerosis, but methodological issues related to the design of the trials cannot be excluded. In particular, none of the trials used markers of oxidative stress as entry criteria,107 nor was baseline antioxidant status considered a prerequisite for inclusion in the trial. In the Heart Protection Study, for instance, baseline levels of vitamin E were in the normal
range, suggesting that patients included in this trial probably did not need any antioxidant treatment.\textsuperscript{108} We recently underscored that at least one third of the patients included in the trials with vitamin E assumed that statins are also antioxidants, and this could have masked the effects of vitamin E.\textsuperscript{109} Finally, the analysis of the trials with vitamin E was complicated by concomitant adverse side effects related to bleeding in the brain. Even if not all of the trials with vitamins examined this issue, there is evidence that cerebral hemorrhage may complicate vitamin supplementation. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study\textsuperscript{110,111} was one of the first showing that in male smokers 50 mg/d vitamin E or 20 mg/d $\beta$-carotene increased the risk of hemorrhagic stroke (Table 3). Notably, the increase in bleeding tendency in the brain circulation was provided.

The increase in bleeding tendency in the brain circulation could be attributed to an antiplatelet effect of vitamin E. The interplay between vitamin E and platelet function has been tested in healthy subjects or in patients at risk of cardiovascular disease such as those with diabetes or dyslipidemia. Among the 6 trials performed in healthy subjects,\textsuperscript{116–121} the amount of daily doses of vitamin E ranged from 200 to 2000 IU, and follow-up lasted from 2 to 5 weeks. The results are not consistent with an inhibitory effect of vitamin E supplementation.

As shown in Table 4, among the 7 trials\textsuperscript{122–128} in patients at risk of cardiovascular disease, 5 were performed in patients with diabetes mellitus. In these 5 trials,\textsuperscript{122–126} the daily amount of vitamin E ranged from 100 to 600 IU, and follow-up lasted from 2 to 10 weeks. An antiplatelet effect was observed in all but 1 study; however, that study included patients who were also taking aspirin.\textsuperscript{125} In the remaining 2 studies performed in smokers or patients with dyslipidemia, inhibition of or no change in platelet function, respectively, was reported.\textsuperscript{127,128}

Globally considered, trials exploring the effect of vitamin E on platelet function inconsistently showed an inhibitory property in healthy subjects. The positive data obtained in diabetic patients are of some interest but are flawed by several methodological reasons, including nonsystematic analysis of oxidative stress and antioxidant status, open design, and small sample size.

The consequence of this argument is that inhibition of platelet function can hardly be considered a key element accounting for bleeding complications occurring after vitamin E administration. An alternative possibility is provided by a recent study showing that in vivo vitamin E exerts an anticoagulant effect because its administration is associated with reduced venous thromboembolism.\textsuperscript{129} Thus, vitamin E interferes with vitamin K–dependent clotting factor activation and inhibits the monocyte expression of tissue factor, a glycoprotein that converts factor X to Xa.\textsuperscript{130}

Vitamin C is another antioxidant molecule that has been investigated to see whether it possesses antiplatelet activity. Vitamin C is a direct antioxidant because it quenches superoxide radicals.\textsuperscript{131} On platelet activation, platelets release superoxide radicals that in turn seem to be responsible for propagating platelet activation.\textsuperscript{132} Quenching superoxide radicals may therefore represent a tool to investigate the validity of such an assumption. Four studies\textsuperscript{133–135} investigated whether short-term treatment with vitamin C, given either intravenously or orally, affects platelet function (Table 5). Globally considered, the studies showed inhibition of platelet function after intravenous\textsuperscript{133,135} and oral\textsuperscript{134,136} administration.
Platelet adhesion
Glycoprotein VI binds to the collagen of the exposed vessel wall and glycoprotein lb-V-IX binds to collagen-bound von Willebrand factor resulting in adhesion of platelets to the site of injury.

Platelet activation
Activation of platelets bound to the injured vessel wall causes a conformational transition in glycoprotein lb/llla (2llb3) that increases its affinity for fibrinogen and von Willebrand factor. Three outside-in signals of particular relevance are mediated by adenosine diphosphate (ADP), thrombin and TXA2.

Platelet recruitment
The recruitment phase depends upon the release of pro-aggregating substances able to induce the activation of new platelets approaching the site of thrombus growth. Among them ADP and superoxide anion (O2-). O2- is a functionally relevant scavenger of nitric oxide (NO) produced by the NADPH oxidase system. The scavenging of NO by O2- prevents its participation in the late disaggregation of thrombus. Molecules such as CD40 ligand (CD40L) participate in the platelet–platelet synapse to create a protected environment in the intersices of the clot that stabilizes the thrombus. Polyphenols inhibit NADPH oxidase-dependent O2- so enhancing NO bioavailability resulting in inhibition of platelet recruitment.

Figure. Platelet adhesion, activation, and recruitment.
of vitamin C. Although the effect achieved after vitamin C given orally is difficult to explain, the inhibition observed after intravenous vitamin C administration raises important questions about the role of reactive oxygen species in platelet activation. Thus, in vivo vitamin C behaves as an antioxidant only if supraphysiological concentration, ie, ≈1 mmol/L, is reached; this concentration is achievable only when vitamin C is given intravenously. It is therefore plausible that intravenous administration of vitamin C actually exerted an antioxidant effect that resulted in platelet reactive oxygen species inhibition and ultimately reduced platelet activation. Further study, however, is needed to support this hypothesis.

**Mixture of Other Vitamins**

The effect of a mixture of vitamins such as vitamins E, C, and β-carotene and/or selenium or polyunsaturated fatty acids with n-3 has been investigated in 5 studies. The studies were conducted predominantly in healthy subjects or in patients with dyslipidemia and had a randomized controlled design. Overall, an inhibitory effect on platelet function was observed in all but 1 study. Folic acid, vitamin B₆, and vitamin B₁₂ have also been thought to affect platelet function, but the results of interventional trials consistently did not support this hypothesis. Studies have been done in healthy subjects or in patients at risk of dementia. The study by van Wyk et al showed a weak inhibitory effect on agonist-induced platelet aggregation by 200 mg/d vitamin B₆; the other studies provided negative results.

**Conclusions**

Although epidemiological and interventional studies have demonstrated that diets such as the Mediterranean or Eskimo diet prevent cardiovascular disease, it is unclear whether these diets may actually affect platelet function. Appropriate study design and laboratory tests are needed to explore this issue in the future. There is evidence, however, that nutrients such as fish oil and polyphenol-rich nutrients may exert an antiplatelet activity. As far as fish oil is concerned, the majority of the ex vivo studies consistently showed an inhibition of platelet function. The plausibility of such an effect is based on the assumption that n-3 accumulation on platelet membrane results in lowering platelet thromboxane A₂ formation (see the Figure). As far as polyphenol-rich nutrients are concerned, ex vivo studies with grape juice and chocolate suggest a potential antiplatelet effect. As in the case of fish oil, these findings are biologically plausible because polyphenols possess an antioxidant property. Thus, they inhibit NADPH oxidase–dependent platelet superoxide anion formation or enhance platelet nitric oxide generation and/or bioactivity; both of these effects might have potential biological implications by modulating platelet recruitment (see the Figure). Despite this, methodological weaknesses related to study design, sample size, and follow-up duration and a lack of pharmacokinetic and pharmacodynamic studies preclude definite conclusions.

Therefore, at this moment, any nutrient or supplements should not be considered an antiplatelet tool potentially usable for clinical purpose in healthy subjects or in patients at risk of cardiovascular disease. Conversely, careful attention should be given to the bleeding complications that may potentially occur after administration of the supplements to male subjects.

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**Disclosures**

None.

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