

openheart Targeting aspirin resistance with nutraceuticals: a possible strategy for reducing cardiovascular morbidity and mortality

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CLINICAL IMPACT OF ASPIRIN RESISTANCE

The failure of daily aspirin therapy to achieve an adequate or 'normal' suppression of platelet aggregation, as assessed *ex vivo*, is known as 'aspirin resistance'.^{1,2} A substantial fraction of patients classified as aspirin-resistant are in fact poorly compliant.³ In other cases, an increase in platelet turnover, often seen in association with systemic inflammation, as found in smokers and patients with diabetes, may render a once-daily administration schedule inadequate.^{2,4-6} (Administering aspirin twice daily can result in greater platelet inhibition but may increase the risk for gastrointestinal bleeding.) When adverse pharmacokinetic factors impede the delivery of aspirin to platelets, an increase in dose can be helpful.^{7,8} Concurrent administration of ibuprofen or other cyclooxygenase-1 (COX-1) inhibitors may prevent aspirin from acetylating the active site of COX-1.⁹ But in some patients, even when platelet cyclooxygenase is fully inhibited, platelet aggregation remains anomalously high; this might be described as inherent aspirin resistance. Inherent aspirin resistance presumably reflects genetic or metabolic factors that alter the expression or function of platelet proteins such that platelets can aggregate effectively in the absence of thromboxane.

Although low-dose daily aspirin regimens reduce the risk for cardiovascular events by about 25% in patients with cardiovascular disease,¹⁰ meta-analyses found that subjects who were resistant to ongoing aspirin therapy, as opposed to those who were sensitive, are about three times more likely to experience cardiovascular events.^{11,12} This greatly increased risk is disproportionate to the benefit achievable with aspirin treatment, and evidently reflects the fact that aspirin resistance is serving as a marker for

metabolic factors, which themselves greatly increase cardiovascular risk. Nonetheless, there is strong evidence that intensified platelet-stabilising therapy can markedly improve outcomes in patients diagnosed with aspirin resistance. A number of controlled trials have defined groups of patients who are resistant to aspirin-clopidogrel therapy, and have randomised them to either continue with this standard care or to receive tailored platelet-stabilising regimens intended to achieve better control of platelet aggregation (entailing dosage increases or addition of additional agents such as integrin alpha-IIb beta-3 antagonists). A recent meta-analysis of such trials found that risk for subsequent death or stent occlusion was only one-quarter as great in patients receiving tailored therapy (OR=0.25, 95% CI 0.13 to 0.49), and risk for total vascular events was only 40% as high (OR=0.40, 95% CI 0.20 to 0.77).¹³ Hence, additional or intensified measures for stabilising platelets appear to have important life-saving efficacy in aspirin-resistant patients.

The possibility of employing safe nutraceutical measures for this purpose should be considered. Agents that may have potential in this regard include the following:

Spirulina/Phycocyanin: targeting NADPH oxidase

The Nox2-dependent form of NADPH oxidase is markedly activated when platelets interact with collagen via their chief collagen receptor, glycoprotein VI (GPVI). This event is the initial stimulus to thrombus formation when arterial plaque bursts and platelets are thereby exposed to collagen in the subendothelial ground substance. Interaction of collagen with GPVI leads to a series of intracellular tyrosine phosphorylation reactions, catalysed by an Src-like kinase and Syk, that induce formation of a signalling



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complex centred around the protein 'linker for activated T cells' (LAT).¹⁴ This complex confers an activating phosphorylation on phospholipase C-gamma, which, by generating diacylglycerol and inosine-1,4,5-trisphosphate, induces a spike in intracellular free calcium as well as activation of protein kinase C (PKC), key triggers for platelet aggregation.¹⁵ The concurrent activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase)—likely downstream from PKC activation—serves to potentiate this signalling pathway by generating hydrogen peroxide in the microenvironment of the GPVI-LAT signalling complex; this hydrogen peroxide oxidises active site cysteine groups in the tyrosine phosphatase SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase), reversibly inhibiting it, and thereby prolonging the half-lives of the tyrosine phosphorylations which SHP-2 targets.^{16–18} Studies show that agents that inhibit Nox2 activity decrease the aggregatory response of platelets to collagen exposure; moreover, platelets that are genetically deficient in Nox2 are less responsive to collagen.^{19–21} Conversely, platelets deficient in peroxiredoxin II or glutathione peroxidase activity are hyper-responsive to collagen.^{16–22} In C57BL/6J mice, susceptibility to induced carotid or venous thrombosis increases during ageing, a phenomenon associated with increased expression of NADPH oxidase components. When these mice are bioengineered to overexpress glutathione peroxidase, this age-related increase in thrombotic activity is abolished; treatment with the NADPH oxidase inhibitor apocynin has a similar effect.²³ Platelets from patients determined to be aspirin-resistant showed greater expression of NADPH oxidase components and greater NADPH oxidase activity when stimulated; the NADPH oxidase inhibitors apocynin and diphenyleneiodonium (DPI) diminished the aggregatory responses of these platelets to collagen and epinephrine, whereas they had little effect on platelets from aspirin-sensitive patients.²⁴

Much remains to be learnt regarding the full impact of NADPH oxidase activation on platelet function. Other physiological agonists that promote platelet aggregation—thrombin, thromboxane, epinephrine and (adenosine diphosphate (ADP)—signal via G-protein mechanisms (as opposed to tyrosine phosphorylation); they provoke at most a mild activation of NADPH oxidase.²⁵ Nonetheless, some studies report that NADPH oxidase inhibition blunts the response of platelets to thrombin or epinephrine; there seems to be agreement that such inhibition fails to influence the proaggregatory impact of ADP.^{21–26–27} How superoxide generation influences thrombin and epinephrine signalling remains unclear. Potentially, NADPH oxidase-generated superoxide could influence platelet function by interfering with the antiaggregatory impact of nitric oxide (NO) (by scavenging NO or promoting uncoupling of NO synthase, as discussed below). Also, superoxide can interact spontaneously with arachidonic acid to generate F2-isoprostanes, which can promote platelet aggregation by serving as agonists for

the thromboxane receptor (thereby bypassing the inhibitory impact of aspirin on thromboxane synthesis).^{28–30}

These considerations suggest that targeting Nox2-dependent NADPH oxidase complexes might be a worthwhile strategy for stabilising platelets, particularly in patients with aspirin resistance.¹⁹ In that regard, the administration of potent doses of lipophilic statins such as atorvastatin or rosuvastatin can exert a platelet-stabilising effect within 2 hours, and this effect may reflect NADPH oxidase inhibition; statins can downregulate NADPH oxidase activity by suppressing isoprenylation and membrane association of Rac1, an essential component of Nox2-dependent NADPH oxidase.^{31–35}

Nutraceutical strategies that target Nox2 may also represent a practical option for platelet stabilisation. Free bilirubin functions physiologically within cells to inhibit certain NADPH oxidase complexes, including those that are Nox2-dependent^{36–39}; it therefore is not surprising that exposure to high physiological levels of free bilirubin has been reported to decrease collagen-triggered platelet aggregation.⁴⁰ (As might be expected, bilirubin did not influence the aggregatory response to ADP.) This phenomenon may contribute to the relative protection from cardiovascular events enjoyed by individuals with high-normal plasma levels of free bilirubin, including people with Gilbert syndrome.^{41–43} Phycocyanobilin (PhyCB), a protein-bound chromophore in cyanobacteria such as spirulina, is a close chemical relative of bilirubin, and has been found to share bilirubin's ability to inhibit NADPH oxidase complexes, likely explaining the profound antioxidant/anti-inflammatory effects of orally administered spirulina or PhyCB-enriched spirulina extracts in rodent studies.^{44–47} Hence, adequate intakes of spirulina or of PhyCB-rich extracts may have clinical potential as a platelet-stabilising strategy. Indeed, exposure of platelets to phycocyanin, the spirulina protein, which features PhyCB as a covalently attached chromophore, has been reported to reduce their responsiveness to collagen.^{48–49}

High-dose biotin: mimicking NO

NO produced by healthy vascular endothelium has a platelet-stabilising effect. Moreover, platelets express the endothelial isoform of nitric oxide synthase (eNOS), which is activated by the increase in cytoplasmic free calcium as well as the PI3K/Akt activation associated with platelet aggregation; hence, the NO produced within stimulated platelets acts as a feedback brake on aggregation.⁵⁰ The effect of NO in this regard is mediated by direct stimulation of the soluble guanylate cyclase (sGC), resulting in increased synthesis of cyclic guanosine monophosphate (cGMP). The consequent rise in platelet cGMP boosts the activity of cGMP-dependent protein kinase I (cGKI), which then phosphorylates a protein (IRAG) associated with the inositol trisphosphate receptor in the platelet endoplasmic reticulum (ER), diminishing the ability of inositol trisphosphate to provoke release of calcium from the ER, and thereby

opposing aggregation.^{51–52} Additionally, cGKI may work in an additional, still poorly understood way to oppose ADP-mediated activation of Rap1b, the G-protein that enables platelet aggregation by activating the fibrinogen-binding activity of integrin alpha-IIb beta-3.⁵³

Not surprisingly, agents capable of mimicking and potentiating NO's activation of sGC have platelet-stabilising activity.^{54–57} In this regard, supraphysiological concentrations of the vitamin biotin can directly stimulate sGC activity, and orally administered high-dose biotin exerts antihypertensive effects in rats that reflect this stimulation of sGC.^{58–60} Since biotin is well tolerated in daily doses as high as 300 mg, it may have clinical potential as a platelet-stabilising agent.^{61–63}

Citrulline and high-dose folate: restoring coupling of eNOS

When vascular endothelium is under chronic oxidative stress—as it frequently is in patients at increased cardiovascular risk—eNOS can become ‘uncoupled’ owing to oxidation of its cofactor tetrahydrobiopterin (BH4) and/or increased production of asymmetric dimethylarginine (ADMA); the uncoupled enzyme produces superoxide rather than NO.^{64–67} Peroxynitrite, which evolves from the spontaneous interaction of superoxide and NO, is a key mediator of BH4 oxidation.⁶⁶ This uncoupling of eNOS might also occur in platelets, either owing to elevated plasma levels of ADMA or oxidation of platelet tetrahydrobiopterin.^{68–70} Oxidation of platelet BH4 may be common in patients with unstable angina, possibly reflecting repeated episodes of oxidative stress triggered by interaction of platelets with the coronary subendothelium.^{70–71} The platelets of smokers and patients with diabetes and metabolic syndrome may also be under chronic oxidative stress. In these conditions, platelet production of NO has been reported to be subnormal and superoxide production elevated, likely reflecting eNOS uncoupling.^{72–76} Hyperglycaemia can boost mitochondrial production of superoxide in diabetic platelets.⁷⁷ The excess exposure to free fatty acids and glucose typically seen in type 2 diabetes and metabolic syndrome may activate PKC via increased diacylglycerol synthesis; PKC, in turn, can stimulate NADPH oxidase activity.^{78–79} Likewise, semistable toxins in cigarette smoke such as acrolein can stimulate PKC, boosting oxidative stress.^{80–82} Not surprisingly, patients with diabetes, metabolic syndrome or tobacco addiction are more likely to be classified as aspirin-resistant, and aspirin therapy appears to have a limited impact on risk for coronary events in patients with diabetes.^{83–93} In oxidatively stressed platelets, aspirin therapy leads to an increase in isoprostane production that would be expected to partially offset the benefit stemming from inhibition of thromboxane synthesis.⁹⁴

Administration of citrulline in multigram daily doses can oppose ADMA-mediated uncoupling of eNOS by boosting intracellular levels of arginine.^{95–98} High-dose folate promotes restoration of normal levels of tetrahydrobiopterin in oxidatively stressed endothelium. This

reflects the ability of intracellular reduced folates to scavenge peroxynitrite-derived radicals, thereby protecting BH4 from oxidation; moreover, high-dose folate boosts endothelial expression of dihydrofolate reductase, an enzyme that can reduce oxidised BH4 (dihydrobiopterin) back to BH4.^{99–103} It is as yet unknown whether high-dose folate can induce dihydrofolate reductase in megakaryocytes (and hence increase its expression in platelets). In any case, when eNOS is uncoupled in vascular endothelium and/or platelets, administration of citrulline and of high-dose folate may help to restore physiologically appropriate production of NO,¹⁰⁴ although the direct impact of high-dose folate on platelets may hinge to some extent on its capacity to boost platelet dihydrofolate reductase activity. These agents would not be expected to influence platelet function in healthy subjects whose eNOS activity is properly coupled.

N-acetylcysteine: boosting glutathione levels

Like reduced folates, reduced glutathione can scavenge peroxynitrite-derived radicals, thereby protecting BH4 from oxidation.^{105–107} Indeed, in patients with type 2 diabetes, daily parenteral administration of glutathione was found to increase the eNOS activity of their platelets.¹⁰⁸ Moreover, through interaction with glutathione peroxidase and glutaredoxin, glutathione can oppose the signalling effects of hydrogen peroxide, either by eliminating hydrogen peroxide or by rereducing oxidised cysteinyl groups.^{109–111} Cysteine availability is rate-limiting for glutathione synthesis, and tissue glutathione levels can be boosted by increased cysteine intake, most conveniently achieved with supplemental N-acetylcysteine (NAC).^{112–115} Tissue glutathione levels tend to decline with ageing, reflecting inducibility of the rate-limiting enzyme for glutathione synthesis, gamma-glutamyl synthetase; youthful levels of glutathione can be restored with NAC supplementation.^{113–116–118} Although the impact of NAC ingestion on platelet reactivity *in vitro* or *ex vivo* has received little study, NAC concentrations comparable to those achievable in plasma with oral administration have been reported to decrease oxidant stress in human platelets, and decrease their aggregability in response to thrombin and ADP; this effect is contingent on conversion of NAC to glutathione.^{119–120} The impact of supplemental NAC on platelet function *ex vivo* merits study, most notably in patients such as those with diabetes, whose platelets may be under increased oxidative stress.

Glycine: hyperpolarising platelet membranes

Glycine-gated chloride channels are expressed by various tissues, including platelets.^{121–126} High-normal plasma levels of glycine boost the open probability of these channels, and in most tissues this induces an influx of chloride ions, leading to hyperpolarisation of the plasma membrane. Notably, platelets appear to express such channels, and activation of this channel with glycine reduces the responsiveness of platelets to both collagen and ADP.¹²⁶ Indeed, previous studies have shown that

plasma membrane potential can regulate platelet responsiveness; most such studies, although not all,¹²⁷ have concluded that depolarised platelets are sensitised to aggregation provoked by collagen, thrombin, ADP and epinephrine—although depolarisation per se does not increase calcium influx or elevate intracellular levels of free calcium.^{128–131} Analogously, platelets are responsive to endothelium-derived hyperpolarising factor, which suppresses ADP-induced platelet adhesion to endothelial cells.¹³² How membrane polarisation influences platelet activation remains unclear. When rats were fed glycine-enriched diets (2.5%–5.0%), the amplitude of platelet aggregation *ex vivo* in whole blood, in response to collagen or ADP, was reduced by approximately 50%.¹²⁶ Hence, dietary glycine supplementation, which has been found to be feasible and well tolerated, may have potential as a platelet-stabilising strategy.

Moreover, it should be noted that glycine is a substrate for glutathione synthesis, and supplemental glycine may amplify the impact of concurrent supplemental NAC on tissue glutathione levels.^{118 133}

Taurine: boosting hydrogen sulfide production

There are several reports that supplemental taurine can exert a platelet-stabilising effect in normally nourished humans and rats^{134–137}; however, a null effect was seen in one clinical study.¹³⁸ How taurine might influence platelet function is unclear. A recent clinical study has found that plasma concentrations of hydrogen sulfide (H₂S) are markedly enhanced by taurine supplementation (1.6 g daily); concurrent rodent studies suggest that this phenomenon reflects increased vascular induction of enzymes that generate H₂S.¹³⁹ There is clear evidence that H₂S has a stabilising effect on platelets; the basis of this effect remains unclear, and it does not appear to reflect an upregulation of NO bioactivity.^{140–144} Future studies assessing the impact of dietary taurine on platelet function should evaluate the possible contribution of H₂S production to any platelet-modulatory effects observed.

Long-chain omega-3 fats: more than thromboxane antagonists

Decades ago, the prolonged bleeding times and superior cardiovascular health of Eskimos following their traditional lifestyle motivated the first studies demonstrating that diets high in long-chain omega-3s from fish oil could reduce the aggregability of platelets.^{145–147} This phenomenon was first attributed to the ability of eicosapentaenoic acid (EPA) to compete with arachidonic acid for access to COX-1, diminishing thromboxane synthesis.^{145 147} However, it was soon discovered that the antithrombotic impact of omega-3-rich fish is complementary to that of aspirin, implying that thromboxane antagonism is not the only mechanism responsible for the antiaggregatory impact of fish oil.^{148–151} Enrichment of platelet membrane lipids with docosahexaenoic acid (DHA) has been reported to decrease the aggregatory response to collagen.^{152–154} The clinical impact

of DHA supplementation per se on platelet function is the subject of conflicting reports.^{155 156} How DHA might influence platelet function remains unclear. The impact of long-chain omega-3 on platelet response to collagen is notable—after 28 days of supplementation with 3.4 g EPA+DHA daily, the aggregatory response to collagen was inhibited by about 50%.¹⁵⁰

SUMMING UP

A significant proportion of patients at risk for thrombotic episodes fail to achieve an adequate control of platelet aggregability when placed on aspirin therapy. These individuals are at greatly increased risk for cardiovascular events—in large part because of the metabolic factors that destabilise their platelets—but clinical studies demonstrate some ancillary measures that promote greater platelet stability can notably decrease their risk. Nutraceutical measures, because of their relative safety, affordability and broader protective metabolic impacts, may have particular merit for this purpose.

Nox2-dependent superoxide production, which plays a key role in collagen-triggered GPVI signalling and acts in other ways to promote aggregation, may be suppressible with the spirulina chromophore PhyCB. Platelet exposure to episodic or chronic oxidative stress, associated with diabetes, metabolic syndrome, acute coronary syndrome and smoking, can uncouple platelet eNOS, diminishing the platelet-stabilising activity of NO while further adding to the burden of oxidative stress. Supplemental citrulline, high-dose folate and NAC may have potential for recoupling eNOS activity in platelets. High-dose biotin should mimic the platelet-stabilising actions of NO by directly stimulating sGC, and supplemental taurine may help to stabilise platelets by boosting production of H₂S. The platelet-stabilising activity of glycine may reflect hyperpolarisation of platelet membrane potential, which downregulates platelet aggregation for unknown reasons. Long-chain omega-3 fatty acids, while decreasing platelets production of thromboxane, appear to work in additional ways to stabilise platelets. These measures can be expected to be safe and reasonably well tolerated. Functional foods providing at least several of the agents discussed here may ultimately represent a feasible and practical strategy for optimising platelet activity with nutraceuticals. Such foods moreover may contribute more generally to vascular and metabolic health.

Contributors All authors contributed to the final manuscript.

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REFERENCES

- Han YL. Aspirin resistance in coronary heart disease: Current understandings and strategies. *J Transl Int Med* 2016;4:7–10.
- Floyd CN, Ferro A. Mechanisms of aspirin resistance. *Pharmacol Ther* 2014;141:69–78.
- Dawson J, Quinn T, Rafferty M, et al. Aspirin resistance and compliance with therapy. *Cardiovasc Ther* 2011;29:301–7.
- Larsen SB, Grove EL, Kristensen SD, et al. Reduced antiplatelet effect of aspirin is associated with low-grade inflammation in patients with coronary artery disease. *Thromb Haemost* 2013;109:920–9.
- Di Minno MN, Lupoli R, Palmieri NM, et al. Aspirin resistance, platelet turnover, and diabetic angiopathy: a 2011 update. *Thromb Res* 2012;129:341–4.
- Paikin JS, Hirsh J, Ginsberg JS, et al. Multiple daily doses of acetyl-salicylic acid (ASA) overcome reduced platelet response to once-daily ASA after coronary artery bypass graft surgery: a pilot randomized controlled trial. *J Thromb Haemost* 2015;13:448–56.
- Bode-Böger SM, Böger RH, Schubert M, et al. Effects of very low dose and enteric-coated acetylsalicylic acid on prostacyclin and thromboxane formation and on bleeding time in healthy subjects. *Eur J Clin Pharmacol* 1998;54:707–14.
- DiNicolantonio JJ. Aspirin 50 versus 100 mg: a case of wrong dosing. *Am J Cardiol* 2011;108:1840.
- Catella-Lawson F, Reilly MP, Kapoor SC, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med* 2001;345:1809–17.
- Anon. Collaborative overview of randomised trials of antiplatelet therapy-I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. *BMJ* 1994;308:81–106.
- Krasopoulos G, Brister SJ, Beattie WS, et al. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. *BMJ* 2008;336:195–8.
- Li J, Song M, Jian Z, et al. Laboratory aspirin resistance and the risk of major adverse cardiovascular events in patients with coronary heart disease on confirmed aspirin adherence. *J Atheroscler Thromb* 2014;21:239–47.
- Li J, Jian Z, Song M, et al. Tailored antiplatelet therapy and clinical adverse outcomes. *Heart* 2014;100:41–6.
- Watson SP, Auger JM, McCarty OJ, et al. GPVI and integrin alphaIIb beta3 signaling in platelets. *J Thromb Haemost* 2005;3:1752–62.
- Pasquet JM, Gross B, Quek L, et al. LAT is required for tyrosine phosphorylation of phospholipase cgamma2 and platelet activation by the collagen receptor GPVI. *Mol Cell Biol* 1999;19:8326–34.
- Jang JY, Min JH, Chae YH, et al. Reactive oxygen species play a critical role in collagen-induced platelet activation via SHP-2 oxidation. *Antioxid Redox Signal* 2014;20:2528–40.
- Jang JY, Min JH, Wang SB, et al. Resveratrol inhibits collagen-induced platelet stimulation through suppressing NADPH oxidase and oxidative inactivation of SH2 domain-containing protein tyrosine phosphatase-2. *Free Radic Biol Med* 2015;89:842–51.
- Wang SB, Jang JY, Chae YH, et al. Kaempferol suppresses collagen-induced platelet activation by inhibiting NADPH oxidase and protecting SHP-2 from oxidative inactivation. *Free Radic Biol Med* 2015;83:41–53.
- Violi F, Pignatelli P. Platelet NOX, a novel target for anti-thrombotic treatment. *Thromb Haemost* 2014;111:817–23.
- Pastori D, Pignatelli P, Carnevale R, et al. Nox-2 up-regulation and platelet activation: Novel insights. *Prostaglandins Other Lipid Mediat* 2015;120:50–5.
- Chlopicki S, Olszanecki R, Janiszewski M, et al. Functional role of NADPH oxidase in activation of platelets. *Antioxid Redox Signal* 2004;6:691–8.
- Jang JY, Wang SB, Min JH, et al. Peroxiredoxin II is an antioxidant enzyme that negatively regulates collagen-stimulated platelet function. *J Biol Chem* 2015;290:11432–42.
- Dayal S, Wilson KM, Motto DG, et al. Hydrogen peroxide promotes aging-related platelet hyperactivation and thrombosis. *Circulation* 2013;127:1308–16.
- Stef G, Csiszar A, Ziangmin Z, et al. Inhibition of NAD(P)H oxidase attenuates aggregation of platelets from high-risk cardiac patients with aspirin resistance. *Pharmacol Rep* 2007;59:428–36.
- Krötzig F, Sohn HY, Gloe T, et al. NAD(P)H oxidase-dependent platelet superoxide anion release increases platelet recruitment. *Blood* 2002;100:917–24.
- Begonja AJ, Teichmann L, Geiger J, et al. Platelet regulation by NO/cGMP signaling and NAD(P)H oxidase-generated ROS. *Blood Cells Mol Dis* 2006;36:166–70.
- Begonja AJ, Gambaryan S, Geiger J, et al. Platelet NAD(P)H-oxidase-generated ROS production regulates alphaIIb beta3-integrin activation independent of the NO/cGMP pathway. *Blood* 2005;106:2757–60.
- Khasawneh FT, Huang JS, Mir F, et al. Characterization of isoprostane signaling: evidence for a unique coordination profile of 8-iso-PGF(2alpha) with the thromboxane A(2) receptor, and activation of a separate cAMP-dependent inhibitory pathway in human platelets. *Biochem Pharmacol* 2008;75:2301–15.
- Schwedhelm E, Bierend A, Maas R, et al. Redox-generated isoprostanes are associated with residual platelet activity in aspirin-treated patients with stable coronary heart disease. *J Thromb Haemost* 2010;8:2662–70.
- Pignatelli P, Carnevale R, Di Santo S, et al. Inherited human gp91phox deficiency is associated with impaired isoprostane formation and platelet dysfunction. *Arterioscler Thromb Vasc Biol* 2011;31:423–34.
- Violi F, Carnevale R, Pastori D, et al. Antioxidant and antiplatelet effects of atorvastatin by Nox2 inhibition. *Trends Cardiovasc Med* 2014;24:142–8.
- Pignatelli P, Carnevale R, Pastori D, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. *Circulation* 2012;126:92–103.
- Pignatelli P, Carnevale R, Di Santo S, et al. Rosuvastatin reduces platelet recruitment by inhibiting NADPH oxidase activation. *Biochem Pharmacol* 2012;84:1635–42.
- Matetzky S, Fefer P, Shenkman B, et al. Statins have an early antiplatelet effect in patients with acute myocardial infarction. *Platelets* 2011;22:103–10.
- Adam O, Laufs U. Antioxidative effects of statins. *Arch Toxicol* 2008;82:885–92.
- Lanone S, Bloc S, Foresti R, et al. Bilirubin decreases nos2 expression via inhibition of NAD(P)H oxidase: implications for protection against endotoxic shock in rats. *FASEB J* 2005;19:1890–2.
- Matsumoto H, Ishikawa K, Itabe H, et al. Carbon monoxide and bilirubin from heme oxygenase-1 suppresses reactive oxygen species generation and plasminogen activator inhibitor-1 induction. *Mol Cell Biochem* 2006;291:21–8.
- Jiang F, Roberts SJ, Datla S, et al. NO modulates NADPH oxidase function via heme oxygenase-1 in human endothelial cells. *Hypertension* 2006;48:950–7.
- Datla SR, Dusting GJ, Mori TA, et al. Induction of heme oxygenase-1 in vivo suppresses NADPH oxidase derived oxidative stress. *Hypertension* 2007;50:636–42.
- Kundur AR, Bulmer AC, Singh I. Unconjugated bilirubin inhibits collagen induced platelet activation. *Platelets* 2014;25:45–50.
- Schwertner HA, Vítek L. Gilbert syndrome, UGT1A1*28 allele, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. *Atherosclerosis* 2008;198:1–11.
- Horsfall LJ, Nazareth I, Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. *Circulation* 2012;126:2556–64.
- Kundur AR, Singh I, Bulmer AC. Bilirubin, platelet activation and heart disease: a missing link to cardiovascular protection in Gilbert's syndrome? *Atherosclerosis* 2015;239:73–84.
- McCarty MF. Clinical potential of Spirulina as a source of phycocyanobilin. *J Med Food* 2007;10:566–70.
- Zheng J, Inoguchi T, Sasaki S, et al. Phycocyanin and phycocyanobilin from Spirulina platensis protect against diabetic nephropathy by inhibiting oxidative stress. *Am J Physiol Regul Integr Comp Physiol* 2013;304:R110–R120.
- Romay C, González R, Ledón N, et al. C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 2003;4:207–16.
- Liu Q, Huang Y, Zhang R, et al. Medical Application of Spirulina platensis Derived C-Phycocyanin. *Evid Based Complement Alternat Med* 2016;2016:7803846.
- Hsiao G, Chou PH, Shen MY, et al. C-phycocyanin, a very potent and novel platelet aggregation inhibitor from Spirulina platensis. *J Agric Food Chem* 2005;53:7734–40.
- Chiu HF, Yang SP, Kuo YL, et al. Mechanisms involved in the antiplatelet effect of C-phycocyanin. *Br J Nutr* 2006;95:435–40.

50. Freedman JE, Sauter R, Battinelli EM, *et al.* Deficient platelet-derived nitric oxide and enhanced hemostasis in mice lacking the NOSIII gene. *Circ Res* 1999;84:1416–21.
51. Antl M, von Brühl ML, Eiglsperger C, *et al.* IRAG mediates NO/cGMP-dependent inhibition of platelet aggregation and thrombus formation. *Blood* 2007;109:552–9.
52. Schinner E, Salb K, Schlossmann J. Signaling via IRAG is essential for NO/cGMP-dependent inhibition of platelet activation. *Platelets* 2011;22:217–27.
53. Danielewski O, Schultess J, Smolenski A. The NO/cGMP pathway inhibits Rap 1 activation in human platelets via cGMP-dependent protein kinase I. *Thromb Haemost* 2005;93:319–25.
54. Wu CC, Ko FN, Kuo SC, *et al.* YC-1 inhibited human platelet aggregation through NO-independent activation of soluble guanylate cyclase. *Br J Pharmacol* 1995;116:1973–8.
55. Teng CM, Wu CC, Ko FN, *et al.* YC-1, a nitric oxide-independent activator of soluble guanylate cyclase, inhibits platelet-rich thrombosis in mice. *Eur J Pharmacol* 1997;320:161–6.
56. Friebe A, Müllershausen F, Smolenski A, *et al.* YC-1 potentiates nitric oxide- and carbon monoxide-induced cyclic GMP effects in human platelets. *Mol Pharmacol* 1998;54:962–7.
57. Roger S, Badier-Commander C, Paysant J, *et al.* The anti-aggregating effect of BAY 41-2272, a stimulator of soluble guanylyl cyclase, requires the presence of nitric oxide. *Br J Pharmacol* 2010;161:1044–58.
58. Vesely DL. Biotin enhances guanylate cyclase activity. *Science* 1982;216:1329–30.
59. Singh IN, Dakshinamurti K. Stimulation of guanylate cyclase and RNA polymerase II activities in HeLa cells and fibroblasts by biotin. *Mol Cell Biochem* 1988;79:47–55.
60. Watanabe-Kamiyama M, Kamiyama S, Horiuchi K, *et al.* Antihypertensive effect of biotin in stroke-prone spontaneously hypertensive rats. *Br J Nutr* 2008;99:756–63.
61. Mock DM. Biotin. In: Ziegler EE, LJr F, *Present Knowledge in Nutrition*. 7th ed. Washington, D.C: ILSI Press, 1996:220–35.
62. Sedel F, Papeix C, Bellanger A, *et al.* High doses of biotin in chronic progressive multiple sclerosis: a pilot study. *Mult Scler Relat Disord* 2015;4:159–69.
63. Tourbah A, Lebrun-Frenay C, Edan G, *et al.* MD1003 (high-dose biotin) for the treatment of progressive multiple sclerosis: A randomised, double-blind, placebo-controlled study. *Mult Scler* 2016;22:1719–31.
64. Antoniadis C, Shirodaria C, Leeson P, *et al.* Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur Heart J* 2009;30:1142–50.
65. Mohan S, Fung HL. Mechanism of cellular oxidation stress induced by asymmetric dimethylarginine. *Int J Mol Sci* 2012;13:7521–31.
66. Milstien S, Katusic Z. Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem Biophys Res Commun* 1999;263:681–4.
67. Crabtree MJ, Tatham AL, Hale AB, *et al.* Critical role for tetrahydrobiopterin recycling by dihydrofolate reductase in regulation of endothelial nitric-oxide synthase coupling: relative importance of the de novo biopterin synthesis versus salvage pathways. *J Biol Chem* 2009;284:28128–36.
68. de Meirelles LR, Mendes-Ribeiro AC, Santoro MM, *et al.* Inhibitory effects of endogenous L-arginine analogues on nitric oxide synthesis in platelets: role in platelet hyperaggregability in hypertension. *Clin Exp Pharmacol Physiol* 2007;34:1267–71.
69. Peng L, Xia K, Zhao Z, *et al.* OS 32-09 the beneficial effect of losartan on platelet aggregation results from the reduction of endogenous asymmetric dimethyl arginine in spontaneously hypertensive rats. *J Hypertens* 2016;34:e391–e392.
70. Kanaya S, Ikeda H, Haramaki N, *et al.* Intraplatelet tetrahydrobiopterin plays an important role in regulating canine coronary arterial thrombosis by modulating intraplatelet nitric oxide and superoxide generation. *Circulation* 2001;104:2478–84.
71. Freedman JE, Ting B, Hankin B, *et al.* Impaired platelet production of nitric oxide predicts presence of acute coronary syndromes. *Circulation* 1998;98:1481–6.
72. Mazzanti L, Mutus B. Diabetes-induced alterations in platelet metabolism. *Clin Biochem* 1997;30:509–15.
73. Rabini RA, Staffolani R, Fumelli P, *et al.* Decreased nitric oxide synthase activity in platelets from IDDM and NIDDM patients. *Diabetologia* 1998;41:101–4.
74. Schaeffer G, Wascher TC, Kostner GM, *et al.* Alterations in platelet Ca²⁺ signalling in diabetic patients is due to increased formation of superoxide anions and reduced nitric oxide production. *Diabetologia* 1999;42:167–76.
75. Takajo Y, Ikeda H, Haramaki N, *et al.* Augmented oxidative stress of platelets in chronic smokers. Mechanisms of impaired platelet-derived nitric oxide bioactivity and augmented platelet aggregability. *J Am Coll Cardiol* 2001;38:1320–7.
76. Leoncini G, Signorello MG, Piana A, *et al.* Hydrogen peroxide formation in platelets of patients with non-insulin-dependent diabetes mellitus. *Platelets* 1998;9:213–7.
77. Yamagishi SI, Edelstein D, Du XL, *et al.* Hyperglycemia potentiates collagen-induced platelet activation through mitochondrial superoxide overproduction. *Diabetes* 2001;50:1491–4.
78. Pirags V, Assert R, Haupt K, *et al.* Activation of human platelet protein kinase C-beta 2 in vivo in response to acute hyperglycemia. *Exp Clin Endocrinol Diabetes* 1996;104:431–40.
79. Kimura M, Ishizawa M, Miura A, *et al.* Platelet protein kinase C isoform content in type 2 diabetes complicated with retinopathy and nephropathy. *Platelets* 2001;12:138–43.
80. Shih RH, Cheng SE, Hsiao LD, *et al.* Cigarette smoke extract upregulates heme oxygenase-1 via PKC/NADPH oxidase/ROS/PDGFR/PI3K/Akt pathway in mouse brain endothelial cells. *J Neuroinflammation* 2011;8:104.
81. Asano H, Horinouchi T, Mai Y, *et al.* Nicotine- and tar-free cigarette smoke induces cell damage through reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase. *J Pharmacol Sci* 2012;118:275–87.
82. Horinouchi T, Higashi T, Mazaki Y, *et al.* Carbonyl Compounds in the Gas Phase of Cigarette Mainstream Smoke and Their Pharmacological Properties. *Biol Pharm Bull* 2016;39:909–14.
83. Kumbhani DJ, Marso SP, Alvarez CA, *et al.* State-of-the-Art: Hyporesponsiveness to oral antiplatelet therapy in patients with type 2 diabetes mellitus. *Curr Cardiovasc Risk Rep* 2015;9:4.
84. Simpson SH, Abdelmoneim AS, Omran D, *et al.* Prevalence of high on-treatment platelet reactivity in diabetic patients treated with aspirin. *Am J Med* 2014;127:95.e1–95.e9.
85. Liu L, Gao YH, Cao J, *et al.* High prevalence of aspirin resistance in elderly patients with cardiovascular disease and metabolic syndrome. *J Geriatr Cardiol* 2016;13:531–6.
86. Santilli F, Vazzana N, Liani R, *et al.* Platelet activation in obesity and metabolic syndrome. *Obes Rev* 2012;13:27–42.
87. Çağırıcı G, Özdemir O, Geyik B, *et al.* The prevalence of aspirin resistance in patients with metabolic syndrome. *Turk Kardiyol Dem Ars* 2009;37:461–6.
88. Anfossi G, Russo I, Trovati M. Resistance to aspirin and thienopyridines in diabetes mellitus and metabolic syndrome. *Curr Vasc Pharmacol* 2008;6:313–28.
89. Eskandarian R, Darabian M, Heshmatnia J, *et al.* Acetyl salicylic acid resistance in patients with chronic stable angina and the correlation with coronary risk factors. *Saudi Med J* 2012;33:39–43.
90. Kaplon-Cieślicka A, Rosiak M, Postuła M, *et al.* Predictors of high platelet reactivity during aspirin treatment in patients with type 2 diabetes. *Kardiol Pol* 2013;71:893–902.
91. Belch J, MacCuish A, Campbell I, *et al.* The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. *BMJ* 2008;337:a1840.
92. Ogawa H, Nakayama M, Morimoto T, *et al.* Low-dose aspirin for primary prevention of atherosclerotic events in patients with type 2 diabetes: a randomized controlled trial. *JAMA* 2008;300:2134–41.
93. Saito Y, Okada S, Ogawa H, *et al.* Low-Dose Aspirin for Primary Prevention of Cardiovascular Events in Patients With Type 2 Diabetes Mellitus: 10-Year Follow-Up of a Randomized Controlled Trial. *Circulation* 2017;135:659–70.
94. Cangemi R, Pignatelli P, Carnevale R, *et al.* Platelet isoprostane overproduction in diabetic patients treated with aspirin. *Diabetes* 2012;61:1626–32.
95. Schwedhelm E, Maas R, Freese R, *et al.* Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* 2008;65:51–9.
96. Waugh WH, Daeschner CW, Files BA, *et al.* Oral citrulline as arginine precursor may be beneficial in sickle cell disease: early phase two results. *J Natl Med Assoc* 2001;93:363–71.
97. Romero MJ, Platt DH, Caldwell RB, *et al.* Therapeutic use of citrulline in cardiovascular disease. *Cardiovasc Drug Rev* 2006;24:275–90.
98. McCarty MF. Asymmetric Dimethylarginine Is a Well Established Mediating Risk Factor for Cardiovascular Morbidity and Mortality—Should Patients with Elevated Levels Be Supplemented with Citrulline? *Healthcare* 2016;4:40.
99. Rezk BM, Haenen GR, van der Vijgh WJ, *et al.* Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant

- activity. Identification of the antioxidant pharmacophore. *FEBS Lett* 2003;555:601–5.
100. Antoniadou C, Shirodaria C, Warrick N, *et al.* 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. *Circulation* 2006;114:1193–201.
 101. Chalupsky K, Cai H. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 2005;102:9056–61.
 102. Gao L, Chalupsky K, Stefani E, *et al.* Mechanistic insights into folic acid-dependent vascular protection: dihydrofolate reductase (DHFR)-mediated reduction in oxidant stress in endothelial cells and angiotensin II-infused mice: a novel HPLC-based fluorescent assay for DHFR activity. *J Mol Cell Cardiol* 2009;47:752–60.
 103. Siu KL, Miao XN, Cai H. Recoupling of eNOS with folic acid prevents abdominal aortic aneurysm formation in angiotensin II-infused apolipoprotein E null mice. *PLoS One* 2014;9:e88899.
 104. McCarty MF. High-dose folate may improve platelet function in acute coronary syndrome and other pathologies associated with increased platelet oxidative stress. *Med Hypotheses* 2007;69:12–19.
 105. Pryor WA, Squadrito GL. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 1995;268(5 Pt 1):L699–L722.
 106. Koppal T, Drake J, Butterfield DA. In vivo modulation of rodent glutathione and its role in peroxynitrite-induced neocortical synaptosomal membrane protein damage. *Biochim Biophys Acta* 1999;1453:407–11.
 107. Cheung PY, Wang W, Schulz R. Glutathione protects against myocardial ischemia-reperfusion injury by detoxifying peroxynitrite. *J Mol Cell Cardiol* 2000;32:1669–78.
 108. Martina V, Bruno GA, Zumpano E, *et al.* Administration of glutathione in patients with type 2 diabetes mellitus increases the platelet constitutive nitric oxide synthase activity and reduces PAI-1. *J Endocrinol Invest* 2001;24:37–41.
 109. Dickinson DA, Forman HJ. Glutathione in defense and signaling: lessons from a small thiol. *Ann N Y Acad Sci* 2002;973:488–504.
 110. Shelton MD, Chock PB, Miesal JJ. Glutaredoxin: role in reversible protein S-glutathionylation and regulation of redox signal transduction and protein translocation. *Antioxid Redox Signal* 2005;7:348–66.
 111. Chen CY, Willard D, Rudolph J. Redox regulation of SH2-domain-containing protein tyrosine phosphatases by two backdoor cysteines. *Biochemistry* 2009;48:1399–409.
 112. Richman PG, Meister A. Regulation of gamma-glutamyl-cysteine synthetase by nonallosteric feedback inhibition by glutathione. *J Biol Chem* 1975;250:1422–6.
 113. Suh JH, Wang H, Liu RM, *et al.* (R)-alpha-lipoic acid reverses the age-related loss in GSH redox status in post-mitotic tissues: evidence for increased cysteine requirement for GSH synthesis. *Arch Biochem Biophys* 2004;423:126–35.
 114. Atkuri KR, Mantovani JJ, Herzenberg LA, *et al.* N-Acetylcysteine—a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol* 2007;7:355–9.
 115. Dodd S, Dean O, Copolov DL, *et al.* N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert Opin Biol Ther* 2008;8:1955–62.
 116. Rebrin I, Sohal RS. Pro-oxidant shift in glutathione redox state during aging. *Adv Drug Deliv Rev* 2008;60:1545–52.
 117. Dröge W, Kinscherf R, Hildebrandt W, *et al.* The deficit in low molecular weight thiols as a target for antiageing therapy. *Curr Drug Targets* 2006;7:1505–12.
 118. Sekhar RV, Patel SG, Guthikhonda AP, *et al.* Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. *Am J Clin Nutr* 2011;94:847–53.
 119. Gibson KR, Neilson IL, Barrett F, *et al.* Evaluation of the antioxidant properties of N-acetylcysteine in human platelets: prerequisite for bioconversion to glutathione for antioxidant and antiplatelet activity. *J Cardiovasc Pharmacol* 2009;54:319–26.
 120. Gibson KR, Winterburn TJ, Barrett F, *et al.* Therapeutic potential of N-acetylcysteine as an antiplatelet agent in patients with type-2 diabetes. *Cardiovasc Diabetol* 2011;10:43.
 121. Wheeler MD, Thurman RG. Production of superoxide and TNF-alpha from alveolar macrophages is blunted by glycine. *Am J Physiol* 1999;277(5 Pt 1):L952–L959.
 122. Wheeler M, Stachlewitz RF, Yamashina S, *et al.* Glycine-gated chloride channels in neutrophils attenuate calcium influx and superoxide production. *Faseb J* 2000;14:476–84.
 123. Wheeler MD, Ikejima K, Enomoto N, *et al.* Glycine: a new anti-inflammatory immunonutrient. *Cell Mol Life Sci* 1999;56:843–56.
 124. Yamashina S, Konno A, Wheeler MD, *et al.* Endothelial cells contain a glycine-gated chloride channel. *Nutr Cancer* 2001;40:197–204.
 125. Froh M, Thurman RG, Wheeler MD. Molecular evidence for a glycine-gated chloride channel in macrophages and leukocytes. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G856–G863.
 126. Schemmer P, Zhong Z, Galli U, *et al.* Glycine reduces platelet aggregation. *Amino Acids* 2013;44:925–31.
 127. MacIntyre DE, Rink TJ. The role of platelet membrane potential in the initiation of platelet aggregation. *Thromb Haemost* 1982;47:22–6.
 128. Friedhoff LT, Kim E, Priddle M, *et al.* The effect of altered transmembrane ion gradients on membrane potential and aggregation of human platelets in blood plasma. *Biochem Biophys Res Commun* 1981;102:832–7.
 129. Ishikawa Y, Sasakawa S. Membrane potential of stored platelets and its effect on platelet functions. *Thromb Res* 1987;45:265–73.
 130. Palés J, López A, Gual A. Platelet membrane potential as a modulator of aggregating mechanisms. *Biochim Biophys Acta* 1988;944:85–9.
 131. Albarrán L, Dionisio N, López E, *et al.* The membrane potential modulates thrombin-stimulated Ca²⁺ mobilization and platelet aggregation. *Arch Biochem Biophys* 2013;538:130–7.
 132. Krötz F, Riexinger T, Buerkle MA, *et al.* Membrane-potential-dependent inhibition of platelet adhesion to endothelial cells by epoxyeicosatrienoic acids. *Arterioscler Thromb Vasc Biol* 2004;24:595–600.
 133. Jackson AA, Persaud C, Meakins TS, *et al.* Urinary excretion of 5-L-oxoproline (pyroglutamic acid) is increased in normal adults consuming vegetarian or low protein diets. *J Nutr* 1996;126:2813–22.
 134. Hayes KC, Pronczuk A, Addesa AE, *et al.* Taurine modulates platelet aggregation in cats and humans. *Am J Clin Nutr* 1989;49:1211–6.
 135. Franconi F, Miceli M, Bennardini F, *et al.* Taurine potentiates the antiaggregatory action of aspirin and indomethacin. *Adv Exp Med Biol* 1992;315:181–6.
 136. Franconi F, Bennardini F, Mattana A, *et al.* Plasma and platelet taurine are reduced in subjects with insulin-dependent diabetes mellitus: effects of taurine supplementation. *Am J Clin Nutr* 1995;61:1115–9.
 137. Park IS, Kang YH, Kang JS. Effects of taurine on plasma and liver lipids, erythrocyte ouabain sensitive Na efflux and platelet aggregation in Sprague Dawley rats. *Nutr Res Pract* 2007;1:200–5.
 138. Spohr C, Brøns C, Winther K, *et al.* No effect of taurine on platelet aggregation in men with a predisposition to type 2 diabetes mellitus. *Platelets* 2005;16:301–5.
 139. Sun Q, Wang B, Li Y, *et al.* Taurine supplementation lowers blood pressure and improves vascular function in prehypertension: randomized, double-blind, placebo-controlled study. *Hypertension* 2016;67:541–9.
 140. Zagli G, Patacchini R, Trevisani M, *et al.* Hydrogen sulfide inhibits human platelet aggregation. *Eur J Pharmacol* 2007;559:65–8.
 141. Pircher J, Fochler F, Czermak T, *et al.* Hydrogen sulfide-releasing aspirin derivative ACS14 exerts strong antithrombotic effects in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 2012;32:2884–91.
 142. Grambow E, Mueller-Graf F, Delyagina E, *et al.* Effect of the hydrogen sulfide donor GYY4137 on platelet activation and microvascular thrombus formation in mice. *Platelets* 2014;25:166–74.
 143. Gao L, Cheng C, Sparatore A, *et al.* Hydrogen sulfide inhibits human platelet aggregation in vitro in part by interfering gap junction channels: effects of ACS14, a hydrogen sulfide-releasing aspirin. *Heart Lung Circ* 2015;24:77–85.
 144. Zhong L, Lv L, Yang J, *et al.* Inhibitory effect of hydrogen sulfide on platelet aggregation and the underlying mechanisms. *J Cardiovasc Pharmacol* 2014;64:481–7.
 145. Dyerberg J, Bang HO, Stoffersen E, *et al.* Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 1978;2:117–9.
 146. Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet* 1979;2:433–5.
 147. Siess W, Roth P, Scherer B, *et al.* Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. *Lancet* 1980;1:441–4.
 148. Thorngren M, Gustafson A. Effects of 11-week increases in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation. *Lancet* 1981;2:1190–3.

149. Thorngren M, Gustafson A, Wohlfart G. Effects of acetylsalicylic acid on platelet aggregation before and during increase in dietary eicosapentaenoic acid. *Haemostasis* 1983;13:244–7.
150. Larson MK, Shearer GC, Ashmore JH, *et al.* Omega-3 fatty acids modulate collagen signaling in human platelets. *Prostaglandins Leukot Essent Fatty Acids* 2011;84:93–8.
151. Block RC, Kakinami L, Jonovich M, *et al.* The combination of EPA+DHA and low-dose aspirin ingestion reduces platelet function acutely whereas each alone may not in healthy humans. *Prostaglandins Leukot Essent Fatty Acids* 2012;87:143–51.
152. Croset M, Lagarde M. In vitro incorporation and metabolism of icosapentaenoic and docosahexaenoic acids in human platelets-effect on aggregation. *Thromb Haemost* 1986;56:57–62.
153. Srivastava KC. Docosahexaenoic acid (C22:6 omega 3) and linoleic acid are anti-aggregatory, and alter arachidonic acid metabolism in human platelets. *Prostaglandins Leukot Med* 1985;17:319–27.
154. Cheryk LA, Conquer JA, Holub BJ, *et al.* Docosahexaenoic acid and docosapentaenoic acid incorporation into human platelets after 24 and 72 hours: inhibitory effects on platelet reactivity. *Platelets* 1999;10:203–11.
155. Nelson GJ, Schmidt PS, Bartolini GL, *et al.* The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. *Lipids* 1997;32:1129–36.
156. Véricel E, Colas R, Calzada C, *et al.* Moderate oral supplementation with docosahexaenoic acid improves platelet function and oxidative stress in type 2 diabetic patients. *Thromb Haemost* 2015;114:289–96.