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The cardiovascular benefits of dark chocolate

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Abstract

Dark chocolate contains many biologically active components, such as catechins, procyanidins and theobromine from cocoa, together with added sucrose and lipids. All of these can directly or indirectly affect the cardiovascular system by multiple mechanisms. Intervention studies on healthy and metabolically-dysfunctional volunteers have suggested that cocoa improves blood pressure, platelet aggregation and endothelial function. The effect of chocolate is more convoluted since the sucrose and lipid may transiently and negatively impact on endothelial function, partly through insulin signalling and nitric oxide bioavailability. However, few studies have attempted to dissect out the role of the individual components and have not explored their possible interactions. For intervention studies, the situation is complex since suitable placebos are often not available, and some benefits may only be observed in individuals showing mild metabolic dysfunction. For chocolate, the effects of some of the components, such as sugar and epicatechin on FMD, may oppose each other, or alternatively in some cases may act together, such as theobromine and epicatechin. Although clearly cocoa provides some cardiovascular benefits according to many human intervention studies, the exact components, their interactions and molecular mechanisms are still under debate.

Abbreviations:

BP, blood pressure

COX, cyclooxygenase

ET-1, endothelin-1

FMD, flow-mediated dilation

PDE, phosphodiesterase

1. Relevant components of chocolate and their bioavailability

After consumption of dark chocolate, the various components are digested and absorbed by distinct pathways. The main ingredients of interest are theobromine, catechins, procyanidins, sucrose and lipid, and each of these can exert complementary or opposing effects on endothelial function and cardiovascular biomarkers.

Theobromine is a xanthine alkaloid and is also one of the compounds derived from caffeine metabolism. It is resistant to cocoa processing, found at high levels in dark chocolate, and has been used as a marker to indicate the cocoa content of chocolates (Cooper et al., 2008). Bioavailability studies on pure theobromine show efficient absorption into the blood with a half-life of 7.2 h (Lelo et al., 1986). A 40 g portion of dark chocolate contains a mean of 240 mg theobromine (Cooper et al., 2008) which is absorbed in the small intestine to give a predicted C_{max} of 20-25 µM (Lelo et al., 1986).

Catechins are flavan-3-ols found at high levels in dark chocolate. A 40 g portion of dark chocolate provides a mean of 31 mg (-)-epicatechin and 9 mg of (+)-catechin (Cooper et al., 2008). A detailed recent pharmacokinetic study on pure (-)-epicatechin indicated a half-life of 1.5 h, a T_{max} of 2 h, and no plateauing of the maximum plasma concentration up to a dose of 200 mg (Barnett et al., 2015). A 40 g portion of procyanidin-rich chocolate would achieve a plasma C_{max} of 0.2 µM (Wang et al., 2000), but most of the epicatechin in plasma is conjugated as sulfate, glucuronide and methyl derivatives (Actis-Goretta et al., 2012). Procyanidins are oligomeric flavonoids consisting of covalently-linked epicatechin and catechin moieties, and procyanidins containing 2 to 10 epicatechin "units" can be readily measured in cocoa and dark chocolate using a multi-lab validated method (Robbins et al., 2013). The amount present in chocolate varies depending on the processing method (Cooper et al., 2007). Procyanidins are very poorly absorbed as the intact molecules (Holt et al., 2002), but studies on ^{14}C-radiolabelled procyanidin B2 in rats show that >80% of the label is absorbed in the colon after metabolism by the microbiota into lower molecular weight compounds (Stoupi et

al., 2010). Often the catechin and procyanidins contents are grouped together as total “cocoa flavonoids”.

Sucrose is not present in cocoa but is added during the manufacture of dark chocolate. Amounts are typically in the 15-30% range depending on the type of chocolate. Sucrose is efficiently hydrolysed into glucose and fructose in the small intestine by the brush border enzyme sucrase-isomaltase (EC3.2.1.10), and the resulting products absorbed into the blood by the sugar transporters SGLT1 (SLC5A1), GLUT2 (SLC2A2) and GLUT5 (SLC2A5) (Blakemore et al., 1995; Kellett et al., 2008; Kellett and Brot-Laroche, 2005). Pure sucrose gives a glycaemic index of ~60-70 % of that of glucose (Foster-Powell et al., 2002; Jenkins et al., 1981). Although fructose contributes a modest ~15% to post-prandial glycaemic responses, its swift transit across the gut wall supplies the liver with lipogenic precursors that amplify the proatherogenic milieu in the vasculature.

Cocoa effectively consists of a non-fat component together with the lipid component, cocoa butter, although other fats are sometimes added as a substitute. Cocoa butter contains predominantly stearic acid (C18:0), palmitic acid (C16:0) and oleic acid (C18:1) (Padilla et al., 2000) in the form of triglycerides. Dietary triglycerides are hydrolysed by lipases in the gut into free fatty acids and 2-monoglycerides, which are absorbed both by passive diffusion and by a family of fatty acid transport proteins (FATP). In the enterocyte, triglycerides are synthesised and packaged into chylomicrons which mainly enter the lymphatic system. After hepatic processing, there is a transient postprandial increase in triacylglycerols and a change in the pattern of lipoproteins (Lopez-Miranda et al., 2007). Procyanidins are known to moderately decrease lipid release from the enterocyte to the blood through limiting dietary triglyceride absorption and restriction of chylomicron assembly by effects on key enzymes central to the processes (reviewed in Blade et al., 2010).

After consumption of dark chocolate, the blood will contain elevated levels of theobromine, epicatechin, glucose, fructose and triglycerides, all of which will add to the post-prandial effects of chocolate on the vascular system. Based on bioavailability studies, the direct effects of theobromine

and epicatechin will be short-lived, but any changes in gene expression or cell signalling derived from these bioactive substances could last much longer. Sugar and fat are used as energy and any excess is stored in the body, giving rise to both short and long term effects. These complex interactions must be taken into account when considering the acute and chronic effects of dark chocolate consumption.

2. Human intervention studies on cocoa

There are now numerous studies on the effect of cocoa or chocolate on multiple biomarkers in healthy volunteers, at-risk groups and patients (Berends et al., 2015; Ellam and Williamson, 2013). In a recent study, cocoa dose-dependently improved FMD, blood ET-1 levels, pulse wave velocity, and blood pressure (Grassi et al., 2015). The sugar and fat from the chocolate may affect the response of physiological and biochemical markers. After administration of glucose to healthy volunteers, postprandial FMD was transiently decreased by >20%. This decrease was almost completely blocked in volunteers who consumed dark chocolate, both when given simultaneously and when they had previously consumed 100 g of dark chocolate for the preceding 3 days, but not if white chocolate was substituted (Grassi et al., 2012). In addition, 3 days of dark chocolate decreased the baseline FMD by almost 1% and blood ET-1 levels were decreased in comparison to white chocolate (Grassi et al., 2012). In chocolate, the "negative" effects of the constituent sugar and fat are counteracted by the presence of the cocoa flavonoids and theobromine, which can result in less dramatic effects of chocolate on biomarkers compared to cocoa alone, although this depends on the control or placebo used. For cocoa, the beneficial effects are manifest by improved vascular function and lowered blood pressure (Grassi et al., 2015).

Since the explosion of interest in cocoa and health over the last decade, a major issue in conducting a study has become the incorporation of a suitable control or placebo. Previously white chocolate or a "chocolate" but without cocoa solids have occasionally been used. However, most studies do not prove which ingredients are responsible for a biological activity, since all dark chocolates contain theobromine, catechins and procyanidins in addition to numerous other components such as

magnesium. One option, as presented by Rull et al. (2015), is to compare low and high "cocoa flavonoid"-containing matrices. This study highlights theobromine as an important mediator of the physiological effects based on the fact that high and low "cocoa flavonoid" doses elicited similar effects. Nonetheless, studies on epicatechin alone (Barnett et al., 2015; Schroeter et al., 2006), although less common, so far have indicated an important role on FMD but also suggested effects on other biomarkers principally related to signalling pathways governing the vasodilatory actions of insulin in the endothelium (Monahan, 2012). In one such recent study of 37 healthy older adults (Dower et al., 2015), supplementation of pure epicatechin did not improve FMD but reduced insulin resistance while it had no effect on any other marker of cardiometabolic health. These data suggest that the combination of theobromine and epicatechin may be important for the optimal effects of chocolate and cocoa and this should be a topic and focus for future research.

3. Targets in vivo

The prevailing balance between nitric oxide concentrations and other endothelial factors is of critical importance to maintain endothelial integrity and vascular tone. Endothelial dysfunction, characterized by reduced nitric oxide production through NOS enzymes and exaggerated release of ET-1 through the MAPK pathway, is a key feature of human insulin-resistant states. Oral administration of 200 mg of (-)-epicatechin augmented endogenous NO and suppressed ET-1 levels in healthy men (Loke, 2008).

Although the PI3K signalling pathway mediating insulin stimulation of nitric oxide production in endothelial cells is overlapping with pathways responsible for insulin activation of glucose transport in metabolic tissues up to the step of Akt activation, the haemodynamic role of insulin driven by capillary recruitment precedes the induction of glucose uptake (Muniyappa et al., 2007). This demonstrates that the vascular effects of insulin are primary and do not simply arise as a consequence of changes in cellular metabolism. However, glucose released to the blood following a meal serves as the leading signal for secretion of insulin from the pancreas. Improvement of pancreatic β-cell

function as well as induction of the Akt /PI3K and ERK1/2 pathways has been suggested to play a role in effects on insulin resistance of cocoa flavanols (Grassi et al., 2008, Granado-Serrano et al., 2010). Controlling postprandial blood glucose concentrations is thought to be beneficial for the insulin resistant endothelium as regulating the glucose-insulin cycle can help avoid undesirable insulin bursts and prolong favourable NO levels. The speculation of the authors is that retention of procyanidins in the gut due to poor bioavailability may elicit such effects through their interactions with glucose transporters (Kerimi & Williamson, unpublished data). Prominent GLUT4 translocation in the muscle facilitating central glucose clearance is reliant on NO and enhanced insulin signalling, as shown in some animal studies, and may be one of the plausible mechanisms underlying effects of procyanidins (Yamashita et al., 2012, Pinent et al., 2012).

NO, once formed in endothelial cells, diffuses freely into adjacent VSMC, where it promotes vasorelaxation and inhibits migration, and into platelets, where it prevents their activation and aggregation. Platelets contribute to the early inflammatory events involved in the formation of plaques and also to the thrombogenic process subsequent to the rupture of advanced, unstable plaques (Muniyappa et al., 2007). Intake of 100 mg of flavanols consistently induced a variable but significant 3-11% reduction in platelet aggregation in numerous studies (reviewed in Habauzit & Morand 2012). Inhibition of thromboxane A2 formation from eicosanoids through antagonism of thromboxane A2 receptors and restriction of ADP induced aggregation were evidenced in vivo and ex-vivo by (+)-catechin, (-)-epicatechin and their metabolites 4-O-methyl-epicatechin and 3-O-methyl-catechin, following erythrocyte haemolysis and collagen exposure (Heptinstall et al. 2006) but only at supra-physiological doses. Augmentation of the eicosanoid pathway poses a double edged sword for cardiovascular health as the balance between prostacyclin, thromboxanes and leukotrienes drives vascular tone, permeability and recruitment of immune cells to the vascular wall (Fernandez-Murga et al., 2011). On the other hand, ADP restricts adenylate cyclase activity and enhances PDE activity reversing the inhibitory effect of cAMP generated through exposure to NO and prostacyclin stemming from insulin action (Cohen & Tong 2010). Rull et al. (2015) demonstrated a similar role for theobromine mainly through PDE inhibition.

Procoagulant activity following platelet aggregation events propagates formation of fibrin and resulting deposits that occlude the blood vessels are linked to clinical manifestations such as unstable angina, heart attack, and stroke. Platelet activation gives rise to interactions with leukocytes mainly via P-selectin (CD62P) which becomes exposed on the platelet surface and allows the platelets to attach to leukocytes via PSGL-1 receptors. Such interactions contribute to further fibrin production and also leukocyte involvement in inflammatory processes. Inhibition of platelet activation has been used for a long time in an effort to prevent and treat cardiovascular disease. However, limited efficacy in some patients, drug resistance, and side effects are limitations of this approach. In a recent mechanistic study epicatechin metabolites at low physiologically relevant concentrations were shown to attenuate the aforesaid interactions between circulating monocytes and TNF-α challenged vascular endothelial cells by regulating genes involved in cell adhesion and transendothelial migration mainly through NF-κB and MAPK signalling pathways by modulating phosphorylation of p65 and p38 (Claude et al., 2014). Esser et al. (2014) found that increased flavanol content did not further magnify effects on markers of endothelial health after daily intake of dark chocolate for 4 weeks. Lower numbers of leukocytes, decreased leukocyte adhesion molecule expression and decreased plasma soluble adhesion molecules were reported in overweight but apparently healthy men independent of flavanol dose implying that either the maximal beneficial effects were reached with the normal concentration or that the effects were due to other constituents. In support of this notion, Claude et al (2014) also noted a bell-shaped dose response effect of flavanols in vitro while Rull et al (2015) reported a flavanol-independent mechanism regarding the platelet aggregation protective role of chocolate.

These observations highlight the complex interplay of different chocolate constituents and the apparent difficulty when dissecting mechanisms. Pure epicatechin studies in contrast to cocoa randomised controlled trials do not show an effect on BP. Hooper et al. (2012) extrapolated that improvements in BP required consumption of 50–100 mg epicatechin containing cocoa/chocolate with no further reductions above 100 mg. In the study of Dower et al. (2015), a dose of 100 mg of

epicatechin did not produce a statistically significant effect; in agreement with the findings of Rull et al. (2015) where only a small tendency was noted for a 10-fold higher dose. The beneficial effects of cocoa flavanols on FMD, BP, and insulin resistance are thought to be partly mediated through the release of NO (Ellam & Williamson, 2013). As epicatechin has been shown to increase NO products acutely (Loke et al., 2008), the acute versus long term variable effects of high/ low flavanol chocolate supplementation should be considered while the length of a study is crucial when assessing chronic effects on several biomarkers after repeated doses.

In perspective, the insulin sensitizing effects of cocoa and epicatechin as supported by in vitro and animal experiments (Corti et al., 2009) may be due to improvements of glucose metabolism and insulin related NO availability, rather than antioxidant properties resulting from inhibition of NADPH oxidase and subsequent reduction in nitrogen reactive species which consume nitric oxide through its reaction with superoxide (Fernandez-Murga et al., 2011). Of interest, both lines of evidence heavily rely on the health status of volunteers and animal strains used since some beneficial effects of epicatechin gain significance only in an immune-compromised setting. Low doses of epicatechin cannot explain direct antioxidant effects as high doses of compounds with strong antioxidant activity have largely failed to mitigate disease progression and mechanistic studies point more towards interactions with key regulatory systems (Ramirez-Sanchez et al., 2013) that aid recovery from oxidative stresses following metabolic disorders and cardiovascular events and which deplete inherent antioxidant mediators like glutathione (Cohen & Tong, 2010). The anti-inflammatory action of flavanols in such situations is thought to be mediated through the NF-κB pathway and downstream genes such as COX-2 or IL-6, reduction of circulating cytokines, and inhibition of the eicosanoid pathway as mentioned above.

Mechanistic evidence for an effect of cocoa flavanols on blood lipids is lacking. According to a meta-analysis (Jia et al., 2010), only eight randomised controlled trial studies including 215 participants were found that assessed the short term changes of the lipid profile post cocoa ingestion. Small changes in total cholesterol and LDL but no effects on HDL were concluded and these did not follow

a dose-response. The changes reported were limited to participants with cardiovascular risk. Neufingerl et al. (2013) recently reported that theobromine independently increased serum HDL concentrations in a 2-center double-blind randomised placebo-controlled study of 152 healthy men without any cocoa or flavanol interaction effects or changes in BP or heart rate. The main effect on increasing HDL was attributed to the significant increase in apolipoprotein A-I levels, the major component of HDL particles.

4. Concluding remarks

Over the last two decades, biomedical interest in naturally occurring bioactives has led to a wealth of data in the literature detailing the chocolate content of flavanols as well as an array of evidence linking them with different protective pathways in cardiovascular-related syndromes. As for many of the studies based on the influence of the diet and given the complexity of the chocolate matrix, it is difficult to ascertain the main determinant of the observed benefit, or if there is a causal relationship. Differences in the backgrounds of cohorts, the length of study and absence of suitable placebos further complicate consistency and interpretation of documented effects on measures that only constitute surrogate markets. As suggested by some studies focused on blood pressure measurements, lipids, and diabetes, it might be that the benefits may be emphasized in individuals with some level of dysfunction. Moreover, knowledge on the molecular action of flavanols is still scarce while clinical studies on individual components, apart from flavanols, is rather limited. Based on these facts the bigger picture is far from complete and future research in the area is necessary to elucidate the role of individual components regarding the health effects of chocolate consumption.

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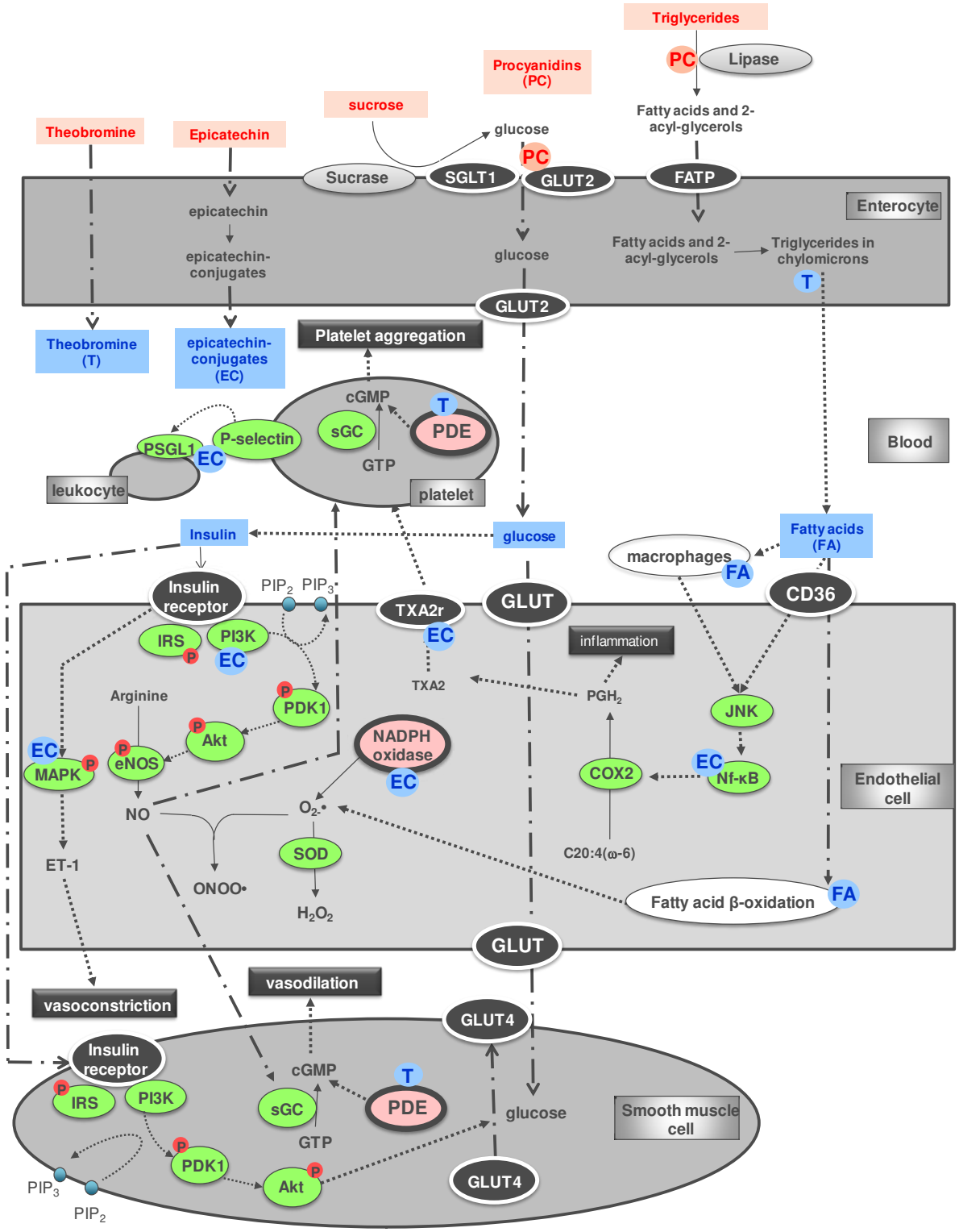
Triglycerides
PC
Lipase
Procyanidins (PC)
sucrose
Fatty acids and 2-acyl-glycerols
Theobromine
Epicatechin
glucose
PC
Sucrase
SGLT1
GLUT2
FATP
Enterocyte
epicatechin
epicatechin-conjugates
glucose
Fatty acids and 2-acyl-glycerols
Triglycerides in chylomicrons
T
GLUT2
Theobromine (T)
epicatechin-conjugates (EC)
Platelet aggregation
cGMP
T
sGC
PDE
GTP
PSGL1
P-selectin
EC
leukocyte
platelet
Blood
Insulin
glucose
macrophages
FA
Fatty acids (FA)
Insulin receptor
PIP2 PIP3
TXA2r
GLUT
EC
CD36
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PI3K
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inflammation
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PDK1
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JNK
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eNOS
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NADPH oxidase
EC
COX2
EC
Nf-κB
Endothelial cell
NO
O2-
C20:4(ω-6)
ET-1
SOD
ONOO•
H2O2
Fatty acid β-oxidation
FA
GLUT
vasoconstriction
vasodilation
GLUT4
Insulin receptor
cGMP
T
IRS
PI3K
sGC
PDE
glucose
Smooth muscle cell
GTP
PDK1
Akt
GLUT4
PIP3
PIP2

Figure 1.

Legends

Figure 1: Proposed modulation of cardiovascular metabolism by components of chocolate.
The chocolate components are absorbed from the gut lumen through the enterocyte and into the blood. The resulting metabolites affect processes in the endothelium, smooth muscle cells and platelets, both directly and indirectly. Green ovals: metabolic enzymes; black ovals: receptors/transporters; pink ovals: key target enzymes; components in chocolate shown in red; components after absorption shown in blue; interaction points shown as blue dots; phosphorylation shown as red dots; solid arrows show chemical reactions; dotted arrows show signalling interactions; dot and dash arrows show diffusion or movement of molecules; fatty acids (FA) in the blood can be in different chemical forms.
PDE, phosphodiesterase; sGC, soluble guanyl cyclase; SOD, superoxide dismutase; PC, procyanidins; EC, epicatechin conjugates; COX2, cyclo-oxygenase 2.

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