The Anti-inflammatory Properties of Cocoa Flavanols

Carlo Selmi, MD, PhD,*† Tin K. Mao, PhD,* Carl L. Keen, PhD,‡ Harold H. Schmitz, PhD,§ and M. Eric Gershwin, MD*

Abstract: Signs of chronic or acute inflammation have been demonstrated in most cardiovascular diseases of multifactorial pathogenesis, including atherosclerosis and chronic heart failure. The triggers and mechanisms leading to inflammation may vary between clinical conditions but they share many common mediators, including specific patterns of eicosanoid and cytokine production. Certain cocoa-based products can be rich in a subclass of flavonoids known as flavanols, some of which have been found in model systems to possess potential anti-inflammatory activity relevant to cardiovascular health. Indeed, experimental evidence demonstrates that some cocoa-derived flavanols can reduce the production and effect of pro-inflammatory mediators either directly or by acting on signaling pathways. However, it should be noted that the evidence for any beneficial effects of cocoa flavanols in providing a meaningful anti-inflammatory action has been gathered predominantly from in vitro experiments. Therefore, additional research in well-designed human clinical experiments, using cocoa properly characterized in terms of flavanol content, would be a welcome addition to the evidence base to determine unambiguously if this benefit does indeed exist. If so, then flavanol-rich cocoa could be a potential candidate for the treatment, or possibly prevention, of the broad array of chronic diseases that are linked to dysfunctional inflammatory responses.

Key Words: inflammation, flavonoids, procyanidins, eicosanoids, nitric oxide

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The Spaniards, both men and women, that are accustomed to the country, are very greedy of this Chocolate. They say they make diverse sorts of it, some hot, some cold, and some temperate, and put therein much of that “chilli;” yea, they make paste thereof, the which they say is good for the stomach and against the catarrh (Jose de Acosta, circa 1590)

Chocolate and cocoa products derived from the seeds of Theobroma cacao are very popular foods in many different cultures around the world. Historically, cocoa was first described as both a food and medicine by the Mayan of Central America, and later imported into Spain by Hernando Cortez. In recent years, a growing evidence base has been building which suggests that flavanol-rich cocoa products can influence several physiologic functions, especially vascular health.

Cocoa flavanols are a subclass of a large polyphenolic class of compounds found in plants, known as flavonoids. The structures of selected flavanols are depicted in Figure 1. Interestingly, a significant proportion of the flavanol content of fresh cocoa comprises larger molecular weight flavanol oligomers known as procyanidins (Table 1), having as many as 10 subunits. These large oligomeric forms are not found in tea or wine. Cocoa flavanols exhibit many biologic actions in model systems that could be relevant to human health, including actions on endothelium-derived nitric oxide (NO) synthase, NO metabolism, cytokine production, and eicosanoid metabolism.

It is generally accepted that inflammation plays a significant role in the initiation and promotion of various cardiovascular disorders that are mediated, in part, by cytokines and eicosanoids, platelets, and NO. For example, human atherosclerotic plaques express a cytokine profile dominated by pro-inflammatory types, which include interferon-γ, interleukin (IL)-1β and β, tumor necrosis factor (TNF)-α, and IL-6. For these reasons, molecules involved in the inflammatory cascade accompanying most cardiovascular diseases should be regarded as promising potential targets in the prevention and treatment of such conditions. Data from numerous studies suggest that cocoa-derived flavanols specifically, and polyphenols more broadly, can effectively influence and positively modify the inflammatory process, and thus potentially provide a benefit to individuals with cardiovascular risk factors.

INFLAMMATION IN CARDIOVASCULAR DISEASES: AN OVERVIEW

There is increasing evidence that inflammation plays a crucial role in the induction and perpetuation of several cardiovascular conditions. This assumption might seem obvious when diseases with a clear inflammatory component, such as vasculitis or autoimmune myopathies, are considered. We will discuss briefly the inflammation...
pathways involved in atherosclerosis-related coronary artery disease (CAD) and chronic heart failure.

Immune cells dominate early atherosclerosis plaque formation, producing molecules that promote inflammation which can, in turn, accelerate plaque deposition and trigger acute CAD. In fact, many of the cells found within the plaque (mostly T cells, macrophages, and mast cells) seem to be activated, thus producing pro-inflammatory cytokines. This event leads eventually to the local recruitment of other immune cells, triggering the immune cascade, with production of eicosanoids, NO, and resultant changes in microvascular tone among other factors that ultimately lead to tissue injury. The primum movens of the inflammatory cascade in atherosclerosis is still poorly understood. However, it has been shown that hypercholesterolemia can lead to the focal activation of endothelial cells by inducing the secretion of phospholipids, which bring about hemodynamic changes and accumulation of lipids that in turn trigger inflammation. On the basis of these observations, novel therapeutic approaches using immunomodulatory interventions (immunosuppressants, vaccines) have been attempted with encouraging results in both prevention and cure of atherosclerosis and CAD.

The detection of high serum levels of pro-inflammatory cytokines in patients with chronic heart failure emanating from various underlying heart diseases, has prompted a radical change of thinking with regard to the pathogenesis of this condition. In fact, patients with chronic heart failure show an imbalance in their cytokine network with a predominant pro-inflammatory pattern, particularly with the production of adhesion molecules, TNF-α and IL-6. Restoring this inflammatory imbalance might prove critical for achieving an effective treatment for patients with this debilitating condition. Also, we note that long-term exposure to pro-inflammatory cytokines can be functionally and structurally detrimental to the myocardium and, accordingly, elevated levels of these soluble mediators of inflammation are also associated with a poor prognosis in chronic heart failure. So far, several immunomodulatory therapeutic approaches have been attempted with conflicting results. Although antiTNF-α therapy has shown no efficacy in clinical trials, promising results have been reported using intravenous immunoglobulin therapies.

In this review article, we summarize the available evidence for the influence of cocoa flavanols on various mediators of inflammation, including eicosanoids, NO, platelets, and cytokines, with particular regard to the cardiovascular implications of the findings.

### EFFECTS OF COCOA FLAVANOLS ON EICOSANOIDS

Eicosanoids are lipid products derived from the metabolism of arachidonic acid, which play a significant role as mediators of the acute inflammatory cascade. They act on vascular permeability and tone and in the recruitment of various inflammatory cells with differing effects. Two major enzyme pathways lead to the

### TABLE 1. Composition (%) of Flavanols and Related Procyanidins in Brazilian Cocoa Beans. Modified from Adamson et al

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>% Contribution by Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer</td>
<td>9.82</td>
</tr>
<tr>
<td>Dimer</td>
<td>13.25</td>
</tr>
<tr>
<td>Trimer</td>
<td>9.85</td>
</tr>
<tr>
<td>Tetramer</td>
<td>10.49</td>
</tr>
<tr>
<td>Pentamer</td>
<td>10.51</td>
</tr>
<tr>
<td>Hexamer</td>
<td>12.68</td>
</tr>
<tr>
<td>Heptamer</td>
<td>7.98</td>
</tr>
<tr>
<td>Octamer</td>
<td>8.44</td>
</tr>
<tr>
<td>Nonamer</td>
<td>11.56</td>
</tr>
<tr>
<td>Decamer</td>
<td>5.42</td>
</tr>
</tbody>
</table>

![Chemical structures of flavan-3-ols and procyanidins.](image)
production of eicosanoids, the cyclooxygenases (COX) for prostaglandins (PG) and thromboxanes (Tx) and the lipoxygenases for leukotrienes (LT).

The cyclooxygenase pathway includes the constitutive COX-1 and the inducible COX-2, both leading to the production of PG (D_2, E_2, F_2) and Tx (A_2). Although prostacyclin’s properties are essentially anti-inflammatory, including vasodilation and inhibition of platelet aggregation, Tx_A2 promotes platelet aggregation and vasoconstriction. An imbalance in PG1_2/TxA2 production is an early event in the formation of thrombi in the coronary and cerebral blood vessels resulting in myocardial infarction and stroke, thus making intervention at this level a promising approach. The effects of cocoa products on the PG1_2/TxA2 balance were investigated in subjects consuming a diet rich in cocoa powder and dark chocolate which supplied 466 mg flavanols per day for 4 weeks. Results indicated that such a diet did not alter urinary thromboxane and prostacyclin concentrations. However, we note that urine samples were analyzed 24 hours after the intake of cocoa products, thus possibly allowing plasma flavanol levels to return to baseline values. Although the effect of cocoa flavanols on COX remains to be completely understood, certain flavanols have been shown to effectively inhibit the enzyme activities of both isoflavones.

In the LT-producing pathway, 5-lipoxygenase is a critical enzyme in the synthesis of these pro-inflammatory eicosanoids. The effects of LT can vary significantly. In fact, whereas LTB_4 is a potent chemotactic agent for neutrophils, cysteinyl-containing LTC_4, D_4, and E_4 each cause intense vasoconstriction, bronchospasm, and increased vascular permeability. Accordingly, an overproduction of LT of the latter type is also regarded as a key event in the bronchial inflammation observed in asthma, thus suggesting a rationale for novel and effective antieicosanoid treatments. The effects of flavanol-rich cocoa products on LT patterns were demonstrated in subjects consuming high flavanol chocolate. These individuals had lower levels of plasma leukotrienes (LTC_4 + LTD_4 + LTE_4) and increased levels of plasma prostacyclin 2 hours after treatment resulting in a 52% decrease in the PG1_2/LT ratio. We should also note that, unlike the study mentioned earlier, in this case the authors analyzed samples from a time point compatible with the peak plasma concentrations of flavanols. Further studies have demonstrated that the effects of cocoa flavanols on LT synthesis are partly mediated by the inhibition of human 5-lipoxygenase. It is also interesting to note that cocoa flavanols also reduced the enzyme activities of soybean 15-lipoxygenase L-1 and the mammalian leukocyte-derived and platelet-derived 12-lipoxygenases, thus implying a possible general lipoxygenase-inhibitor effect.

We submit that, when considered collectively, the available data (summarized in Table 2) support the concept that cocoa-derived flavanols can modulate the synthesis and effects of eicosanoids and possibly exert anti-inflammatory effects in humans.

<table>
<thead>
<tr>
<th>TABLE 2. Synopsis of the Anti-inflammatory Effects of Cocoa Flavanols on Eicosanoid Production, Platelet Activation, and NO-dependent Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eicosanoid production:</td>
</tr>
<tr>
<td>Thromboxane A_2: Prostacyclin ratio</td>
</tr>
<tr>
<td>Leukotriene (LTC_4 + LTD_4 + LTE_4):</td>
</tr>
<tr>
<td>Prostacyclin ratio</td>
</tr>
<tr>
<td>Lipoxigenase activity</td>
</tr>
<tr>
<td>Platelet activation:</td>
</tr>
<tr>
<td>Gp Ib/IIa expression</td>
</tr>
<tr>
<td>P-selectin expression</td>
</tr>
<tr>
<td>Microparticle formation</td>
</tr>
<tr>
<td>Primary hemostasis</td>
</tr>
<tr>
<td>Nitros oxide-dependent activities:</td>
</tr>
<tr>
<td>Vasodilation</td>
</tr>
<tr>
<td>Nitrosylated and nitrosated species</td>
</tr>
<tr>
<td>eNOS activity</td>
</tr>
<tr>
<td>Peroxynitrite-induced oxidation</td>
</tr>
<tr>
<td>Nitration of tyrosine</td>
</tr>
<tr>
<td>iNOS-derived NO</td>
</tr>
</tbody>
</table>

However, more research is clearly warranted to unambiguously determine the real influence of frequent consumption of flavanol-rich cocoa on eicosanoid metabolism.

EFFECTS OF COCOA FLAVANOLS ON PLATELETS

The intrinsic property of an intact endothelium is to maintain blood in a fluid, clot-free state in normal vessels, mediated in part, by endothelial-derived PG1_2 and NO. After a vascular injury, however, extracellular matrix constituents, normally sequestered beneath an intact endothelium, become exposed to circulating platelets. After adhesion to the extracellular matrix, platelets secrete adenosine diphosphate (ADP) and Tx_A2, which promote their aggregation, thus initiating the thrombotic cascade. Eventually, erythrocytes and lymphocytes also adhere to aggregated platelets, thereby contributing to the inflammatory response that accompanies thrombosis. An abnormal activation of hemostatic processes can result in thrombus formation after relatively minor injury or even in uninjured vasculature. As such, platelets are key players in early atherosclerotic lesions.

The effects of flavanol-rich cocoa consumption on platelet activation in healthy human subjects have been extensively investigated (Table 2). First, Rein et al demonstrated that ingestion of cocoa beverage containing a total of 897 mg of cocoa flavanols, including the higher molecular weight procyanidins, resulted in an inhibition of platelet activation and function. In the same study, it was shown that flavanol-rich cocoa suppressed ex vivo expression of two important mediators of platelet aggregation, glycoprotein Ib/IIa and P-selectin, on un-stimulated and ADP-induced platelets.

In vitro cultures of blood cells demonstrated that such inhibition of activation-dependent platelet surface antigens was partly caused by the cocoa procyanidin trimers and pentamers. In addition, acute exposure to...
flavanol-rich cocoa also modulated the formation of platelet microparticles, which are hemostatically active membrane vesicles formed during physiologic platelet activation.\textsuperscript{42} More importantly, flavanol-rich cocoa consumption was able to suppress platelet function after 6 hours as indicated by a prolonged primary hemostasis.\textsuperscript{37} It should be noted that the amount of cocoa flavanols present in the cocoa used in this study is much greater than the amount found in typical cocoa or chocolate beverages commonly available to the public. Subsequent work by the same group demonstrated that the consumption of chocolate containing 220 mg of total flavanols also exerted similar effects on platelet function.\textsuperscript{39} Long-term ingestion of flavanol-rich cocoa (234 mg per day for 4 wk) also significantly reduced P-selectin expression, and ADP-induced or collagen-induced platelet aggregation.\textsuperscript{41} Finally, flavanol-rich cocoa was recently discovered to exert inhibitory effects on platelet function qualitatively similar to aspirin, but less potent.\textsuperscript{40}

**NO-MEDIATED EFFECTS OF FLAVANOLS**

The role of NO in inflammatory processes is complex and involves 2 main pathways. First, NO plays an important role in vascular function,\textsuperscript{43} particularly as a potent vasodilator to maintain vascular tone, as demonstrated in animal models lacking isoforms of NO synthase (NOS).\textsuperscript{44} Interestingly, endothelium-derived NO also inhibits leukocyte recruitment\textsuperscript{45} and platelet aggregation\textsuperscript{46} to the site of inflammation. Furthermore, in situ neutrophils and macrophages can significantly increase local NO concentrations through the inducible isoform of NOS (iNOS), producing significantly higher amounts compared with the constitutive endothelial NOS (eNOS).\textsuperscript{47} The resulting picture therefore indicates that low NO concentrations produce vasodilation, whereas high concentrations cause excessive vasorelaxation that contributes to hypotension eventually leading to systemic shock. Second, NO is a free radical that reacts with superoxide to form more active intermediates, such as peroxynitrite, responsible for toxic protein nitrosylation and/or nitration. Therefore, prolonged exposure to high concentrations of iNOS-derived NO are potentially detrimental to the host during infectious processes.\textsuperscript{48} Studies have been conducted to determine the effects of cocoa flavanols on both aspects of NO activity.

Because it is now clear that NO is an essential signaling molecule in vascular physiology, a study was conducted to examine the acute vascular effects of a single dose of a cocoa drink containing 176 mg of flavanols on healthy individuals who consumed flavanol-rich cocoa for 5 days (821 mg of flavanols/d) displayed a marked tendency to vasodilation,\textsuperscript{50} mediated in part by NO because the administration of \(N^G\)-nitro-L-arginine methyl ester (\(L\)-NAME; an arginine analog that blocks NO synthesis) completely reversed the vasodilation response. An in vitro study was also performed to evaluate the effects of cocoa flavanols on endothelium-dependent relaxation (EDR), which demonstrated that larger molecular weight cocoa flavanols, that is, the procyanidins, particularly the tetramer through decamer, induced EDR in rabbit aortic rings, whereas the monomer, dimer, and trimer fractions failed to elicit an EDR.\textsuperscript{10} Similar to what was observed in humans, this effect was abolished by \(L\)-NAME.

Flavonoids are considered capable of protecting against the cytotoxic effects of peroxynitrite, either by efficiently scavenging its precursors (NO and superoxide) or by preventing its interaction with cell targets.\textsuperscript{51} In vitro studies have shown that cocoa flavanols protect against peroxynitrite-induced oxidation of dihydroorhodamine 123 and the nitration of tyrosine.\textsuperscript{52,53} In particular, the tetramer fraction was more efficient at protecting against oxidation and nitration reactions than the monomeric flavanol (-)-epicatechin. In a more recent study, an aqueous extract of cocoa inhibited NO production in murine macrophages challenged by lipopolysaccharide and interferon-\(\gamma\).\textsuperscript{17}

**EFFECTS OF COCOA FLAVANOLS ON CYTOKINE PRODUCTION**

Emerging evidence suggests that certain flavanols from cocoa may reduce the production of specific pro-inflammatory cytokines, although stimulating the production of anti-inflammatory cytokines such as tumor growth factor (TGF)-\(\beta\) and IL-4. The modulatory effects of the cocoa flavanols on peripheral white blood cells are illustrated in Table 3.

**Pro-inflammatory Cytokines**

Cocoa flavanols share the capability to effectively suppress the production of cytokines that promote inflammation. The case of IL-2 illustrates this concept. The stimulation of resting T cells by mitogenic lectins, such as phytohemagglutinin (PHA), activates a cascade of signaling events that includes up-regulation of transcription factors (ie, NF-\(\kappa\)B, AP-1, and NF-AT), all leading to the transcription and secretion of IL-2.\textsuperscript{54} In vitro, pentamer, hexamer, and heptamer cocoa flavanol fractions dramatically inhibited IL-2 mRNA expression by cultured PHA-stimulated peripheral blood mononuclear cells (PBMC).\textsuperscript{12,55} Also, cacao liquor polyphenol suppressed IL-2 production from PHA-stimulated PBMC at both the mRNA and the protein level.\textsuperscript{13} In this latter case, the preparation used contained approximately 50% polyphenols, 3% of which were in the form of (-)-epicatechin.

IL-\(\beta\) and TNF-\(\alpha\), are considered to be the major cytokines that mediate inflammation each demonstrating...
effects that lead to endothelial cell activation, leukocyte recruitment and activation, and systemic acute-phase reaction induction.

In particular, both cytokines induce the synthesis of endothelial adhesion molecules and chemical mediators, including other cytokines, chemokines, growth factors, eicosanoids, and NO. These events combine to increase the thrombogenicity of the endothelium. Furthermore, IL-1β induces the production of proatherogenic C-reactive protein, a recently validated marker for CAD. TNF-α, on the other hand, is critical in the hemodynamic signs of septic shock including severe hypotension, tachyarrhythmia, and metabolic acidosis.

Our group investigated the effects of cocoa flavanols and their related procyanidins on the production of proinflammatory cytokines by PBMC. We demonstrated a biphasic effect with the smaller flavanols fractions (monomer through tetramer) exhibiting an anti-inflammatory response by suppressing IL-1β mRNA expression and protein secretion and the larger molecular weight fractions (pentamer through decamer) stimulating IL-1β production. Interestingly, the trimeric through decameric fractions were the most active on TNF-α production, causing as much as a 4-fold increase compared with controls, whereas the monomeric and dimeric flavanols fractions showed only slight increases in this cytokine production. Thus, although not all cocoa flavanol fractions elicit a common anti-inflammatory response, we suggest that the inhibition of IL-2, IL-1β, and TNF-α might be dependent on the degree of polymerization of the flavanols as well as the target cytokine. We are aware, however, that further studies are needed to determine which particular cocoa flavanols combination will lead to the most beneficial effects by suppressing pro-inflammatory cytokine production.

Anti-inflammatory Cytokines

Our in vitro study has also demonstrated that cocoa flavanols can modulate the levels of anti-inflammatory cytokines, being particularly effective on IL-4 and TGF-β production. IL-4-mediated effects include the promotion of immunoglobulin E production by B cells, hematopoiesis, and development of effector T-cell responses. CD4+ T lymphocytes secrete IL-4, which in turn activates B cells and promotes IgG1 and IgE responses while inhibiting macrophage function. Similar to what was observed with TNF-α, we demonstrated that large cocoa flavanol fractions (pentamer through decamer) can enhance IL-4 protein levels from resting PBMC, whereas the smaller molecular weight fractions (monomer through tetramer) were nonstimulatory. However, in PHA-stimulated cells the monomer was the only flavanol fraction able to induce IL-4. TGF-β is a pleiotropic cytokine involved in regulating the repair and regeneration of tissue after injury. The most abundant of the three isoforms, TGF-β1, has received attention as the mediator of cardiovascular protection because Grainger and Metcalf proposed the “protective cytokine” hypothesis. This hypothesis was based on the evidence that TGF-β1 actively maintains the normal physiologic phenotype of endothelial cells and smooth muscle cells in the arterial wall, thereby inhibiting the activation of endothelial cells, and suppressing migration, dedifferentiation, and proliferation of smooth muscle cells induced by atherogenic agents. In support of TGF-β1 as an inhibitor of atherogenesis, in vivo studies have shown decreased levels of the active form of TGF-β1 in subjects with advanced atherosclerosis. On the other hand, excess production of TGF-β1 can cause extracellular matrix accumulation that is unfavorable in the injured vessel wall, consequently leading to cardiac fibrosis. Interestingly, increases in the active form of TGF-β1 are associated with the occurrence and severity of CAD. Furthermore, a correlation exists between a high-producing TGF-β1 genotype and an early onset of CAD after cardiac transplantation.

On the basis of this evidence, a variety of agents have been suggested to augment the production of TGF-β1, although the use of TGF-β1 antagonists such as decorin might prove beneficial in the treatment of fibrotic cardiovascular diseases. Resveratrol, a dietary plant polyphenol, was reported to exert a protective effect against dysfunction in vascular smooth muscle cells, in part due to the inhibition of TGF-β1 expression. Our study of the effects of cocoa flavanols on TGF-β1 secretion demonstrated 2 different responses based on baseline cytokine production in healthy subjects. Individuals with low baseline levels of TGF-β1 were stimulated by all flavanol fractions to produce large amounts of the cytokine, whereas elevated baseline TGF-β1 production was inhibited by cocoa flavanols.

Last, although IL-5 is not a prototypical anti-inflammatory cytokine it is able to play a role in eosinophil infiltration and maturation during allergic inflammation. Furthermore, the cytokine’s capacity to

### TABLE 3. Anti-inflammatory Effects of Specific Cocoa Flavanols on Cytokine Production

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Flavanol(s)</th>
<th>Effect</th>
<th>Level of Detection</th>
<th>PBMC Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>5,6,7-mer</td>
<td>Inhibition</td>
<td>mRNA</td>
<td>PHA-stimulated</td>
<td>49, 49</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1,2,3,4-mer</td>
<td>Inhibition</td>
<td>Protein/mRNA</td>
<td>PHA-stimulated</td>
<td>49, 54</td>
</tr>
<tr>
<td>IL-4</td>
<td>5,6,7,8,9,10-mer</td>
<td>Stimulation</td>
<td>Protein</td>
<td>Resting</td>
<td>49, 57</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>Monomer</td>
<td>Stimulation (low producers)</td>
<td>Protein</td>
<td>Resting</td>
<td>67</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>Protein</td>
<td>Inhibition (high producers)</td>
<td>Protein</td>
<td>Resting</td>
<td>67</td>
</tr>
<tr>
<td>Interleukin-5</td>
<td>1,2,3,4,5-mer</td>
<td>Stimulation</td>
<td>Protein</td>
<td>PHA-stimulated</td>
<td>74</td>
</tr>
</tbody>
</table>

All cocoa flavanols stimulate the secretion of TNF-α.

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induce the maturation of IgA-producing plasma cells suggests that the induction of IL-5 might in fact protect against inflammation associated with chronic infections observed in periodontal diseases, which have been repeatedly associated with CAD in epidemiologic studies. In particular, chronic periodontal infections might produce an inflammatory immune response that may contribute to coronary atherogenesis, possibly mediated by an abnormal humoral response involving IgA. Smaller molecular weight cocoa flavanols (monomer through pentamer) can enhance mitogen-induced secretion of IL-5 whereas larger molecular weight flavanol fractions (hexamer through decamer) inhibit IL-5 production in PHA-stimulated PBMC from healthy subjects. The increment in IL-5 suggests that specific cocoa flavanols may preferentially stimulate IgA, which could in turn reduce the risk for dental caries and periodontal disease.

Taken together, these data suggest that cocoa flavanols display the capacity to modulate cytokine levels thus potentially attenuating inflammatory processes. However, it should be emphasized that no individual cocoa flavanol should be regarded as the sole agent responsible for beneficial effects. Rather, depending on the cytokine of interest, it is more likely that specific fractions or possibly an undetermined mixture of flavanols will produce the observed results desired in inflammatory mediated diseases, such as atherosclerosis and CAD. These findings in cultured PBMC treated with cocoa flavanols are potentially intriguing and warrant further studies.

**COCOA FLAVANOLS AND THE NUCLEAR FACTOR-KB PATHWAY**

Nuclear Factor-kB (NF-kB) is one of the most important inducible transcription factors in mammals; in particular, it regulates the expression of genes involved in controlling the inflammatory response, cellular proliferation/growth, and cell adhesion. NF-kB consists of different combinations of Rel proteins (ie, p65 or RelA, RelB, c-Rel, p50, and p52) in various heterodimers and homodimers, with p50/p65 representing the most abundant activated form of NF-kB. In resting cells, the inactive form of NF-kB resides in the cytoplasm bound to an inhibitory protein known as IκB. Upon cellular activation by extracellular stimuli, the dissociation of IκB from NF-kB allows the activated free dimer to translocate into the nucleus where it induces the transcription of several genes by binding to κB motifs found in the promoter or enhancer region. As an activator of many inflammatory processes, including pro-inflammatory cytokines, PGs, and NO, the modulation of the NF-kB signaling pathway is a potential key target for modulating inflammation. Several chemoprotective phytochemicals [ie, curcumin, epigallocatechin gallate (EGCG), resveratrol] have been shown to inhibit COX-2 and iNOS expression by preventing NF-kB activation. A recent investigation has shed light on possible molecular mechanisms by which cocoa flavanols might regulate NF-κB activation. (−)-Epicatechin, (+)-catechin, and dimeric flavanols are able to inhibit phorbol myristate acetate (PMA)-induced NF-kB activation at multiple steps in specific experimental settings. These compounds were not only incorporated into cultured T cells, but they also exhibited a dose-dependent accumulation within the nuclei. Cells pretreated with flavanols or flavanol dimers could inhibit the PMA-induced NF-kB-DNA binding activity resulting in a significant IL-2 decrease. The reduction in DNA binding activity was not a consequence of lower levels in p50 or p65 subunits but was rather determined by blocking the binding of active NF-kB to the DNA κB motifs. These compounds were also capable of interfering at the early stages of NF-kB activation because pretreatment with flavanols reduced PMA-stimulated intracellular antioxidants, an important event in the initiation of NF-κB activation. Further downstream in the NF-kB activation cascade, flavanols can also directly interact with IκB by preventing its phosphorylation thus preventing the release of activated NF-kB. In summary, our data ultimately suggest plausible molecular mechanisms underlying the previously presented anti-inflammatory effects of cocoa flavanols on the production of cytokines, eicosanoids, and NO. A scheme of the proposed mechanisms is depicted in Figure 2.

**FURTHER CONSIDERATIONS AND CONCLUSIONS**

A growing body of evidence suggests that flavanols present in fresh cocoa and certain finished cocoa-derived products may modulate other steps of the inflammation cascade. In particular, the anti-inflammatory actions of certain cocoa flavanols and/or flavanol-rich cocoa includes their ability to alter eicosanoid and cytokine production, inhibit platelet aggregation, and promote favorable levels of NO. However, most of the data supporting the hypothesis that cocoa flavanols can impart a meaningful anti-inflammatory action are obtained from in vitro studies. Additional in vivo research is therefore needed to clarify the potential of cocoa flavanols in this context. Several studies have demonstrated that epicatechin is rapidly absorbed in humans, with plasma levels detected after 30 minutes and reaching a peak at 2 to 3 hours after ingestion. However, plasma epicatechin concentrations typically return to baseline values within 6 to 8 hours after consumption of flavanol-rich chocolate. It has been proposed that, in the acidic environment of the gastric milieu, a significant proportion of flavanol oligomers are hydrolyzed into monomers and dimers, enhancing their absorption in the small intestine whereas possibly modifying their biologic activity. However, it should be noted that Rios et al demonstrated that most ingested high molecular weight flavanol oligomers seem to reach the small intestine intact and are available for absorption. More research is needed to
define the fate of flavanols during digestion and/or absorption.

In conclusion, cocoa-derived products that are sufficiently rich in flavanols have the potential to positively modulate the inflammatory status that characterizes several chronic and acute cardiovascular diseases. However, further studies are needed, especially in the context of well-designed clinical trials, to determine whether the potential observed thus far in experiments can, in fact, translate to providing advantages in the context of treating and/or preventing morbidity and mortality associated with cardiovascular diseases.

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