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REVIEWS: CURRENT TOPICS

Mechanisms by which cocoa flavanols improve metabolic syndrome and related disorders☆

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Abstract

Dietary administration of cocoa flavanols may be an effective complementary strategy for alleviation or prevention of metabolic syndrome, particularly glucose intolerance. The complex flavanol composition of cocoa provides the ability to interact with a variety of molecules, thus allowing numerous opportunities to ameliorate metabolic diseases. These interactions likely occur primarily in the gastrointestinal tract, where native cocoa flavanol concentration is high. Flavanols may antagonize digestive enzymes and glucose transporters, causing a reduction in glucose excursion, which helps patients with metabolic disorders maintain glucose homeostasis. Unabsorbed flavanols, and ones that undergo enterohepatic recycling, will proceed to the colon where they can exert prebiotic effects on the gut microbiota. Interactions with the gut microbiota may improve gut barrier function, resulting in attenuated endotoxin absorption. Cocoa may also positively influence insulin signaling, possibly by relieving insulin-signaling pathways from oxidative stress and inflammation and/or *via* a heightened incretin response. The purpose of this review is to explore the mechanisms that underlie these outcomes, critically review the current body of literature related to those mechanisms, explore the implications of these mechanisms for therapeutic utility, and identify emerging or needed areas of research that could advance our understanding of the mechanisms of action and therapeutic potential of cocoa flavanols.

Keywords: Epicatechin; Procyanidins; Diabetes; β-Cells; GLP-1; Endotoxin

1. Introduction

1.1. Metabolic syndrome

Metabolic syndrome is a cluster of related conditions that increases an individual's risk for developing cardiovascular disease and Type 2 diabetes mellitus (T2DM) [1,2]. The components of metabolic syndrome include abdominal obesity, dyslipidemia, elevated blood pressure, insulin resistance, glucose intolerance, β-cell loss, low-grade chronic inflammation and a prothombotic state [1–3]. The prevalence of obesity, cardiovascular disease and diabetes has been increasing in the United States and worldwide for the past several decades. Approximately one in ten adults in the United States has diabetes, one in three has a cardiovascular disease and one in three is obese [4,5]. Many individuals with metabolic syndrome will progress to the full expression of these diseases. The prevalence of metabolic syndrome is now greater than 34% in the U.S. [6]. Increasing attention has been directed toward finding novel strategies to prevent, slow the onset and/or progression of and potentially reverse metabolic syndrome [7].

1.2. Flavanols and metabolic syndrome

Dietary flavanols offer an interesting potential complementary strategy that may improve this complex, multifaceted syndrome. First, flavanols may help reduce glucose excursion by slowing digestion and enhancing the incretin response. Second, flavanols may help reduce systemic endotoxin exposure *via* improvement in gut barrier function. While flavanols from a variety of dietary sources appear promising, cocoa flavanols represent an emerging approach for intervention in metabolic syndrome. Following an overview of polyphenols, this review will focus on flavanols found in cocoa. Cocoa bioavailability will be briefly reviewed, followed by a summary of the primary research utilizing cocoa, and lastly, the hypothesized mechanisms by which cocoa flavanols improve metabolic syndrome will be discussed.

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2. Cocoa flavanols

2.1. Flavanols

Polyphenols are secondary metabolites found ubiquitously in plants. One prominent subclass of polyphenols is the flavonoids. The basic flavonoid skeleton consists of two benzene rings linked by a 3 carbon heterocyclic (*O*-containing) ring (Fig. 1A). Flavonoids are further divided into subclasses based on the nature of the heterocyclic ring and substituents: flavanols, flavonols, flavones, flavanones, isoflavones and anthocyanins [8]. Flavanols are hydroxylated at C3 in the heterocyclic ring (Fig. 1B) and are thus sometimes referred to as *flavan-3-ols*. This hydroxyl group may be modified by an addition of a gallate group. Flavanols may exist as monomers, or as oligomers/polymers [with various degrees of polymerization (DP)] comprised of flavanol monomer residues (known as *proanthocyanidins*). Major dietary flavanol monomers include (+)-catechin (+C), (−)-catechin (−C), (−)-epicatechin (EC) (Fig. 1C) and others. Cocoa is unique in that it is the only significant dietary source of −C. Procyanidins (PCs, as opposed to prodelphinidins) specifically refer to proanthocyanidins with predominantly catechin and epicatechin monomer residues [9]. A representative cocoa procyanidin dimer is shown in Fig. 2. Although largely beyond the scope of this review, PCs may also contain either A- or-B-type linkages [10]. Cocoa, the focus of this review, contains PCs with B-type linkages.

2.2. Dietary sources of flavanols

Significant levels of flavanols are found in a variety of dietary plants including tea, apples, grapes, cocoa, berries, plums, apricots and nuts [9,11–13]. The flavanol content is higher in certain foods such as grapes, tea and cocoa, compared to other plants, and thus the body of literature focuses on these products. Cocoa is generally regarded as the most concentrated dietary source of flavanols with the strongest antioxidant potential [7,14].

Although many potentially bioactive compounds are found in cocoa, many of the health benefits associated with its consumption are likely due to its high flavanol content. Cocoa is composed of flavanol monomers, oligomers, and polymers [15]. The most common monomers found in cocoa are epicatechin (up to 35% of polyphenol content) [16,17], as well as (±)-catechin. It is important to note that cocoa is one of the few foods with appreciable levels of (−)-catechin, which is produced by epimerization of (+)-catechin during fermentation. Cocoa contains PCs composed of up to 12 monomeric residues [18], although larger species likely exist but are not easily measured by common chromatographic methods. There can be great variability in cocoa phenol content from *Theobroma cacao* plants of different origins [16] and the polyphenol content of cocoa powder is largely dependent on processing methods.

The impacts of tea and grape seed on metabolic syndrome have been extensively reviewed and analyzed [19–22]. Furthermore, there is a large body of literature regarding the effects of cocoa on cardiovascular disease [23–25]. However, the potential link between cocoa and improvements to metabolic syndrome and, specifically, glucose homeostasis and diabetes is a newer, less-studied area and warrants further investigation and a review of the current literature. Therefore, this review focuses specifically on the potential mechanisms by which cocoa flavanols improve metabolic syndrome, particularly glucose homeostasis and diabetes.

2.3. Bioavailability of cocoa flavanols

Understanding flavanol bioavailability is critical for identifying flavanol bioactivities [13]. Bioavailability of cocoa flavanols from food is a multistep process including digestion and release of flavanol from its food matrix, solubilization and absorption into enterocytes, xenobiotic metabolism in the enterocytes, liver and colon and, lastly, elimination [26]. While an exhaustive discussion of flavanol bioavailability is beyond the scope of this review, unique aspects of cocoa flavanol bioavailability warrant mention as they pertain to mechanism.

Potential PC instability during gastric transit has been suggested as a factor limiting bioavailability of orally administered flavanols. PCs could be hydrolyzed to form monomers (or partially hydrolyzed to form monomers and smaller PCs) in the low pH conditions of gastric juice. Spencer *et al.* [27] reported that PC oligomers (up to DP 6) were degraded to monomeric flavanol residues when incubated in an acidic solution (pH~2.0) for up to 3.5 h. However, there are conflicting reports on this phenomenon in both animals and humans [28–33]. Tsang *et al.* [30] found that polyphenols from grape seed extract (catechin, epicatechin PC dimers, trimers and tetramers) were intact in the GI tract after an oral gavage in Sprague–Dawley rats. They concluded that there was neither a sizeable increase in monomers nor a concomitant decrease in oligomers, suggesting that the oligomers were stable through gastric transit [30]. Rios *et al.* [28] reported that PCs were intact after being ingested with a meal in humans. After participants drank a 500-ml cocoa beverage, the pH of the stomach was elevated, keeping the cocoa powder protected from an extremely acidic environment (such as the environment utilized in the study conducted by Spencer *et al.* [27]). Further, the *in vivo* study showed that the 500-ml beverage was emptied from the stomach in about 50 min, whereas the incubation study lasted up to 3.5 h [28]. Therefore, it appears that PCs, as well as monomeric flavanols, remain intact during gastric transit. Some depolymerization may occur, but the amount is so small that any increase in monomer concentration would be negligible [9,30]. Therefore, gastric degradation is unlikely to limit flavanol bioavailability and bioactivity.

Bioavailability is thought to reduce potential flavanol bioactivity *in vivo*. Monomers (catechin and epicatechin) are relatively well absorbed compared to PCs [28,34,35]. They first appear in the circulation 30–60 min after ingestion [36] and reach peak plasma concentrations at 2–3 h [28]. Epicatechin appears in greater concentrations in human plasma than catechin. Holt *et al.* [37] reported that there is a preferential absorption of epicatechin. When catechin and epicatechin were given to participants in equal concentrations, there was 5.92-μM epicatechin but only 0.16-μM catechin in the plasma 2 h after ingestion [37]. Furthermore, the (+)-catechin is more bioavailable than (−)-catechin, which predominates in fermented cocoa [38]. Dimeric, trimeric and tetrameric PCs are also absorbed in their intact form but at a much lower rate compared to the monomers [9]. Interestingly, Deprez *et al.* [39] showed that (+)-catechin and PC dimers and trimers had similar permeability coefficients as mannitol (an indicator of paracellular transport) in Caco-2 monolayers. Therefore, these smaller flavanols are likely entering the bloodstream *via* paracellular diffusion [39,40]. Polymers larger than tetramers are generally not absorbed intact [9] and proceed to the colon, along with unabsorbed fractions of monomers and smaller PCs. Approximately 5–10% of polyphenols can be absorbed in the small intestine while the remaining 90–95% proceed to the colon [41]. Poor PC bioavailability therefore is likely a main factor that limits bioactivity in peripheral tissues, particularly for larger PCs. Their relatively low bioavailability indicates that the gut may be the primary location of action for cocoa PCs due to the high concentrations present there compared to levels in circulation [9,42]. Concentrations of flavanols in the blood and tissues are typically less than 5 μM [37,43–45], which are at the lower end of concentrations typically used *in vitro* to asses bioactivity in cell models [46]. However, when the intestinal lumen or epithelial surface is the site of action (such as inhibition of digestive enzymes or absorption transporters, modulation of gut barrier integrity, *etc.*), bioavailability is not a limiting factor.