Effect of cocoa/chocolate ingestion on brachial artery flow-mediated dilation and its relevance to cardiovascular health and disease in humans

Kevin D. Monahan*
Penn State Heart and Vascular Institute, Pennsylvania State University College of Medicine, Hershey, PA 17033, United States

**A R T I C L E I N F O**

Article history:
Available online 7 March 2012

Keywords:
Flavonoid
Flavanol
Chocolate
Endothelium
Endothelial function

**A B S T R A C T**

Prospective studies indicate that high intake of dietary flavanols, such as those contained in cocoa/chocolate, are associated with reduced rates of cardiovascular-related morbidity and mortality in humans. Numerous mechanisms may underlie these associations such as favorable effects of flavanols on blood pressure, platelet aggregation, thrombosis, inflammation, and the vascular endothelium. The brachial artery flow-mediated dilation (FMD) technique has emerged as a robust method to quantify endothelial function in humans. Collectively, the preponderance of evidence indicates that FMD is a powerful surrogate measure for firm cardiovascular endpoints, such as cardiovascular-related mortality, in humans. Thus, literally thousands of studies have utilized this technique to document group differences in FMD, as well as to assess the effects of various interventions on FMD. In regards to the latter, numerous studies indicate that both acute and chronic ingestion of cocoa/chocolate increases FMD in humans. Increases in FMD after cocoa/chocolate ingestion appear to be dose-dependent such that greater increases in FMD are observed after ingestion of larger quantities. The mechanisms underlying these responses are likely diverse, however most data suggest an effect of increased nitric oxide bioavailability. Thus, positive vascular effects of cocoa/chocolate on the endothelium may underlie (i.e., be linked mechanistically to) reductions in cardiovascular risk in humans.

© 2012 Elsevier Inc. All rights reserved.

**Introduction**

Cardiovascular disease (CVD) is the leading cause of death in developed countries [1]. Thus, effective strategies to prevent, delay, or reverse CVD and its sequelae are critical to identify. Diet may be important in this context. Previous research indicates that cardiovascular-related morbidity and mortality are reduced in individuals who consume a high concentration of flavonoids in their diets [2–5]. Specific subclasses of flavonoids, such as flavanols, may be particularly effective in reducing cardiovascular risks [3]. Consistent with this view, increased dietary intake of cocoa/chocolate, foods that are a rich source of flavanols, is associated with reduced rates of cardiovascular and all-cause mortality in humans.

**Epidemiological evidence linking cocoa/chocolate intake with cardiovascular outcomes**

Numerous lines of evidence link increased cocoa/chocolate consumption with reduced rates of cardiovascular events, such as myocardial infarction and stroke, as well as reduced rates of cardiovascular and all-cause mortality in humans. Prospective epidemiological studies indicate that in adults free of CVD at study entry, those who habitually consumed the largest quantities of cocoa/chocolate had the lowest subsequent rates of cardiovascular and all-cause mortality over the follow-up period. Specifically in the Zutphen Elderly Study, cardiovascular-related mortality was approximately 50% lower in older men ingesting the highest compared to the lowest tertile of cocoa over 15 years of follow-up [6]. The Iowa Women’s Health Study found a similar association with chocolate intake and cardiovascular mortality over a 16 year follow-up period, although after multivariate adjustment this effect was not as pronounced (p = 0.06) [3]. Similarly, rates of myocardial infarction and stroke have been reported to be lower in individuals who habitually consume the largest quantities of chocolate [7]. These studies provide strong evidence for the previously developed view that improved cardiovascular health in the Kuna Indians may be the result of high levels of habitual cocoa intake [8,9]. However, an important limitation of these studies is the fact that none were randomized trials. In fact at present there are no randomized controlled trials that have determined the effect of cocoa/chocolate intake on key cardiovascular outcomes such as cardiovascular-related mortality, myocardial infarction, or stroke in humans. Additionally, other recent reports indicate that the risk of developing heart failure [10], as well as mortality after first myocardial infarc-
Mechanisms underlying the cardioprotective effect of cocoa/chocolate

Various mechanisms are likely to contribute to the cardioprotective effect of dietary cocoa/chocolate ingestion in humans. These include blood pressure lowering effects [6], antioxidant effects [12], anti-inflammatory effects [13], anti-platelet effects [14], and effects on blood lipids [15], as well as improved vascular health [16]. The remainder of this review will focus on the possibility that cocoa/chocolate exerts positive effects on the endothelium, which contributes to improved vascular health in humans.

Central role of the endothelium in CVD development and progression

The vascular endothelium is a continuous layer of cells anatomically situated at the intima-lumen interface of all blood vessels of the body. Originally the endothelium was believed to serve as a simple barrier between the vasculature and the bloodstream. However, this overly simplistic view of endothelium and its function in the human vasculature has evolved dramatically in the past decades. Today it is recognized that the endothelium is a highly dynamic organ that contributes critically to the maintenance (in health) or loss (in disease) of vascular homeostasis [17–20]. These effects of the endothelium are mediated by the synthesis and release of various paracrine and autocrine factors, such as nitric oxide (NO), prostacyclin, endothelium-derived hyperpolarizing factor, endothelin-1, and cell adhesion molecules [19,20]. Vascular homeostasis is the net result of effects exerted by these paracrine/autocrine factors on key variables such as vascular tone, vascular permeability, and platelet aggregation/thrombosis. In disease there is an undesirable phenotypic change in the endothelium such that vasoconstriction, thrombosis, inflammation, and arterial wall proliferation are favored [17]. These changes are consistent with the view that abnormal function of the endothelium is a key early event in the atherosclerotic process [21]. For these reasons, having a method(s) to quantify endothelial function would be of great interest from the perspective of being able to assess and compare group(s) and/or individual’s states of cardiovascular and/or vascular health. Additionally, identifying such a method could provide powerful insight into the effectiveness of therapies aimed at improving endothelial function and thus reducing atherosclerotic/cardiovascular risk in individuals.

The brachial artery flow-mediated dilation technique

Twenty years ago Celermajer et al. first described the method of brachial artery flow-mediated dilation, from hereon referred to as FMD,1 to quantify endothelial function in humans [22]. The development of the FMD technique provided a novel way to quantify endothelial function non-invasively in humans. Since this original report, thousands of studies have used the FMD technique to assess within as well as between group(s) differences in endothelial function. The FMD method relies on the use of a high-resolution ultrasound system to image a conduit artery, usually the brachial artery. The ultrasound transducer is positioned proximal to the antecubital crease and a longitudinal image of the brachial artery is obtained. After obtaining ‘baseline’ images a blood pressure cuff positioned on the upper forearm, is inflated (250 mm Hg) for 5-min before being rapidly deflated. After cuff deflation a hyperemia occurs resulting in a large increase in vascular shear stress, which mediates an increase in brachial artery diameter (i.e., FMD) that is largely endothelium dependent. The fact that dilator responses to an FMD trial are endothelium-dependent is supported by the observation that inhibition of nitric oxide (NO) synthesis nearly abolishes this response [23,24]. FMD is quantified as the percent change in brachial artery diameter from baseline (i.e., period before cuff inflation) to peak increase after cuff release. This method appears to provide quantitative insight into the degree of endothelial function/dysfunction, and thus a relative index of vascular and cardiovascular “health” or lack thereof to a physiological stimulus (e.g. and increase in vascular shear). The reader is referred elsewhere for a more extensive review of the FMD method [25,26].

FMD provides a surrogate measure of cardiovascular endpoints in humans

It is not always practical to follow patients/subjects for years or decades to gain insight into cardiovascular risk and its modification by interventions. Therefore, methods or measures that provide strong surrogates for firm cardiovascular endpoints, such as cardiovascular-related mortality, are critical to identify. In this regard brachial artery FMD may be just such a measure for numerous reasons (Table 1). First, FMD is impaired in individuals at elevated risk of having a cardiovascular event. For instance, FMD is reduced in those with risk factors for CVD, such as smokers [27], older adults [28], and those with elevated total and low-density cholesterol levels [29] compared to levels observed in control subjects. Second, impaired FMD is detectable early in the disease process (i.e., before overt clinical disease is observed) [30]. Low levels of FMD are observed in young adulthood in “healthy” offspring of Type 2 diabetics [31], hypertensives [32], and those with a family history of premature coronary artery disease [33]. These data are consistent with the concept that endothelial dysfunction is not only a marker of established CVD, but that abnormal function of the endothelium may contribute to the genesis of disease [17,18,30,34]. Third, in a vast array of populations low FMD is associated with poorer outcomes (i.e., FMD has prognostic significance). For example in healthy adults low levels of FMD are predictive of incident cardiovascular events (e.g. myocardial infarction, stroke, coronary revascularization) [35–39] and cardiovascular-related death [35,36,38,39]. Additionally in a number of patient populations, such as those with hypertension [40], myocardial infarction [41], heart failure [42,43], coronary artery disease [44,45], chest pain without documented coronary artery disease [46], and vascular disease [47–50] FMD predicts future cardiovascular-related morbidity [40–44,46–50] and mortality [40–42,44,45,47,49,50]. Fourth, FMD is modifiable by therapies that alter prognosis/risk. For instance, statin administration [51], weight loss [52], and endurance exercise [28] are all associated with decreased cardiovascular mortality rates as well as increased FMD. Fifth, when serial measurements of FMD are made, any observed changes in FMD help to identify subsequent risk [44,53,54]. In other words, when FMD increases or decreases over time, these changes correspond to decreased and increased risk, respectively. Lastly, FMD has prognostic significance that extends beyond that provided by assessment of conventional risk factors in humans [55]. Collectively, for these reasons FMD appears to provide a strong surrogate for firm cardiovascular endpoints, such as cardiovascular morbidity and mortality in humans. Thus, assessing FMD and changes in this measure over short timeframes likely provides insight into long-term changes in risk in humans.

---

1 Abbreviations used: FMD, brachial artery flow-mediated dilation; CVD, cardiovascular disease; NO, nitric oxide; eNOS, endothelial derived nitric oxide synthase.
Effect of acute and chronic ingestion of cocoa/chocolate on FMD

Ingestion of a high flavanol cocoa drink was first demonstrated to acutely increase FMD in coronary artery disease patients and patients with cardiovascular risk factors about a decade ago [16]. Since this original observation, numerous studies have reported that FMD increases acutely after cocoa/chocolate ingestion in healthy young [56,57] and older adults [58], smokers [59–61], obese [62,63], coronary artery disease patients [16], and diabetics [64]. Similar effects of cocoa/chocolate on FMD are observed in the setting of prolonged (chronic) intake (≥1 week) [60,64–70]. Although most studies have observed increased FMD after cocoa/chocolate ingestion, it is important to note that not all studies have reported positive effects [71].

Although these prior studies were important, a critical question that remained was what compound(s) or metabolite(s) of cocoa/chocolate is responsible for the observed increase in FMD? In this regard, metabolites of epicatechin measured in the blood appear to increase in a time- and dose-dependent fashion [58]. FMD responses after ingestion follow a similar temporal pattern [16,57,59,60,64]. Thus, it is not surprising that increases in FMD correlate with blood levels of epicatechin metabolites after cocoa ingestion [58]. More conclusive evidence that epicatechin, or one of its metabolites, mediates increased FMD after cocoa/chocolate ingestion is provided by the fact that pure epicatechin ingestion increases FMD (Fig. 1) [56]. Collectively, these studies provide strong evidence that cocoa/chocolate improves FMD in a diverse group of individuals, possibly as a result of epicatechin naturally occurring in cocoa/chocolate.

Cocoa/chocolate dose-dependently increases FMD in humans

The preponderance of data supports the concept that cocoa/chocolate ingestion increases FMD in humans. What is less clear, but of equal importance, is what levels of cocoa/chocolate must be ingested to observe these increases? To date, most studies have focused on effects mediated by very high levels of flavanol intake. However, at least three studies have addressed the dose-dependency of increases in FMD after cocoa ingestion in humans. Balzer et al. reported that acute ingestion of a high flavanol cocoa drink (963 mg total flavanols), as well as a moderate flavanol cocoa drink (371 mg total flavanols), increased FMD in diabetic patients, relative to responses observed after ingestion of a low flavanol cocoa drink (75 mg total flavanols) [64]. Responses were significantly greater when high compared to moderate doses of cocoa were ingested (Fig. 2). Additionally, in a small subgroup of smokers (n = 6) acute ingestion of 28, 36, 179, 330, 485, and 918 mg of total flavanols resulted in a dose-dependent increase in FMD [60]. FMD was significantly elevated when cocoa doses containing above 179 mg of total flavanols were ingested. Consistent with these data, we reported that both acute and chronic ingestion of cocoa/chocolate results in a dose-dependent increase in FMD [64–70].
tate conversion of L-arginine into NO. Along similar lines, cocoa lowers vascular arginase activity [77], which could increase substrate availability (L-arginine) for NO synthesis via eNOS. Antioxidants served by Balzer (180 mg total flavanols) [64] and similar to Heiss FMD at levels of cocoa ingestion that were lower than those observed in healthy older adults (Fig. 3). We observed significant increases in FMD after ingestion of chocolate are dose-dependent and occur after ingestion of rather modest quantities of cocoa (5 g). Collectively, such data would be important from both a primary and secondary disease prevention standpoint.

Future directions/conclusions

Cocoa/chocolate appears to exert cardioprotective effects in humans. Effects exerted by cocoa/chocolate on the endothelium may be a critical mediator of these cardioprotective actions. After cocoa/chocolate ingestion, increases in FMD occur in a dose-dependent fashion. The mechanisms underlying increased FMD after cocoa/chocolate ingestion are unknown, but increased bioavailability of NO appears to be critical. It appears that more studies need to be conducted in vivo to provide more conclusive evidence of the specific mechanisms involved. Additionally, further work is required to determine the minimal doses of cocoa/chocolate needed to exert positive effects for numerous outcome measures. Such studies would help to define specific quantities of cocoa/chocolate that needs to be ingested to observe an increase in FMD in healthy older adults appears to be greater than 2 g and at most 5 g, which is a modest quantity (~1 teaspoon of natural cocoa). Collectively, such data would be important from both a primary and secondary disease prevention standpoint.

Mechanisms by which cocoa/chocolate improves FMD

Numerous mechanisms could underlie increased FMD after cocoa/chocolate intake. Many of these revolve around the concept that cocoa/chocolate increases NO bioavailability. Indirect support for this concept comes from the observation that cocoa/chocolate ingestion increases the pool of circulating metabolites of NO [16, 61, 73]. More direct evidence is provided by the fact that increased FMD after cocoa/chocolate ingestion are reversed by NO synthase inhibition [61, 74]. In vitro flavanols increase endothelial derived NO synthase activity (eNOS) [75, 76], which should facilitate conversion of L-arginine into NO. Along similar lines, cocoa lowers vascular arginase activity [77], which could increase substrate availability (L-arginine) for NO synthesis via eNOS. Antioxidant effects of cocoa/chocolate could also play a role as a result of reduced quenching of NO after synthesis, but before reaching the vascular smooth muscle cells. However, as the antioxidant effect of cocoa/chocolate in vivo is still debated [78], the role it plays in FMD improvements is also unclear. Cocoa/chocolate possesses angiotensin converting enzyme inhibitor like activities [79]. As angiotensin converting enzyme inhibitors are effective at increasing FMD in humans [80], it is possible that similar effects occur after ingestion of cocoa/chocolate in humans. As vascular responses to oral nitroglycerin administration are not altered by cocoa/chocolate ingestion [57, 58, 60, 64] it is unlikely that increased FMD reflects a generalized increase in vascular smooth muscle reactivity. Collectively, increased FMD after cocoa/chocolate ingestion likely occurs as a result of increased bioavailability of NO.

**References**


