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Short communication

Effects of bioactive constituents in functional cocoa products on cardiovascular health in humans

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ABSTRACT

Cocoa manufacturers are producing novel products increasing polyphenols, methylxanthines or dietary fibre to improve purported health benefits. We attempt to explain the contribution of cocoa bioactive compounds to cardiovascular effects observed in previous studies, placing particular emphasis on methylxanthines. We focused on a soluble cocoa product rich in dietary fibre (DFCP) and a product rich in polyphenols (PPCP). Effects of regularly consuming DFCP (providing daily 10.17 g, 43.8 mg and 168.6 mg of total-dietary-fibre, flavanols and methylxanthines, respectively) as well as PPCP (providing daily 3.74 g, 45.3 mg and 109.8 mg of total-dietary-fibre, flavanols and methylxanthines, respectively) on cardiovascular health were assessed in two controlled, cross-over studies in free-living normocholesterolemic and moderately hypercholesterolemic subjects. Both products increased HDL-cholesterol concentrations, whereas only DFCP decreased glucose and IL-1β levels in all subjects. Flavanols appeared to be responsible for the increase in HDL-cholesterol, whereas insoluble-dietary-fibre and theobromine in DFCP were associated with the hypoglycemic and anti-inflammatory effects observed.

1. Introduction

In recent years drastic changes have taken place in dietary habits throughout Mediterranean populations, and a marked increase in cardiovascular disease mortality is occurring (Gomez-Huelgas et al., 2011). In Spain, the consumption of meat, fish, fruit and dairy products has increased at the expense of consuming less vegetables, cereals and legumes (Carbajal, 2013). In contrast, cocoa remains a popular foodstuff worldwide. Soluble cocoa products have been popular in Spain, among other countries, being consumed twice a day, at breakfast and as part of an evening break known as 'merienda'. Cocoa is an important source of flavonoids, mainly flavanols, epicatechin and catechin and low molecular weight procyanidins such as procyanidin B1 and B2, methylxanthines (mainly theobromine), and magnesium; all are biologically active substances that may also affect human health positively (Ellam & Williamson, 2013). Cocoa is also a relevant source of dietary fibre (DF) in contrast to chocolate (Sarriá et al., 2012).

The cardio-protective effects of cocoa are well established as several comprehensive reviews recently published have shown (Arranz et al., 2013; Ellam & Williamson, 2013). In an attempt to improve potential health properties, cocoa manufacturers are

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producing new functional cocoa products enriched with bioactive components, with reduced energy (fat and sugar content). Recently, we have shown that two soluble cocoa products rich in flavanols and DF increased serum HDL-cholesterol concentrations in healthy and moderately hypercholesterolemic (2000–2400 mg/L) subjects, the DF rich product also decreased glucose, IL-1β and IL-10 levels (Martínez-López et al., 2014; Sarriá et al., 2014). IL-1β is a pro-inflammatory cytokine that induces transient and reversible endothelial dysfunction in humans as IL-1β can activate NF-κB, which binds to specific sites on the promoter regions of target genes, and this, in turn, modulates the endothelial synthesis of other proinflammatory cytokines (IL-1, IL-6 and TNF-α) and chemokines such as IL-8 and MCP-1 (Kofler, Nickel, & Weis, 2005). This work elaborates on the relative contribution of bioactive compounds, namely flavonoids, DF and methylxanthines, from soluble cocoa products, to the observed health benefit.

2. Material and methods

2.1. Cocoa powder products

The commercialised soluble cocoa products used in the studies were provided by Nutrexpa S.L. As described in Bravo and Saura-Calixto (1998), cocoa extracts were obtained by washing 1 g of defatted cocoa with 40 mL of 50% aqueous methanol (HPLC grade; Panreac, Castellar del Vallés, Spain) containing 0.8% of 2 mol/L hydrochloric acid (Panreac) for 1 h at room temperature with constant shaking. Afterwards, samples were centrifuged (10 min, 3000*g*) and supernatants were collected. Residues obtained were successively extracted with 40 mL of 70% acetone (v/v; Panreac) in water (1 h, constant shaking). Then samples were centrifuged (10 min, 3000*g*) and supernatants were collected. Finally, supernatants obtained after each extraction step were combined and made up to 100 mL. Polyphenolic and methylxanthine composition was characterised by high-performance liquid chromatography (HPLC) with diode-array detection (DAD) using an Agilent 1200 series equipment (Martin et al., 2008). Quantification was achieved in comparison with known standards [catechin, epicatechin, procyanidin B1 and theobromine were purchased from Sigma–Aldrich (St. Louis, USA), procyanidin B2 from Extrasynthese (Genay, France), and caffeine from Fluka Chemika (Buchs, Switzerland)].

Total polyphenols were measured spectrophotometrically using the Folin–Ciocalteu reagent and gallic acid (Sigma–Aldrich), as standard. Total DF analysis was carried out following the same procedure described in Sarriá et al. (2014). The total polyphenol, flavanol, DF and methylxanthine composition of the soluble cocoa product rich in DF (DFCP) and the soluble cocoa product rich in polyphenols (PPCP) is shown in Table 1.

2.2. Study design

Consecutively, two controlled studies were carried out in free-living healthy and moderately hypercholesterolemic (2000–2400 mg/L; 5.172–6.206 mmol/L) subjects to assess the effects of consuming DFCP (Sarriá et al., 2014) and PPCP (Martínez-López et al., 2014) on markers of cardiovascular health. Each study consisted of a two-week run-in, four-week milk stage (control) and four-week cocoa intervention. The doses of cocoa consumed in both studies corresponded with consumption patterns as recommended by the manufacturer: 15 g per serving of the DFCP twice a day (30 g/day), and half that amount of PPCP, 7.5 per serving of PPCP twice per day (15 g/day) since this product had a strong cocoa flavour. For the duration of the studies, other cocoa products, certain fruits and vegetables rich in polyphenols, and their derived beverages, were restricted to reduce inter-individual differences in polyphenol intake and to be able to attribute the results observed to the cocoa products. The daily consumption of flavanols, DF and methylxanthines provided by DFCP and PPCP is shown in Table 2.

Table 1
Flavanol, dietary fibre and methylxanthine composition of the cocoa product rich in dietary fibre (DFCP) and the product rich in flavanols (PPCP).

	DFCP	PPCP
Total polyphenols (mg equiv gallic acid/g product)	15.75 ± 0.67	34.04 ± 2.28
Total flavanols (mg/g dry matter)	1.46 ± 0.32	3.02 ± 0.35
Epicatechin (mg/g dry matter)	0.31 ± 0.09	1.26 ± 0.18
Catechin (mg/g dry matter)	0.60 ± 0.12	0.47 ± 0.03
Procyanidin B1 (mg/g dry matter)	n.d.	0.20 ± 0.04
Procyanidin B2 (mg/g dry matter)	0.55 ± 0.11	1.09 ± 0.10
Total dietary fibre (%)	33.90 ± 1.80	24.90 ± 1. 64
Soluble dietary fibre (%)	1.68 ± 0.13	3.13 ± 0.59
Neutral sugars (%)	0.69 ± 0.04	2.46 ± 0.43
Uronic acid (%)	0.99 ± 0.09	0.67 ± 0.16
Insoluble dietary fibre (%)	32.22 ± 1.67	21.77 ± 1.05
Neutral sugars (%)	19.06 ± 1.60	10.49 ± 0.96
Uronic acid (%)	1.26 ± 0.07	1.47 ± 0.09
Klason lignin (%)	11.90 ± 0.28	9.81 ± 0.21
Total methylxanthines (mg/g dry matter)	5.62 ± 0.19	7.32 ± 0.93
Theobromine (mg/g dry matter)	5.11 ± 0.14	6.43 ± 0.84
Theophylline (mg/g dry matter)	n.d.	0.01 ± 0.01
Caffeine (mg/g dry matter)	0.51 ± 0.05	0.88 ± 0.08

Values represent mean ± SD, $n = 6$; n.d.: not detected.

2.3. Subjects

The studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Clinical Research Ethics Committee of Hospital Universitario Puerta de Hierro Majadahonda in Madrid (Spain). Volunteer recruitment was carried out through placing advertisements in the Universidad Complutense campus as well as through giving short talks between lectures. The inclusion criteria were: total cholesterol of <2000 and >2000–2400 mg/L (<5.172 and >5.172–6.206 mmol/L) for the normocholesterolemic and hypercholesterolemic groups, respectively, being non-vegetarian, non-smoker, men and women (not including pregnant women), between 18 and 55 y old, not suffering from any chronic pathology or gastrointestinal disorder. None had taken dietary supplements, laxatives, or antibiotics six months before the start of the study. Their body mass index was under 30 kg/m^2.

Fifty subjects were initially accepted to participate in the studies and gave informed written consent; however, only 44 completed both interventions (baseline characteristics are given in Table 3).

Table 2
Daily intake of the cocoa product rich in dietary fibre (DFCP) and the product rich in polyphenols (PPCP) and the corresponding daily intake of bioactive compounds.

	DFCP	PPCP
Cocoa dose (g)	30.0	15.0
Total flavanols (mg)	43.80	45.30
Epicatechin (mg)	9.30	18.90
Catechin (mg)	18.00	7.05
Procyanidin B1 (mg)	0.00	3.00
Procyanidin B2 (mg)	16.50	16.35
Total dietary fibre (g)	10.17	3.74
Soluble dietary fibre (g)	0.50	0.47
Insoluble dietary fibre (g)	9.67	3.27
Total methylxanthines (mg)	168.60	109.80
Theobromine (mg)	153.30	96.45
Theophylline (mg)	0.00	0.15
Caffeine (mg)	15.30	13.20