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# Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease

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#### **KEYWORDS**

Cocoa powder; Oxidized LDL; HDL cholesterol; Polyphenols; CHD **Abstract** Background and Aims: Epidemiological studies suggest that regular consumption of cocoa-containing products may confer cardiovascular protection, reducing the risk of coronary heart disease (CHD). However, studies on the effects of cocoa on different cardiovascular risk factors are still scarce.

The aim of this study was too evaluate the effects of chronic cocoa consumption on lipid profile, oxidized low-density lipoprotein (oxLDL) particles and plasma antioxidant vitamin concentrations in high-risk patients.

Methods and Results: Forty-two high-risk volunteers (19 men and 23 women, mean age  $69.7 \pm 11.5$  years) were included in a randomized, crossover feeding trial. All received 40g of cocoa powder with 500 mL of skimmed milk/day(C + M) or only 500 mL/day of skimmed milk (M) for 4 weeks in a random order. Before and after each intervention period, plasma lipids, oxLDL and antioxidant vitamin concentrations were measured, as well as urinary cocoa polyphenols metabolites derived from phase II and microbial metabolisms. Compared to M, C + M intervention increases HDLc [2.67 mg/dL (95% confidence intervals, CI, 0.58–4.73;

Abbreviations: oxLDL, oxidized Low-Density Lipoprotein; HDLc, High Density Lipoprotein cholesterol; LDLc, Low Density Lipoprotein cholesterol; CHD, Coronary Heart Disease; SFA, Saturated Fatty Acids; MUFA, MonoUnsaturated Fatty Acids; PUFA, PolyUnsaturated Fatty Acids; mDP, mean Degree of Polymerization.

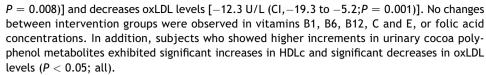
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Conclusions: Consumption of cocoa power with milk modulates the lipid profile in high-risk subjects for CHD. In addition, the relationship observed between the urinary excretion of cocoa polyphenol metabolites and plasma HDLc and oxLDL levels suggests a beneficial role for cocoa polyphenols in lipid metabolism.

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#### Introduction

Atherosclerosis is considered as a low-grade chronic inflammatory process resulting from interactions between plasma lipoproteins, cellular components and the extracellular matrix of the arterial wall [1]. High-density lipoproteins(HDL) exert anti-inflammatory functions, whereas oxidized low-density lipoproteins(oxLDL), very low-density lipoproteins and lipoprotein(a) are atherogenic lipoproteins that play a critical role in pro-inflammatory reactions. Therefore, an increase in serum HDLc concentrations, a reduction in LDLc levels, and an inhibition of oxidation of LDL particles may prevent the onset and progression of atherosclerosis.

A healthy diet and lifestyle modifications are the first step in the management of cardiovascular disease [2]. Indeed, consumption of polyphenol-rich foods has been associated with a reduced risk of coronary heart disease (CHD) [3]. Among these, cocoa (*Theobroma cacao*) and its derivatives represent a rich source of dietary flavonoids [4]. Evidence based on epidemiological studies suggests that consumption of cocoa-containing products may confer cardiovascular protection, reducing the risk of CHD mortality [5]. In addition, cocoa products may reduce blood pressure (BP) [6], increase plasma antioxidant capacity [7], inhibit oxidation of LDL particles in humans *ex vivo* [8] and reduce biomarkers of oxidation such as F2-isoprostanes and malondialdehyde [9].

However, most of these studies have been performed in healthy volunteers, and outcomes in lipid metabolism from cocoa-feeding trials remain scarce. Indeed, some studies have reported that consumption of chocolate reduces serum LDLc levels [10], whereas others have observed a neutral effect on serum total and LDLc concentrations [11,12]. Other studies carried out in healthy subjects [12] and patients with hypercholesterolemia [13] agree that cocoa consumption may actually increase HDLc levels.

To further evaluate the benefits of cocoa, we performed a randomized, crossover, controlled clinical trial to evaluate the effects of regular consumption of cocoa on classical risk factors for CHD in subjects at high risk of this disease.

#### **Methods**

#### Subjects

A total of 47 high-risk subjects (age  $\geq$  55 years) were recruited for the study in the outpatient clinic of our institution. The

subjects included had diabetes mellitus or three or more of the following risk factors: tobacco smoking, hypertension, plasma LDLc  $\geq$  160 mg/dL, plasma HDLc  $\leq$  40 mg/dL for men or  $\leq$ 50 mg/dL for women, overweight or obesity [body mass index (BMI)  $\geq$  25 Kg/m²] and/or family history of premature CHD. Exclusion criteria included documented cardiovascular events and history of allergic reactions to any cocoa components. The Institutional Review Board of the hospital approved the study protocol, and all participants gave written consent. This trial was registered in the Current Controlled Trials at the International Standard Randomized Controlled Trial Number Register in London, as ISRCTN75176807.

#### Study design

The study was designed as a 4-week randomized, crossover and controlled clinical trial. After a 2-week lead-in diet, subjects received two sachets of 20 g of soluble cocoa powder(C) per day, one for breakfast and another for an afternoon snack or after dinner (total/day: 40 g) with 250 mL of skimmed milk each (total/day:500 mL) (C + M intervention) or only 500 mL/day of skimmed milk(M intervention) for 4 weeks in a random order. Half received C + M as the first intervention, and the other half, only M. None received multivitamin or vitamin E supplements. The nutritional composition of the soluble cocoa powder (defatted and sugar-free) used in the study is detailed in Table 1. The total phenolic and total proanthocyanidin content of the soluble cocoa powder was determined by using the Folin-Ciocalteu [14] and Bathe-Smith methods [15], respectively. Individualized phenolic compounds were determined by HPLC. The mean degree of flavanol polymerization (mDP) in the soluble cocoa powder was 8, as estimated by thiolysis.

#### Diet monitoring

All participants in the study followed an isocaloric Mediterranean-type diet and were asked to exclude all other cocoa-containing foods. At the study onset and after each intervention period, a 3-day validated food recall questionnaire was used to assess nutrient intake. Energy and nutrient intake was calculated from Spanish food composition tables [16], using the Professional Diet Balancer software (Cardinal Health Systems, Inc., Edina, MN). Throughout the study, dieticians assessed any adverse effects from the interventions and gave advice on possible remedies.

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