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Natural cocoa consumption: Potential to reduce atherogenic factors?

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Abstract

Short-term consumption of flavanol-rich cocoa has been demonstrated to improve various facets of vascular health. The purpose of the present study was to determine the effect of 4 weeks of natural cocoa consumption on selected cardiovascular disease (CVD) biomarkers in young (19–35 years) women of differing body mass indices (BMI; normal, overweight or obese). Subjects (n=24) consumed a natural cocoa-containing product (12.7 g natural cocoa, 148 kcal/serving) or an isocaloric cocoa-free placebo daily for 4 weeks in a random, double-blind manner with a 2-week washout period between treatment arms. Fasted (>8-h) blood samples were collected before and after each 4-week period. Serum was analyzed to determine lipid profile (chemistry analyzer) and CVD biomarkers (26 biomarkers). EDTA-treated blood was used to assess monocytes (CD14, CD16, v11b and CD62L), while citrate-treated blood was used to measure changes in endothelial microparticles (EMPs; CD42a−/45−/144+) by flow cytometry. Natural cocoa consumption resulted in a significant decrease in haptoglobin (P=.034), EMP concentration (P=.017) and monocyte CD62L (P=.047) in obese compared to overweight and normal-weight subjects. Natural cocoa consumption regardless of BMI group was associated with an 18% increase in high-density lipoprotein (P=.020) and a 60% decrease in EMPs (P=.047). Also, obese subjects experienced a 21% decrease in haptoglobin (P=.034) and a 24% decrease in monocyte CD62L expression in (P=.047) following 4 weeks of natural cocoa consumption. Collectively, these findings indicate that acute natural cocoa consumption was associated with decreased obesity-related disease risk. More research is needed to assess the stability of the observed short-term changes.

Keywords: Flavanol-rich cocoa; Monocyte adhesion molecule; Endothelial microparticle; Inflammation; Chronic disease

1. Introduction

Over the past decade, nutrition researchers have linked short-term consumption of flavanol-rich cocoa to alterations in vascular health and antioxidant defense [1,2]. Animal models have reported that long-term consumption to reverse inflammation and dietary endotoxemia caused by high-fat feeding [3]. The most common effects attributed to short-term natural cocoa intake are improved flow-mediated dilation [4,5], increased vascular compliance [6–8], increased high-density lipoprotein cholesterol (HDL-c) [1,4,7] and decreased monocyte adhesion molecule expression (CD11b and CD62L) [6]. Increased HDL-c coupled with reduced monocyte adhesion may reflect an overall reduction in the capacity to form foam cells, which are a first stage in the development of atherosclerosis [9]. An increase in antioxidant capacity also has the potential to reduce the risk of foam cell formation by delaying the oxidation of low-density lipoprotein (LDL) in the subendothelial space. A decrease is oxidized LDL (oxLDL) decreases the stimuli responsible for increased cell-surface monocyte adhesion molecule expression [10]. Elevated endothelial microparticles (EMPs) are associated with obesity, metabolic syndrome and consumption of a high-fat meal and are an emerging risk factor for cardiovascular disease (CVD) [11,12]. A recent review article indicated the potential for daily flavanol-rich cocoa consumption to be used as an antiobesity treatment [7]. However, there is a critical gap in the existing literature with regard to the capacity to utilize short-term flavanol-rich cocoa consumption as a countermeasure to reduce cardiovascular and metabolic disease risk in individuals of differing body mass indices (BMI).

Of all the naturally occurring compounds found on flavanol-rich cocoa, catechins and polyphenols have been implicated as the primary compounds responsible for improved vascular health [1]. Obese individual are known to have elevated systemic inflammation, and when combined with poor dietary choices, chronic damage to the endothelial wall is common [9,11,12]. Our lab and others have used EMP as an index of endothelial wall damage [13,14]. Specifically, our laboratory has focused on the evaluation of novel disease risk biomarkers with recent efforts focused on circulating biomarkers of systemic inflammation [14–19], altered EMP and alterations in monocyte adhesion molecule expression [14,19]. All of these

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measures could be considered preclinical indicators of the risk of the accumulation of arterial plaques. The purpose of the present study was to examine the effects of 4 weeks of daily flavanol-rich natural cocoa consumption on CVD biomarkers, EMPs and monocytes in women of differing obesity statuses who were weight stable.

2. Materials and methods

2.1. Subjects

The University of North Texas committee for the protection of human subjects approved all procedures used in this report, and the study was carried out in accordance with the Declaration of Helsinki. After being explained the study requirements, interested subjects gave oral consent to participate and signed an informed consent form. We screened 75 women who matched one of the three desired BMI groups. Of the 75 that we screened, 35 met all study requirements and 30 of those had schedules compatible with the study timeline. These 30 individuals were assigned to one of three groups based on BMI: normal weight (BMI=20.0–24.9 kg/m^2), overweight (BMI=25.0–29.9 kg/m^2) and obese (BMI=30.0–39.9 kg/m^2). We excluded subjects with a BMI > 40 kg/m^2. Every subject completed both conditions in a double-blind, crossover design. Condition orders were randomized to minimize carry over and account for potential order effects. Twenty-four of the 30 subjects completed all study requirements and samples. There was 0% attrition in the normal-weight group and 30% attrition in both the overweight and obese groups. These attrition percentages are consistent with what we have observed in other human studies in our laboratory and what others commonly report in the literature [14,17,18,20,21].

2.2. Subject screening

Subjects were assessed inclusion/exclusion criteria using a medical history form, a graded exercise test (Medgraphics Ultima metabolic cart, St. Paul, MN, USA), a whole body dual-energy X-ray absorptiometry (DXA) scan (GE Lunar Prodigy, Piscataway, NJ, USA) and basal metabolic rate test (MedGem, Golden, CO, USA). Subject characteristics are presented in Table 1. In general, the obese subjects had greater body weight, BMI, percent fat, fat mass, lean mass and basal metabolic rate than overweight and normal-weight subjects. Also, obese subjects had lower levels of fitness than normal-weight and overweight subjects. Overall, the fitness level of all the subjects was low and no subjects reported any regular physical activity habits prior to or during completion of the study.

2.3. Natural cocoa treatment

The active and placebo bars used for the present study were manufactured and provided by The Hershey Company (Hershey, PA, USA). According to the manufacturer, the bars did not differ in their respective macronutrient composition, but did differ in magnesium, potassium and catechins in accordance with natural cocoa content. Bars were provided to the study staff packaged in either silver or white wrappers, and the study conditions were not revealed until all tests were completed. Table 2 summarizes the various nutritional components found in the bars. Subjects were asked to avoid consuming chocolate in any form during participation in the study and were asked to consume the bars at the same time each day. Table 3 summarizes the polyphenol content of the natural cocoa and placebo bars. Total flavanols were assessed using three different methods (each analytical method is optimized to assess a specific subgroup of flavanols). The flavanol monomers, epicatechin and catechin, were measured by atmospheric-pressure chemical ionization mass spectrometry [22], and the flavanol dimer through decamer polymers (DP2-10) was measured using mass spectrometry [23,24]. Total flavanols were measured using the colorimetric 4-dimethylaminocinnamaldehyde (DMAC) method. The DMAC assay is unique in that it measures all flavanols including monomers (epicatechin and catechin), gallated flavanols and flavanol polymers (DP2 to DP10) and polymers greater than DP10 [25]

Table 1
Subject characteristics

Characteristic	Normal (n=10)	Overweight (n=7)	Obese (n=7)
Age (y)	21±2	22±3	22±3
Height (cm)	161.1±5.4	164.8±5.8	164.9±6.7
Weight (kg)	56.2±7.5	68.3±19.3[a]	94.6±11.1[b]
BMI (kg/m^2)	21.6±1.9	27.0±1.4[a]	34.9±3.0[b]
DXA body fat (%)	28.6±5.7	34.2±9.9[a]	49.4±3.2[b]
Fat mass (kg)	16.3±4.4	27.5±3.1[a]	45.9±4.8[b]
Lean mass (kg)	38.3±5.5	41.9±5.5[a]	44.3±7.7[a]
Bone mass (kg)	2.5±0.3	3.1±0.3	3.1±0.5
SBP (mm Hg)	104±10	111±9	119±19
DBP (mm Hg)	71±10	69±7	83.8±19
RMR (kcal/d)	1174±171	1438±156[a]	1690±460[a]
VO_{2max} (ml/kg/min)	30.5±5.5	25.3±3.7[a]	20.9±3.1[b]
Total cholesterol (mg/dl)	173.7±4.8	152.7±15.4	153.9±8.6
HDL-c (mg/dl)	43.6±4.2	46.7±5.1	47.0±4.1
Triglycerides (mg/dl)	69.6±5.9	55.1±6.9	109.3±20.3[b]
Glucose (mg/dl)	88.3±2.3	85.4±4.9	85.5±3.7

Values reported as mean±S.D. Body composition measurements were made from a standard whole-body DXA scan. All serum blood measurements were made following a fast of at least 8 h.

[a] Greater than normal (P<.05).

[b] Greater than normal and overweight (P<.05).

2.4. Blood sample collection

Venous blood samples were collected before and after each 4-week supplement condition. Subjects reported to the laboratory between 0600 and 0900 h following an overnight fast (>8 h) and abstention from physical activity (>15 h). Subjects were encouraged to drink plenty of water during the fast in order to ensure hydration and ease of blood sample collection. After 30 min of seated rest, a trained technician collected a venous blood sample into three evacuated tubes (Greiner Vacuette, Monroe, NC, USA) treated with serum-clotting chemical, EDTA or sodium citrate. Sample tubes were mixed 10 times by inversion. EDTA and citrate tubes were held at room temperature until analysis, while serum tubes were centrifuged to separate serum, which was frozen at −80°C until the end of the human subjects trials.

2.5. Traditional disease risk factors

In the present study, total cholesterol, HDL-c, triglycerides and glucose were measured using a series of enzymatic assays (Pointe Scientific) on an automated chemistry analyzer (ChemWell T; Awareness Technologies, Palm City, FL, USA). All samples were analyzed in triplicate.

2.6. Novel CVD biomarker

CVD risk biomarkers were measured using multiplex technique (EMD Millipore Milliplex, Billerica, MD, USA).The panel of 26 serum CVD risk biomarkers included the following: α-2-macroglobulin, adipsin, AGP, C-reactive protein, fetuin A, fibrinogen, haptoglobin, sE-selectin, platelet factor 4, serum amyloid P, von Willebrand factor, BNP, CK-MB, CXCL6/GCP-2, CXCL16, endocan-1, FABP3, FABP4, LIGHT, oncostatin, placental growth factor, troponin I, IL-1β, IL-6, IL-8 and TNF-α. These were biomarkers were measured in duplicate on the same day using a Human CVD Panel 1 (HCVD1MAG-67K), Human CVD Panel 3 (HCVD3MAG-67K) and a Human High-Sensitivity Cytokine Panel (HSTCMAG28SPMX4). After following the manufacturer's protocol for sample processing, samples were acquired using a Luminex MagPix (Austin, TX, USA). All samples were assayed in duplicate on the same day in order to minimize intra-assay and interassay coefficient of variations. After acquisition, unknown values were calculated using a 7-point standard curve method on Milliplex Analyst software (v. 5.1; EMD Millipore).

2.7. Flow cytometry for monocytes

Monocytes were measured to determine relative concentration (CD14 and CD16) and expression of cell-surface adhesion molecules (CD11b and CD62L) using four-color immunofluorescent assays. All antibodies were titered prior to analysis to ensure adequate signal-to-noise ratios (data not shown). Consistent with previous methods utilized in our laboratory [14,21,26], we separated peripheral blood mononuclear cells (PBMCs) from 10 ml of EDTA-treated whole blood. After separation using Leukosep tubes (Griener) and Ficoll-paque, cells were measured for concentration and viability

Table 2
Macronutrient, vitamin and mineral content of the natural cocoa and placebo bar

Analysis	Placebo	% DV	Cocoa	% DV
Calories (kcal)	148	7.4	141	7.1
Fat (g)	5	7.7	6	9.2
Sat fat (g)	3	15.0	4	20.0
Trans-fat (g)	–	–	–	–
Cholesterol (mg)	3	1.0	1	0.3
Sodium (mg)	62	2.6	38	1.6
Carbohydrates (g)	25	8.3	26	8.7
Fiber (g)	1	4.0	4	16.0
Sugar (g)	17		15	
Protein (g)	2	4.0	3	6.0
Vitamin A (IU)	9	0.18	7	0.14
Vitamin C (mg)	0	–	0	–
Calcium (mg)	53	5.3	20	2.0
Iron (mg)	0	–	2	11.1
Magnesium (mg)	7	1.8	73[a]	18.3
Potassium (mg)	81	3.4	202[a]	5.8

[a] Different from placebo. The two bars did not differ in their major macronutrient composition.