Original Communication

Cocoa and unripe banana flour beverages improve anthropometric and biochemical markers in overweight women: A randomised double-blind study

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Abstract: *Objectives*: The objective of this study was to evaluate the effect of a functional cocoa and unripe banana flour (UBF) beverage intake on the anthropometric and biochemical markers of overweight women. *Methods*: This prospective, double-blinded, randomized clinical trial involved 60 female volunteers aged between 20 and 50 years. One group received a cocoa beverage (n = 30) and one group received a cocoa and UBF beverage (n = 30), for 6 weeks. The cocoa beverage showed 3.07 total g dietary fiber/serving and 29.55 mg gallic acid equivalentes/portion (GAE/portion). Cocoa and UBF beverage contained 8.48% resistant starch by weight, 4.37 g dietary fiber/full portion and 69.24 mg GAE/portion. Weight, waist circumference, hip circumference, glucose, triglycerides, total cholesterol and fractions were evaluated. *Results*: The UBF and cocoa beverage reduced waist circumference (−2.03 cm, $P < 0.001$) and the cocoa beverage reduced total cholesterol (−19.3 mg/dL, $P < 0.001$) and the LDL/HDL ratio (−0.32, $P < 0.001$); in addition, both beverages promoted the reduction of body fat percentage (−1.98%, $P = 0.001$ and −1.15%, $P < 0.001$ Cocoa/UPF group and Cocoa group respectively). *Conclusion*: The additional dietary fiber did not further improve health status.

Introduction

Non-communicable chronic diseases (NCDs), including circulatory diseases, cancer, diabetes and chronic respiratory diseases, are now the world's largest health problem, accounting for 63% of global deaths each year [1]. The incidence of obesity is increasing in all countries and, in South America, the prevalence of obesity and central obesity is higher in women and is strongly associated with cardiovascular risk factors [2].

In this context, food has gained prominence as a modifiable risk factor for the prevention and control of NCDs, as well as for the reduction of developed diseases [3]. In this way, some foods stand out because they go beyond the function of nourishment, producing metabolic effects that are beneficial to health and are known as functional foods [4]. Among these foods and compounds with functional properties, foods rich in phenolic compounds, dietary fiber and resistant starch (RA) have been highlighted [5–7].

Cocoa is a food rich in phenolic compounds, and studies identify the benefits of its polyphenols as improved insulin sensitivity and vascular function, reduced cortisol excretion and blood pressure, improved mitochondrial function, and reduced cardiovascular diseases in women, among other effects [8–11]. Therefore, it is a food that should be further studied by the scientific community; the effect of cocoa on anthropometric and biochemical markers is still not well understood, especially in the presence of other functional foods, such as unripe banana flour (UBF), which has similar effects to that of cocoa.

Unripe banana flour is rich in resistant starch, dietary fiber, and has demonstrated nutraceutical and nutritional value, such as glycaemic and insulinaemic response

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control, improvement in lipid profile, satiety increase and, consequently, reduction of energy intake [12, 13].

Evidence is growing of the benefits of cocoa and UBF, but the efficacy of this blend has not yet been investigated in terms of the anthropometric and biochemical indicators of overweight individuals. Thus, combining foods with functional properties to potentiate the effect of both, and reduce the intake dose, may be an interesting approach to reduce the risk of various diseases [14], such as obesity [15]. Investigations of possible synergistic or antagonistic effects among bioactive compounds will contribute to filling a scientific gap.

Thus, the objective of this study was to evaluate the effect of a functional cocoa and unripe banana flour beverage intake on the anthropometric and biochemical markers of overweight women.

Materials and methods

Ethical standards

The study was approved by the Research Ethics Committee (CEP) of Federal University of Alfenas, number 525.888, and performed in accordance with the ethical standards laid down in the Declaration of Helsinki guidelines. All participants signed an informed consent document approved by the Ethics Committee prior to participation.

Participants

Our experimental setup has been described previously [16]. Fifty-two overweight/obese (OW/O) women between the ages of 20 and 50 years were recruited for the study. The participants met two of the following three criteria: body mass index (BMI) 25.0–39.9 kg/m^2, a percentage body fat of > 35%, and waist circumference > 80 cm [17].

Exclusion criteria were: pregnancy or lactation; diagnosis of autoimmune, endocrine, cardiac, cerebrovascular, renal, or hepatic disease, gout, gastrointestinal, inflammatory and/or infectious diseases; hypertension with altered medication in the last three months; use of insulin in the last year; use of lipid-lowering drugs in the last six months; immunosuppressant use in the last five years; use of dietary supplements, laxatives or antibiotics in the last three months; surgical procedure in the last six months; regular use of alcohol and other drugs in the last year; vegans; participation in a weight loss programme in the last six months; use of diuretics, antiobesity, antidepressants, glucocorticoids, antibiotics; allergic reactions to the intervention beverage components.

Participants were recruited at the Federal University of Alfenas (UNIFAL-MG), Alfenas, Minas Gerais, Brazil. The study was conducted at the Central Laboratory of Clinical Analysis "Professor Afranio Caiafa de Mesquita" (LACEN) of the university and at the Outpatient Clinic of the Faculty of Nutrition (FANUT), being accompanied by a university doctor.

Experimental draw

This was a prospective double-blind randomised clinical trial to evaluate the effect of cocoa and UBF in the same beverage on the anthropometric and biochemical parameters of overweight women. The study used a drink, containing only cocoa, to exclude the effect of it together with UBF. Eligible women were randomised into two groups by lot, to ingest one of the two drinks. We determined 30 volunteers per group guaranteeing 80% power and an alpha of 0.05 to obtain meaningful results, and to allow for a possible 15% attrition from the study.

Clinical trial products

Daily servings of powder were given to the volunteers to make at home, preparing the drink with cocoa (39.1 g) and cocoa and UBF (54.1 g). The drinks were homogenized in a blender with 150 mL of water. Each package of the product was delivered to volunteers with instructions for preparation and storage. According to the literature the portions were based on phenolic (catechin, epicatechin, gallocatechin, tannins) content and resistant starch respectively present in cocoa (3 g/portion) and UBF (30 g/portion).

The UBF and powder for preparation of drinks were evaluated for chemical composition according to the methodologies described by the Association of Official Analytical Chemistis (AOAC, 2000) [18]. The content of resistant starch product was determined according of method AOAC (2002) [19], using a commercial Resistant Starch Assay Kit (Megazyme K-Rstar 08/11). The concentration of total phenolics was determined in triplicate according to the methodology described by Singleton, Orthofer and Lamuela-Raventos (1999) [20] using the Folin-Ciocalteau reagent. Absorbance reading at 726 nm was done on ELISA equipment (Thermo Scientific®, model Multiskan GO). The results were expressed in milligrams of gallic acid equivalentes (GAE) using the regression equation from a standard curve of gallic acid with concentrations ranging from 0 to 250 ppm.

The drinks were made from whole milk powder, sucralose, xanthan gum, cocoa powder (Armazem São Vitto Comercio de Produtos Alimentícios, São Paulo, SP, Brazil) and UBF (Relva Verde Produtos Naturais, Londrina, PR, Brazil). Both drinks had the same ingredients, except UBF had excluded from the cocoa drink. It is emphasized that was the same cocoa in the same proportion used in both products.

Daily servings of powder the cocoa beverage was 39.1 g and contained 11.24 g total carbohydrates (g/portion), 8.19 g proteins (g/portion), 11.84 total lipids (g/portion), 3.07 total g dietary fiber/serving, 29.55 mg GAE/portion and 184.37 kcal/portion. Daily servings of powder the cocoa and UBF beverage were 54.1 g and contained 31.22 g total carbohydrates (g/portion), 5.49 g proteins (g/portion), 7.29 g total lipids (g/portion), 8.48% resistant starch by weight, 4.37 g dietary fiber/full portion, to 69.24 mg GAE/portion and 212.73 kcal/portion.

Study protocol

After the sample selection, the participants attended the FANUT outpatient clinic for anthropometric evaluation and were then referred to LACEN for initial biochemical evaluation. Once these baseline assessments were completed, the drinks were delivered to the participants in adequate amount for seven days. The drinks were consumed at breakfast for six weeks, that is, for 42 days. At the end of this period the anthropometric and biochemical measures were re-evaluated.

Participants were instructed to maintain their usual diet and do not initiate or change their practice of physical exercise or diet programmes, and do not intake steroids, multivitamins or antioxidants during the study period. Failure to comply with these conditions would exclude the person from the sample.

At the end of the study, participants with biochemical markers and body composition at risk were referred for medical and nutritional follow-up with professionals from the Federal University of Alfenas.

Compliance

Each week the participants received seven sachets with the individual portions of the beverages. Leftovers were returned to the study and counted at the end of each week. It was assumed that all non-returned portions had been consumed. So, the volunteers consumed a serving once a day for six weeks (42 servings). Compliance was recorded in terms of missed doses from the 42 servings.

Measures

Personal and anthropometric data

During the individualised evaluations, data were collected regarding personal information, schooling, bowel habits, the usual physical activity and presence of comorbidities.

In the anthropometric evaluation, weight, height and waist circumference were measured. The body weight was evaluated by means of a portable digital electronic platform scale, calibrated by INMETRO, brand Kratos (maximum capacity of 150 kg, minimum of 1.25 kg and precision of 50 g). The height was measured using the Alturexata portable stadiometer (maximum range of 213 cm and accuracy of 1 mm). Body circumference (hip, waist and brachial) was obtained using an inelastic and inextensible measuring tape, 1 mm, for comparison of their absolute values. The cut-off points recommended by the World Health Organization (2000) [21] were used to classify the nutritional status, according to the BMI. The bioimpedance measurements were performed using the RJL Systems Quantum BIA-101Q quadrupole apparatus, with the patient in the supine position, after observing all the precautions prior to the preparation for the examination. The device provided the fat percentage directly through equations already programmed by the manufacturers. The volunteers presented with light clothes, preferably bikinis, and the measurements were taken according to recommendations provided by Duarte (2007) [22].

Biochemical evaluation

Blood collection and tests of fasting blood glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), total cholesterol, high density lipoprotein – cholesterol (HDL-c), low density lipoprotein – cholesterol (LDL-c), very low density lipoprotein – cholesterol (VLDL-c) and triglycerides dosages were performed at the Laboratory of Clinical Analyses (LACEN) of UNIFAL-MG. The protocol for the preparation of the biochemical tests was performed according to the recommendation of the Brazilian Society of Cardiology (SBC) [17].

The measurement of serum blood glucose was performed by the automated enzymatic method. Plasma levels of cholesterol were analysed, comprising total cholesterol by the automated colorimetric enzymatic method, HDL cholesterol by the automated direct method and triglycerides by the enzymatic colorimetric method. Blood levels of LDL-c were obtained by calculation using the Friedewald, Levi and Fredrickson (1972) [23] formula: LDL-c = CT-HDL-c – Triacylglycerols (TAG)/5, for samples with lower triglyceride levels to 400 mg/dL and VLDL = TAG/5.

Control and monitoring measures

The volunteers completed a non-consecutive three-day food diary on two occasions, before the study and in its sixth week, to assess whether they had maintained their usual diet during the study, according to the protocol devised by Martins (2011) [24]. Food intake was evaluated using the AVANUTRI Revolution Nutrition Prescription and Evaluation software, version 4.0.

Weekly, at the time of withdrawal of the drinks for consumption the following week, the participants answered a brief questionnaire that assessed their adherence to the protocol, possible intercurrences, and changes in dietary habits and physical activity.

Statistical analysis

The data obtained in the study were analysed using the software SPSS Statistics, version 19. To evaluate the normality of the distribution of the variables, the Shapiro-Wilk W test was applied. The variables were expressed as mean ± standard deviation and median and interquartile range (P25 and P75), since some had a non-parametric distribution.

To compare differences in anthropometric and biochemical data within each group between 0 and 6 weeks, the paired Student's t-test, or its corresponding non-parametric Wilcoxon matched-pairs signed rank, was used. The comparison between the treatment and control groups was performed using Student's t-tests or the corresponding non-parametric Mann Whitney test. All statistical tests were applied with significance level of 5%.

Results

Selection of participants and post-randomisation conditions

The research was advertised on the university Alfenas campus, 151 women expressed interest in the screening evaluation; 25 were excluded due to lack of baseline assessments, 25 because they did not meet the selection criteria and 29 because they fell within the exclusion criteria. Seventy-two women were selected to start the study, and 55 completed all stages of the study. Ten volunteers withdrew from the study and did not attend the anthropometric or biochemical evaluations or refused to undergo the blood tests (Figure 1). Of these 62 volunteers, three were excluded: one volunteer from the Cocoa/UBF group for ingesting medication that caused water retention and two from the Cocoa group who changed their usual dietary intake with a caloric reduction of approximately 50%. Therefore, the data of 52 volunteers, 26 in each group, were analyzed.

When comparing pre-intervention anthropometric and biochemical data between the groups after randomization, it was verified that there was no statistically significant difference for the evaluated variables. Therefore, both groups started the research at the same basal condition.

Profile of participants

The overall profile of study participants is shown in Table 1. The mean age of the Cocoa/UBF group intervention was 38.2 ± 8.0 years and 33.2 ± 8.5 years in the Cocoa group. In relation to marital status, 65.38% (n = 17) of the Cocoa/UBF group and 50.00% (n = 13) of the Cocoa group were married, the others were single or divorced. Regarding the educational level, most of the volunteers had 12 years or more of education, 16 (61.54%) in the Cocoa/UBF group and 18 (69.23%) in the Cocoa group; However, most worked in jobs requiring only high school education (less than 12 years of schooling).

Regarding water intake, only eight (30.76%) women in the Cocoa/UBF group and seven (26.92%) in the Cocoa group group consumed 1.5 L or more of water per day.

Concerning the physical activity profile of the volunteers, only 23.08% (n = 6) of each group were in the habit of regularly performing physical exercises in their leisure time, from 2 to 7 times a week, lasting 40 to 60 minutes.

Only five people in each group (19.23%) consumed the recommended portions of vegetables (3 portions/day); in relation to fruits these values increased to fifteen (57.69%) in the Cocoa/UBF group and eight (30.77%) in the Cocoa group.

Anthropometric evaluation

In relation to the anthropometric variables, the Cocoa/UBF group intervention was associated with a reduction in waist circumference – WC ($P < 0.001$), waist – height ratio – WHR ($P < 0.001$), as well as in percentage of body fat ($P < 0.001$). The intervention with the Cocoa group was associated with a reduction only of percentage of body fat ($P < 0.001$) (Table 2 and Figure 2).

Biochemical evaluation

Regarding the metabolic variables, Cocoa/UBF ($P = 0.014$) and cocoa group ($P = 0.004$) had an increased of glucose levels. The intervention with Cocoa/UBF group did not promote a change in other metabolic variables. The intervention with Cocoa group decresead CT ($P < 0.001$), LDL ($P = 0.001$) and LDL/HDL ratio ($P < 0.001$). However, the changes between the two groups do not differ significantly (Table 3 and Figure 2). The main outcomes before and after the intervention with the beverages are presented in Figure 2.

Control of food consumption

Regarding the macronutrient consumption by the participants before the intervention, there were no statistically significant differences for any of the dietary variables evaluated; therefore, both groups started the research under the same dietary conditions. The food consumption of the participants were evaluated before and during the sixth week of intervention (demonstrated in Table 4).

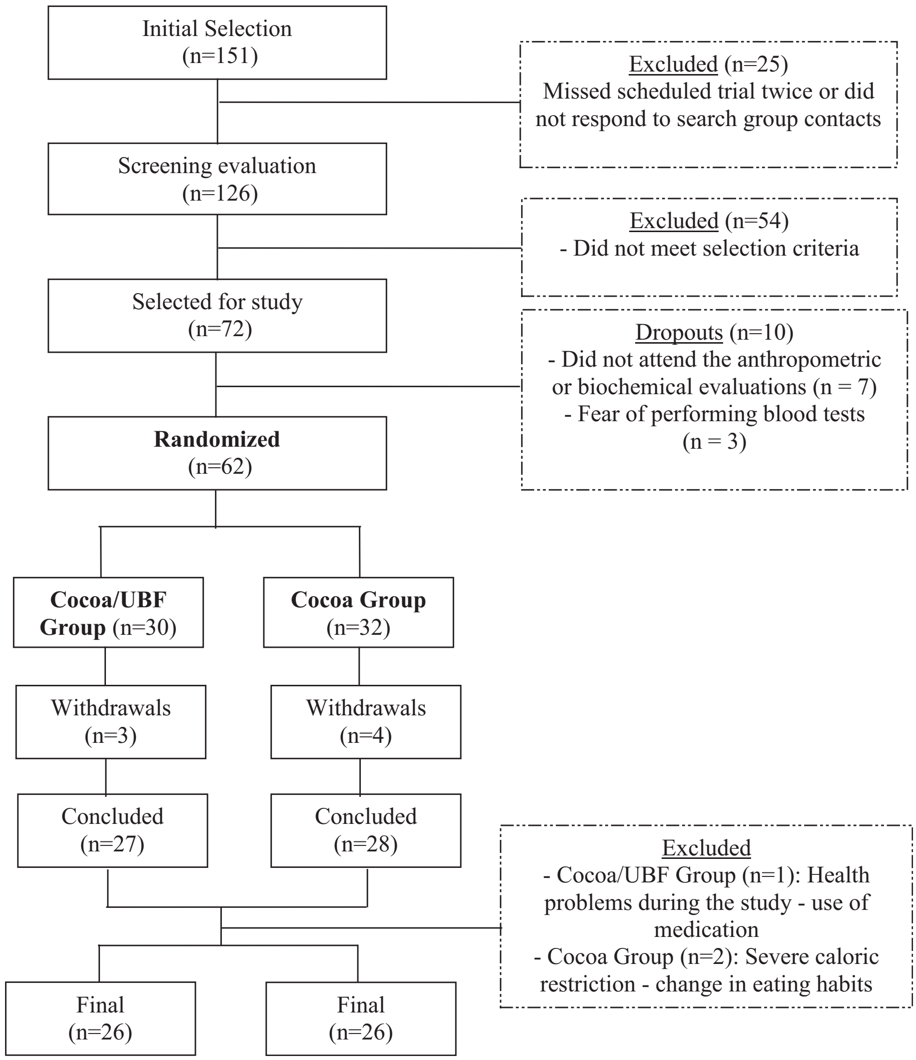

Figure 1. Clinical trial flowchart. Figure Legend: UBF: unripe banana flour.

The Cocoa/UBF group intervention increased dietary fiber intake (P < 0.001), whereas the Cocoa group intervention decreased carbohydrate intake (P = 0.017) and, consequently, reduced caloric intake (P = 0.027).

However, when assessing behaviour regarding food intake during the study (Table 4), comparing the effect (P2) between the two interventions, there was an observed increase only for dietary fiber intake (P < 0.001). This was expected due to the amount of unripe banana flour provided in the Cocoa/UBF group intervention. Additionally, there were no differences were observed in the macronutrient intakes (P > 0.05).

Discussion

The present study focused on the potential benefit of cocoa and UBF in overweight and abdominal obesity women. The combined beverage Cocoa/UBF group reduced waist circumference and Cocoa group reduced the total cholesterol and LDL/HDL ratio; in addition, both beverages decreased body fat percentage. These results indicate that the addition of UBF promoted some different effects, which does not mean that there was the empowerment of the cocoa effects. These results are corroborated with other data observed by the research group [16].

The cocoa beverage showed a better influence on lipid profile than Cocoa/UBF beverage. As the two drinks contained the same amount of cocoa, some aspects should be considered. The first is about cocoa polyphenols, some authors claim that this food has the highest flavonoid content of all foodstuffs, presenting levels higher than those of teas and wine [25, 26]. Martínez-Lópes et al. (2014) [27] conducted a cross-controlled study with healthy and moderately hypercholesterolaemic volunteers who consumed a milk-soluble cocoa product for four weeks and found similar results; cocoa improved HDL levels, but no changes in anthropometric parameters. The Cocoa/UBF

Table 1. Characteristics of study volunteers by intervention group.

Characteristics	Cocoa/UBF group (n = 26)	Cocoa group (n = 26)	P[a]
Age (years)	38.26 ± 8.03	33.26 ± 8.55	0.034
Weight (kg)	77.88 ± 13.36	76.01 ± 10.71	0.581
BMI (kg/m^2)	29.93 ± 5.07	29.09 ± 3.65	0.498
Waist circumference (cm)	94.65 ± 12.16	92.44 ± 8.33	0.449
Schooling (%)			
Elementary school	19.23	19.23	
High school	46.16	42.31	0.544
Higher education	19.23	15.38	
Student	15.38	23.08	
Water intake (%)			
>2 L/day	15.38	3.84	
1.5–2 L/day	15.38	23.08	0.275
1–1.5 L/day	34.62	50.00	
<1 L/day	34.62	23.08	
Physical activity (PA) (%)			
Yes	30.77	26.92	1.000
No	69.23	73.08	
Frequency of PA (%)			
None	69.23	73.08	
4–7 ×/week	7.69	7.69	1.000
2–3 ×/week	15.39	15.39	
1 ×/week	7.69	3.84	
PA duration (%)			
None	69.23	73.08	
<40 minutes	0.00	7.69	0.636
40–60 minutes	26.93	19.23	
>60 minutes	3.84	0.00	

Legend: [a]Results obtained using the t-test or Fisher's Exact Test (P < 0.05). UBF: unripe banana flour.

beverage did not affect lipid profile, since the higher dietary fiber content present in the UBF may have reduced gastric emptying rate, with slower access of cocoa flavonoids in the intestine and reduction of free epicatechin absorption [28]. In addition, UBF dietary fiber may increase the the formation of conjugated metabolites, which may have contributed to reducing the potential of flavonoids [29]. Sarriá et al. (2015) [30] studied the effect of a high-dietary fiber cocoa product and a product rich in polyphenols and reported that cocoa flavonoids are responsible for the increase in HDL cholesterol, while dietary fiber is related to other anti- inflammatory properties which prevent fat accumulation and reduce anthropometric parameters.

Resistant starch present in UBF was associated with a significant reduction in waist circumference observed at our study. Despite the lack of long-term studies, several point out that resistant starch may aid in weight control [31]. Tavares da Silva et al. (2014) [32] supplemented for 45 days the usual diet of women with 20 grams of UBF to be consumed at breakfast along with dairy products or juices,

Table 2. Anthropometric markers at baseline and after intervention by and between groups.

Variables	Cocoa/UBF group (n = 26)					Cocoa group (n = 26)					
	Baseline		After 6 weeks			*Baseline*		After 6 weeks			
	Mean ± sd	Median (P25; P75)	Mean ± sd	Median (P25; P75)	P[1]	Mean ± sd	Median (P25; P75)	Mean ± sd	Median (P25; P75)	P[1]	P[2]
Weight (kg)	76.47 ± 12.93	73.82 (65.5; 85.20)	76.45 ± 13.44	73.58 (65.6; 85.85)	0.921	75.42 ± 11.03	72.9 (67.15; 84.6)	75.75 ± 11.25	72.88 (68.4; 86.5)	0.344	0.839
BMI (kg/m^2)	29.40 ± 4.73	27.56 (25.52; 31.45)	29.39 ± 4.96	27.28 (25.41; 32.10)	0.959	29.01 ± 3.69	27.63 (25.74; 32.46)	29.12 ± 3.70	27.82 (25.95; 32.17)	0.383	0.826
WHR	58.38 ± 8.10	56.90 (51.88; 61.61)	57.15 ± 8.44	55.86 (50.88; 60.68)	0.000	57.24 ± 5.32	56.28 (53.44; 61.8)	56.72 ± 4.56	55.84 (53.27; 61.31)	0.118	0.821

(1) Comparison at the baseline (before the beverage intake) and after 6 weeks of beverage intake at each intervention, results obtained using the paired Student's t-test or Wilcoxon signed ranks test (P < 0.05). (2) Comparison of effects between treatments – comparison of Δ of changes between the two interventions using Student's t-test (P < 0.05). UBF: unripe banana flour; SD: standard deviation; BMI: body mass index; WHR: Waist/height ratio.

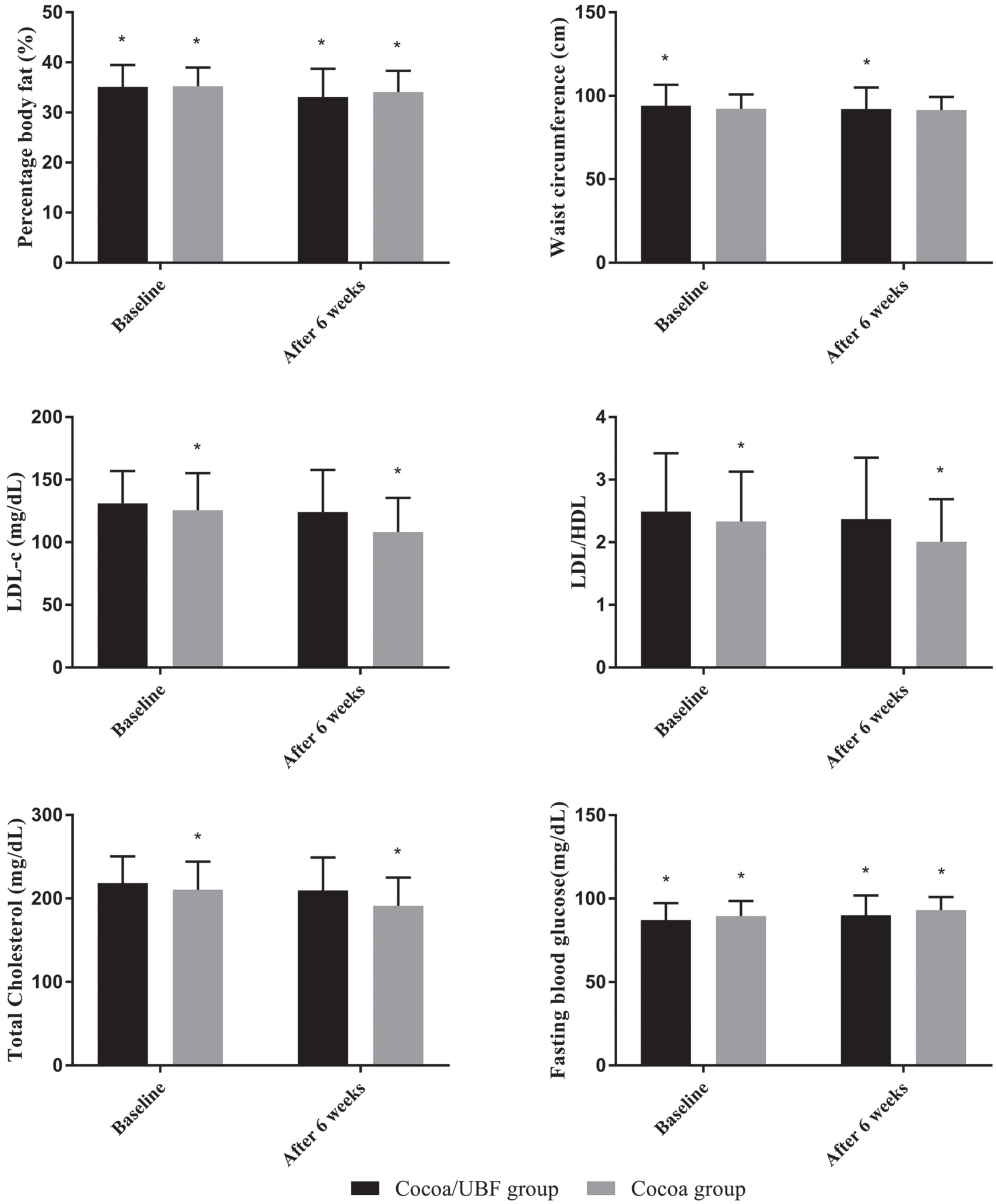

Figure 2. Mean ± sd in percentage body fat, waist circumference, low density lipoprotein – cholesterol (LDL-c), LDL/HDL cholesterol ratio, total cholesterol and fasting blood glucose, comparing the baseline with the six weeks after a daily consumption of cocoa beverage (Cocoa group; n = 26) and a cocoa/unripe banana flour beverage (Cocoa/UBF group; n = 26). *Significant intra-group reduction (P < 0.05).

and also found similar results; there was a significant reduction of hip circumference and a consequent increase in WHR among women with metabolic syndrome.

The increased glycaemia observed in both groups may be related to the sucralose present in the beverages. This ingredient has been incorporated to sweeten the drink without adding calories and with low cost. Sucralose drinks were the most accepted in the sensory analysis prior to the trial. According to Viggiano (2003) [33] and Torloni et al. (2007) [34], sucralose has a high sweetness and there are no contraindications to its use. The amount of sucralose present in the beverage portion met the acceptable daily intake of 15 mg/kg body weight, as determined by the Codex Alimentarius [35]. However, there are more recent

Table 3. Metabolic markers of volunteers at baseline and after intervention by and between groups.

	Cocoa/UBF group (n = 26)					Cocoa group (n = 26)					
	Baseline		After 6 weeks			*Baseline*		After 6 weeks			
Variables	Mean ± sd	Median (P25; P75)	Mean ± sd	Median (P25; P75)	P¹	Mean ± sd	Median (P25; P75)	Mean ± sd	Median (P25; P75)	P¹	P²
INSU (mg/dL)	10.01 ± 5.05	8.2 (7; 12.4)	10.08 ± 6.32	8.45 (6.3; 10.9)	0.893	10.62 ± 4.00	11.05 (6.6; 13.7)	10.35 ± 4.59	9.5 (6.9; 12.9)	0.707	0.859
HOMA–IR	2.16 ± 1.13	1.7 (1.46; 2.81)	2.29 ± 1.60	1.9 (1.28; 2.46)	0.761	2.38 ± 1.03	2.4 (1.29; 3.38)	2.38 ± 1.10	2.24 (1.48; 2.98)	0.991	0.814
HDL-c (mg/dL)	58.15 ± 18.23	52 (45; 74)	57.73 ± 17.39	51.5 (44; 70)	0.688	56.42 ± 11.87	53 (49; 61)	56.38 ± 13.01	53 (46; 64)	0.979	0.753
VLDL-c (mg/dL)	29.21 ± 12.76	27.7 (23; 33)	27.62 ± 13.89	25.1 (18.2; 30.6)	0.230	28.34 ± 13.24	25.9 (20.2; 32.2)	26.38 ± 13.27	21.5 (19.8; 27.4)	0.201	0.742
TAG (mg/dL)	146.04 ± 63.80	138.5 (115; 165)	138.12 ± 69.43	125.5 (91; 153)	0.230	141.69 ± 66.20	129.5 (101; 161)	131.88 ± 66.33	107.5 (99; 137)	0.201	0.742

Legend: (1) Comparison at the baseline (before the beverage intake) and after 6 weeks of beverage intake at each intervention, results obtained using the paired Student's t-Test or Wilcoxon signed ranks test ($P < 0.05$). (2) Comparison of effect between treatments – comparison of Δ of changes between the two interventions using Student's t-test ($P < 0.05$).
UBF: unripe banana flour; SD: standard deviation; INSU: Insulin; HOMA-IR: homeostasis model assessment of insulin resistance; HDL-c: High density lipoprotein; VLDL: Very low density lipoprotein; TAG: Triacylglycerols.

Table 4. Average daily macronutrients, calories and fibres intake by volunteers at baseline and in the sixth week of intervention by and between groups.

	Cocoa/UBF group (n = 26)					Cocoa group (n = 26)					
	Baseline		During the 6th week			*Baseline*		During the 6th week			
Variables	Mean ± sd	Median (P25; P75)	Mean ± sd	Median (P25; P75)	P¹	Mean ± sd	Median (P25; P75)	Mean ± sd	Median (P25; P75)	P¹	P²
PTN (g)	69.36 ± 19.94	68.74 (56.66; 76.33)	67.95 ± 16.82	63.31 (56.53; 74.84)	0.564	69.61 ± 20.04	65.59 (58.89; 76.11)	66.57 ± 21.13	64.6 (53.29; 76.75)	0.418	0.798
CHO (g)	218.66 ± 51.65	217.08 (176.72; 240.62)	214.11 ± 63.19	198.84 (174.04; 230.65)	0.698	214.43 ± 52.71	210.11 (168.83; 254.02)	189.44 ± 61.95	187.17 (153.15; 226.66)	0.017	0.166
LIP (g)	57.86 ± 17.26	58.38 (48.95; 65.63)	57.74 ± 22.53	52.56 (41.19; 72.04)	9.982	55.01 ± 13.24	50.30 (46.59; 65.61)	49.95 ± 12.40	50.59 (39.88; 59.12)	0.090	0.130
CAL (kcal)	1673.02 ± 367.03	1586.69 (1489.92; 1863.13)	1647.91 ± 477.20	1478.82 (1423.1; 1965.63)	0.773	1630.08 ± 305.79	1575.86 (1323; 1887.23)	1479.18 ± 355.69	1372.5 (1235.96; 1753.01)	0.027	0.158
Fibres (g)	11.16 ± 4.88	9.23 (7.6; 13.9)	14.26 ± 4.40	13.43 (10.93; 16.7)	0.000	9.50 ± 2.96	9.24 (7.4; 10.97)	8.82 ± 2.88	8.85 (6.9; 9.6)	0.185	0.000

Legend: (1) Comparison at the baseline (before the beverage intake) and after 6 weeks of beverage intake at each intervention, results obtained using the paired Student's t-test or Wilcoxon signed ranks test ($P < 0.05$). (2) Comparison of effect between treatments – comparison of Δ of changes between the two interventions by Student's t-test or the Mann Whitney test ($P < 0.05$).
UBF: unripe banana flour; SD: standard deviation; PTN: proteins; CHO: carbohydrates; LIP: lipids; CAL: caloric intake.

reports that sucralose stimulates sugar receptors present in the tongue, brain and intestine, which causes an increased appetite for sweets and increased intestinal glucose uptake [36, 37]. Pepino et al. (2013) [38] evaluated the acute effects of sucralose intake on obese subjects and demonstrated that this sweetener increases glycaemic and insulin responses in these individuals. It should be noted that the increase in fasting glycaemia that occurred in both groups was of small magnitude and all values remained within the parameters adequate for glycaemia. In addition, there was no change in insulin and HOMA-IR in both groups.

The participants' food intake did not change for any of the evaluated variables, so both groups started the research under the same conditions, showing an adequate randomisation between them.

It is relevant to note that these results may have been influenced by gender. Only women were selected because although obesity affects people of all ages, several issues are particularly pertinent to women's health, mainly because obesity is more prevalent among women than men [39].

The study of these two beverages brought additional information on the effect of cocoa and UBF interaction on biochemical and anthropometric parameters, especially for six weeks, in overweight women. The results presented are innovative, indicating that, depending on the objective, it may be better to use only the cocoa or to add the UBF to the cocoa. That is, for the anthropometric parameters, the Cocoa/UBF beverage proved to be more effective whereas, for lipid metabolism, cocoa was the best option.

Some limitations of the study should be considered, including the possibility of a placebo effect. The participants' expectations from intaking a food product with functional properties may have resulted in a higher motivation to change their eating habits [40], causing anthropometric alterations. Another issue to be raised concerns the age of the participants, since the group with UBF had a mean age greater than the cocoa group. The ageing process influences the whole organism, such as gastric emptying, intestinal absorption and metabolism [41, 42]; therefore, age may have influenced the findings. In addition, a weakness of the study is the lack of an experimental group with only UBF (without cocoa), because would be useful to distinguish which effect is due to each compound.

Conclusion

The beverage with UBF and cocoa reduced waist circumference and the beverage with only cocoa reduced total cholesterol and the LDL/HDL ratio; in addition, both beverages promoted the reduction of body fat percentage. These results suggest that the additional dietary fiber did not further improve health status. The findings of this study imply the necessity for further investigations of functional foods coexisting in the same product because, depending on the objective, their interaction may be beneficial or not. A better knowledge is required of the mechanism of action of these compounds which may lead to the identification of more strategies in clinical nutrition practice.

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