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ORIGINAL ARTICLE

Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects

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Objective: Impaired endothelial function in obesity may reduce blood flow to sites of metabolism, contributing to impaired fat oxidation and insulin resistance. This study investigated the effects of cocoa flavanols and regular exercise, interventions known to improve endothelial function, on cardiometabolic function and body composition in obese individuals.
Design: Overweight and obese adults were randomly assigned to high-flavanol cocoa (HF, 902 mg flavanols), HF and exercise, low-flavanol cocoa (LF, 36 mg flavanols), or LF and exercise for 12 weeks (exercise duration was 3×45 min per week at 75% of age-predicted maximum heart rate). Body composition was assessed by dual-energy X-ray absorptiometry at 0 and 12 weeks. Brachial artery flow-mediated dilatation (FMD), supine blood pressure (BP) and fasting plasma insulin, and glucose levels were assessed at 0, 6 and 12 weeks, respectively. Insulin sensitivity/resistance was determined using the modified homeostasis model assessment of insulin resistance (HOMA2).
Results: A total of 49 subjects (M = 18; F = 31) completed the intervention. Baseline averages were as follows: body mass index = 33.5 kg/m^2; BP = 123/76 mm Hg; HOMA2 = 2.4; FMD = 4.3%; rate of fat oxidation during exercise = 0.34 g min^{-1}; abdominal fat = 45.7% of total abdominal mass. Compared to LF, HF increased FMD acutely (2 h post-dose) by 2.4% ($P<0.01$) and chronically (over 12 weeks; $P<0.01$) by 1.6% and reduced insulin resistance by 0.31% ($P<0.05$), diastolic BP by 1.6 mm Hg and mean arterial BP by 1.2 mm Hg ($P<0.05$), independent of exercise. Regular exercise increased fat oxidation during exercise by 0.10 g min^{-1} ($P<0.01$) and reduced abdominal fat by 0.92% ($P<0.05$).
Conclusion: Although HF consumption was shown to improve endothelial function, it did not enhance the effects of exercise on body fat and fat metabolism in obese subjects. However, it may be useful for reducing cardiometabolic risk factors in this population.
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Keywords: flavanols; FMD; body composition; insulin resistance

Introduction

Endothelial dysfunction is associated with reduced production of nitric oxide (NO) by the endothelium. There is evidence that, in addition to the well-documented adverse cardiovascular effects, reduced availability of NO can impair metabolic function.[1] Among the proposed mechanisms for this is a reduction in blood flow to areas of high metabolic demand such as skeletal muscle.[1]

Flow-mediated dilatation (FMD) of the brachial artery is used to assess the NO-dependent endothelium-mediated dilatory response of an artery and, as such, provides a marker of endothelial function. In recent years, the consumption of cocoa products containing flavanols and procyanidins (oligomeric flavanols) has been shown to elicit dose-dependent acute improvements in FMD, and regular consumption for up to 14 days provides a sustained improvement in FMD.[2–5] Improvements in FMD are associated with increased skeletal muscle blood flow during exercise,[6] suggesting that improvements in FMD might facilitate increased blood flow and nutrient delivery to sites

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of metabolism, particularly skeletal muscle during exercise, with resultant improvements in metabolic control and body composition. Indirect support for this hypothesis comes from studies that have shown that the consumption of flavanol-containing cocoa products, and L-arginine (the primary substrate for NO), not only improve FMD,[7] but also reduce body fat.[8,9]

Aerobic exercise is strongly advocated for increasing energy expenditure and reducing fat stores, but the amount of exercise required to achieve weight loss may be difficult to achieve for obese individuals who are unaccustomed to exercise.[10] However, if cocoa flavanols can improve circulatory function and fat metabolism, it may result in an augmentation of substrate utilization and/or energy expenditure during exercise. Thus, a less demanding and more achievable exercise regimen may be effective for the treatment of obesity. Such an approach has been used successfully in another recent study where the combination of dietary ω-3 fatty acid supplementation with modest exercise provided cardiovascular and metabolic benefits and improved body composition in an obese population.[11]

The primary aim of this study was to investigate whether cocoa flavanol consumption can improve cardiometabolic function and ultimately body composition in an overweight/obese population. A secondary aim was to determine whether the addition of a moderate exercise program may enhance the impact of such effects on adiposity.

Materials and methods

This study used a randomized, double-blind, placebo-controlled, parallel, 2×2 factorial design incorporating consumption of cocoa beverages and regular exercise. The study was conducted at the Nutritional Physiology Research Centre at the University of South Australia (Adelaide, South Australia, Australia) between September 2005 and December 2006. Ethical approval for the study was obtained from the Human Research Ethics Committees of both the University of South Australia and the University of Adelaide and written informed consent was obtained from all subjects prior to their participation.

Subjects

Sedentary (not exercising >1 per week for the purpose of improving health) male and female volunteers aged 18–65 years with a body mass index (BMI) $>25\,kg/m^2$ were recruited from the general public. People with habitually high cocoa consumption (daily consumption of dark chocolate or $>26\,g$ milk chocolate or powdered cocoa), stage 2 hypertension (resting systolic blood pressure (SBP) $>160\,mm\,Hg$ or diastolic BP (DBP) $>100\,mm\,Hg$), existing cardiovascular disease or who were taking any cardiovascular medications (including aspirin) or fish oil supplements were excluded. Initial screening for suitability to participate was based on diet and lifestyle questionnaires, measurement of height, weight and blood pressure (BP), and a medical clearance for exercise based on electrocardiogram monitoring during a graded submaximal treadmill test.

Intervention

Volunteers were block-matched into two groups according to BMI, gender, age and BP. The groups were then randomized to the daily consumption of either a high-flavanol cocoa (HF) or low-flavanol cocoa (LF; placebo) drink for 12 weeks. Volunteers in each of the two groups were then further randomized to either undertake a program of regular modest exercise for 12 weeks, or to remain sedentary. At baseline (week 0), subjects attended the clinic following an overnight fast ($\geqslant 12\,h$) for collection of blood samples and measurement of BP, FMD, body composition (dual-energy X-ray absorptiometry (DXA)) and fat oxidation during submaximal exercise. Each subject then consumed a single packet of the appropriate beverage product (LF or HF; acute intervention) and FMD was reassessed 2 h later. On the day following the baseline testing, subjects commenced the chronic (12-week) intervention and the baseline tests were repeated after 6 and 12 weeks. Body composition was assessed at baseline and week 12 only. On test days at week 6, cocoa drinks were not consumed until all assessments had been completed. Subjects were instructed not to change their dietary habits during the study, aside from consuming the appropriate drink, restricting caffeine intake to no more than two caffeinated beverages per day and avoiding red wine and dark chocolate throughout the intervention.

Dietary intervention

The cocoa-containing product used in this study was a dairy-based powder mix. Table 1 shows the macro/micronutrient content of the HF and LF drink mixes. They were closely matched for all ingredients, other than the cocoa flavanol content. The cocoa flavanol content refers to the total amount of epicatechin, catechin and procyanidins up to decamers in the drinks. The drink mixes were mixed with 150 ml of cold water immediately prior to consumption.

Table 1 Nutritional contents of high- and low-flavanol cocoa drinks

	High flavanol cocoa drink	*Low flavanol cocoa drink*
Flavanols (mg)	451	18
Energy (kJ)	497	490
Total fat (g)	1.4	1.5
Sat fat (g)	0.8	0.8
Total CHO (g)	17.1	16.5
Sugars (g)	9.4	9.2
Protein (g)	9.4	9.2
Fibre (g)	3	3.9
Caffeine (mg)	18	21
Theobromine (mg)	337	327

Subjects were instructed to consume the beverages twice daily throughout the intervention period, which provided 902 and 36 mg of cocoa flavanols per day in the HF and LF groups, respectively. To monitor compliance with the intervention, subjects recorded their drink consumption in a diary each day. Empty sachets were returned at weeks 6 and 12 and counted as a further check of compliance.

Three-day physical activity[12] and food diaries (analyzed with Foodworks Professional Edition (version 3.02; Xyris Software, Highgate Hill, Queensland, Australia)) were recorded by all subjects during the week prior to commencing the intervention and the final week of the intervention study to identify any changes in background energy intake/expenditure that may have influenced study outcomes. Subjects were instructed to substitute the cocoa beverages for other foods rather than simply adding the cocoa drink on top of their usual energy intake.

Exercise protocol

Volunteers randomized to undertake regular exercise walked or jogged three times per week for 45 min at an exercise intensity that elicited 75% of their age-predicted maximum heart rate (220–age in years) for 12 weeks. The nonexercising volunteers were asked to maintain their normal levels of physical activity. To assist with maintaining compliance, subjects in the exercise group were required to attend at least one supervised exercise session per week and to maintain a training diary.

Assessment of flow-mediated dilatation and blood pressure

Endothelial function was assessed by FMD. The diameter of the brachial artery was measured by a single operator with the use of two-dimensional B-mode ultrasound (LOGIQ 5; GE Medical Systems, Waukesha, WI, USA). Optimal imaging of the artery has been described by Raitakari and Celermajer.[13] For the production of reactive hyperemia, a sphygmomanometer cuff was placed around the midpoint of the forearm (that is, distal to the scanned part of the artery) and inflated to a pressure of 200 mm Hg for 5 min. Images of the artery were taken before cuff inflation, 10 s before cuff release, 10 s after cuff release and then every 30 s for an additional 3 min. On the basis of repeat assessments of FMD on 2 consecutive days in 12 subjects the standard error of the estimate for this technique in our laboratory, expressed as a coefficient of variation, is 5%. Resting BP and heart rate were measured after 10 min lying supine by automated oscillometry using a Spacelabs ambulatory BP monitor (Spacelabs Healthcare, WI, USA). Four readings were taken over a 15 min period, the first value was discarded and BP was calculated as the mean of the remaining three measurements.

Anthropometric assessment

Body mass and waist circumference were assessed at weeks 0, 6 and 12. Body composition was assessed at weeks 0 and 12 only using DXA (Lunar Prodigy; General Electric, Madison, WI, USA). Abdominal fat content was determined using regional analysis of the body segment bordered superiorly and inferiorly by the lowest point of the rib cage and the uppermost aspect of the iliac crests, respectively, and extended laterally to the outer edge of the rib cage.

Fat oxidation

Fat oxidation during exercise was assessed at weeks 0, 6 and 12 by indirect calorimetry (True One 2400; Parvo Medics, Sandy, UT, USA). Subjects walked on a treadmill (Model Q65; Quinton Instruments, Bothell, WA, USA) at a comfortable speed, and at an incline that elicited a heart rate equivalent to 75% of their age-predicted maximum (220−age (year) × 0.75) for 10 min. The same treadmill speed and incline was used at weeks 0, 6 and 12. Fat oxidation was estimated from steady-state measurements of oxygen uptake and carbon dioxide output during the final minute of exercise using the stochiometric equation of Frayn.[14] Both oxygen consumption and the respiratory exchange ratio reached a plateau by the seventh minute of the exercise test in all cases.

Blood sampling and analysis of biomarkers

Fasting blood samples were obtained by venepuncture and plasma was stored at −80 °C until analysis. Fasting plasma glucose and lipid concentrations were determined using a commercial assay kit with a Synchron LX20 autoanalyzer (Beckman Coulter Inc., Fullerton, CA, USA). Fasting plasma insulin concentrations were determined by radioimmunoassay (Human Insulin Specific RIA Kit; Millipore, Billerica, MA, USA) using a Packard Cobra Auto-Gamma counter (PerkinElmer, Meriden, CT, USA). Insulin resistance was estimated from fasting glucose and insulin concentrations using the modified homeostasis model assessment of insulin resistance (HOMA2) as described by Wallace *et al*.[15] As previously reported, this model provides an index of insulin resistance (HOMA2—IR), β-cell function (%B) and insulin sensitivity (%S).[15]

Statistical analysis

Statistical analysis was performed using Statistica for Windows software (version 5.1; StatSoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) with repeated measures was used to determine the effect of the treatments, time of measurement and their interactions on the dependent variables. Where ANOVA showed no interaction effect between exercise and cocoa, factorial analysis was performed for each of these separate treatments to detect independent effects. Where ANOVA showed a statistically significant main effect, pair-wise comparisons were performed using Tukey's Honestly Significant Differences test to determine differences between means. To optimize the analysis of

differences between treatments, where appropriate, a nested ANOVA design was used to examine changes in dependent variables from baseline with the treatment factor (that is, cocoa or exercise) nested in time. Statistical significance was set at an *a* level of 0.05. Data are expressed as mean ± s.e.m. in tables and figures.

Results

General

A total of 98 subjects were screened for inclusion in the study; of these 65 overweight/obese volunteers were enrolled and 49 completed the full 12 weeks of the intervention. Of those that completed, there was an average compliance of 98% for cocoa drink consumption and 86% for the exercise component (that is, of a required three sessions per week of exercise the average achieved was 2.6). Fourteen subjects withdrew during the study due to time restrictions or changes in personal circumstance unrelated to the requirements of the intervention and two subjects were excluded due to noncompliance with study requirements (one failed to comply with exercise protocol and one went on a calorie-restricted diet). Baseline characteristics are shown in Table 2.

Cardiovascular parameters

There were no significant three-way interactions (cocoa × exercise × time) for any of the cardiovascular outcomes (FMD $P=0.957$; SBP $P=0.856$; DBP $P=0.92$; low-density lipoprotein (LDL) $P=0.93$; high-density lipoprotein (HDL) $P=0.17$; triglyceride $P=0.18$). FMD increased significantly in the HF group compared to LF 2 h after consuming a single 450 mg dose of the cocoa drink ($P=0.02$; Figure 1). Resting (preocclusion) vessel diameter did not differ between groups pre- or post-cocoa consumption ($P=0.26$) nor did it change as a result of regular consumption of cocoa for 12 weeks ($P=0.52$). FMD increased significantly at Weeks 6 and 12 relative to baseline in the HF group compared to LF ($P=0.002$, cocoa × time interaction; Figure 1). Thus, chronic HF consumption had a sustained effect on FMD which was still evident at least 12 h after consumption of the previous dose.

BP and HR measurements tended to decrease over time with HF compared to LF treatment (Figure 2), although the differences between treatments were not significant at either 6 or 12 weeks. However, the effects at both the time points were taken into consideration by nesting the effects of cocoa on BP in time. This combined analysis revealed a significant reduction in both diastolic ($P=0.04$) and mean arterial pressure ($P=0.05$) with HF versus LF but there were no differences in systolic BP or HR.

Table 2 Baseline characteristics of all four treatment groups and of the combined cocoa and exercise treatment groups

	LF+Ex	*HF+Ex*	*LF−Ex*	*HF−Ex*
Number (M/F)	4/9	6/7	3/8	4/8
Height (cm)	168.7 ± 2.7	168.7 ± 2.9	165.3 ± 4.1	171.5 ± 3.6
Weight (kg)	94.7 ± 2.2	94.7 ± 5.2	94.6 ± 6.1	97.2 ± 5.8
BMI (kg/m^2)	33.5 ± 1.1	33.2 ± 1.6	34.5 ± 1.8	32.8 ± 1.1
Age (years)	45.2 ± 3.0	45.5 ± 4.0	44.4 ± 4.4	45.3 ± 4.4
Waist (cm)	107 ± 2.0	105 ± 6.0	109 ± 4.0	107 ± 4.0
FMD (%)	5.37 ± 0.68	4.05 ± 0.51	3.65 ± 1.40	4.12 ± 0.75
SBP (mm Hg)	121 ± 3.6	126 ± 2.7	124 ± 1.8	124 ± 3.0
DBP (mm Hg)	74 ± 1.6	78 ± 2.4	77 ± 1.5	76 ± 1.8
HR (bpm)	60 ± 1.4	66 ± 2.1	61 ± 1.9	66 ± 3.1
Total Chol (mmol l^{-1})	5.39 ± 0.25	5.94 ± 0.24	6.1 ± 0.22	5.43 ± 0.37
LDL Chol (mmol l^{-1})	3.25 ± 0.18	3.74 ± 0.2	3.75 ± 0.22	3.26 ± 0.33
HDL Chol (mmol l^{-1})	1.52 ± 0.09	1.53 ± 0.18	1.48 ± 0.13	1.54 ± 0.15
Trigs (mmol l^{-1})	1.28 ± 0.16	1.56 ± 0.14	1.89 ± 0.18	1.37 ± 0.2
Glucose (mmol l^{-1})	5.2 ± 0.1	5.5 ± 0.2	5.1 ± 0.2	5.6 ± 0.1
Insulin (μU ml^{-1})	15.2 ± 1.1	21 ± 2.7	19.2 ± 2.3	19.3 ± 2.4
HOMA2–IR	2.03 ± 0.15	2.70 ± 0.35	2.45 ± 0.29	2.46 ± 0.30
Fat oxid (g min^{-1})	0.28 ± 0.02	0.30 ± 0.13	0.35 ± 0.12	0.42 ± 0.04
%BF (%)	43.8 ± 1.7	43.2 ± 2.4	44.9 ± 2.5	44.1 ± 2.6
%ABF (%)	45.5 ± 1.4	45.0 ± 1.4	48.2 ± 1.8	44.4 ± 1.0

Abbreviations: %ABF, total percentage body fat in abdominal region; %BF, total percentage body fat; BMI, body mass index; Chol, cholesterol; DBP, diastolic blood pressure; Ex, exercise program; Fat oxid, rate of fat oxidation during exercise; FMD, % change in FMD; HF, high-flavanol drink; HOMA2–IR, modified homeostasis model assessment of insulin resistance; HR, heart rate; LF, low-flavanol drink; SBP, systolic blood pressure.

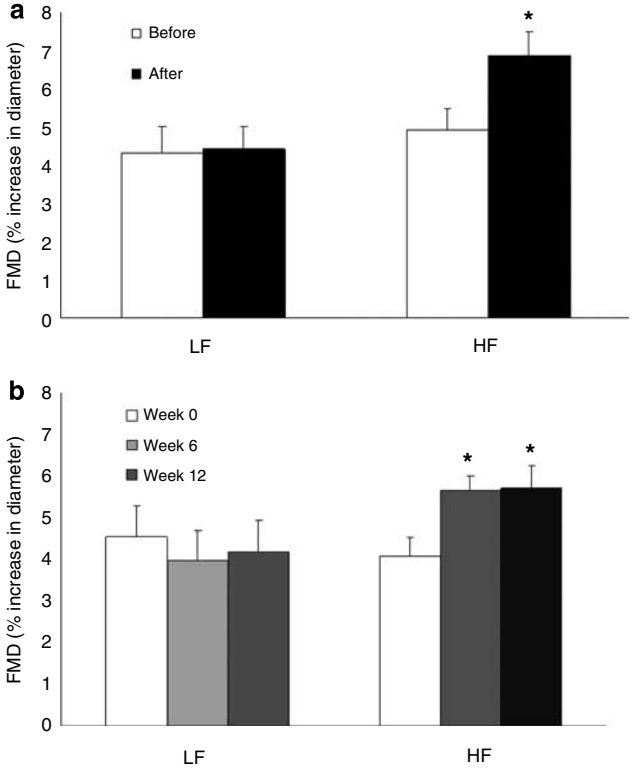

Figure 1 Flow-mediated dilatation, expressed as percent increase in brachial artery diameter: (**a**) before and 2 h after initial cocoa administration at baseline and (**b**) following a 12 h fast at baseline, 6 and 12 weeks. LF, low flavanol; HF, high flavanol (pooled for cocoa treatment combining both exercise and nonexercise groups). Asterisk (*) represents significantly different change from baseline between treatments (panel **a**, $P=0.02$; panel **b**, $P<0.01$).

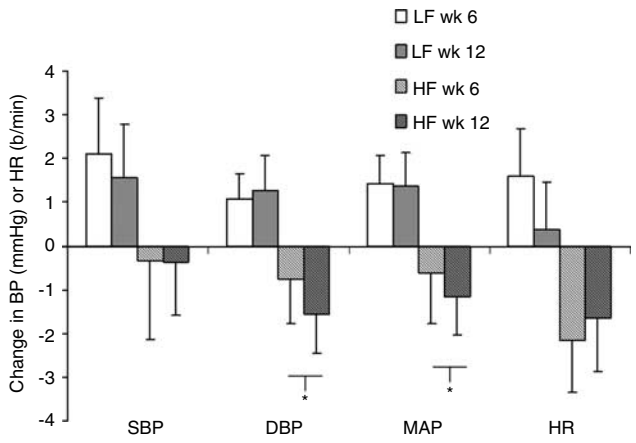

Figure 2 Changes in blood pressure and heart rate from baseline to weeks 6–12. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; LF, low flavanol; HF, high flavanol (pooled for cocoa treatment combining both exercise and nonexercise groups). *HF significantly different from LF by nested analysis of weeks 6 and 12 data ($P<0.05$).

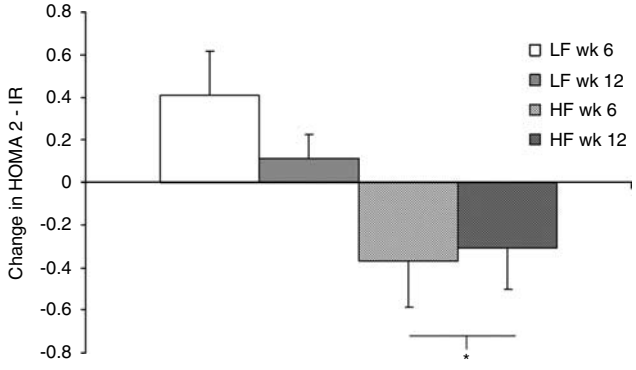

Figure 3 Change in homeostasis model assessment of insulin resistance (HOMA2–IR) from baseline to weeks 6 and 12. LF, low flavanol; HF, high flavanol (pooled for cocoa treatment combining both exercise and nonexercise groups). *HF significantly different from LF by nested analysis of weeks 6 and 12 data ($P<0.05$).

Metabolic and anthropometric parameters

There were no significant three-way interactions (cocoa × exercise × time) for any of the metabolic parameters (fat oxidation during exercise $P=0.95$; percent body fat $P=0.97$; percent abdominal fat $P=0.09$; HOMA2—IR $P=0.79$, %B $P=0.6$, %S $P=0.89$). The addition of gender as a factor in the ANOVA revealed no interaction with the effects of exercise or cocoa on any parameter. Table 3 displays the change data for metabolic and anthropometric assessments in each intervention group. Exercise was independently associated with a significant reduction in abdominal body fat percentage ($P=0.02$). Nesting the effects of cocoa in time revealed a significant improvement in all HOMA2 parameters with HF compared to LF. This difference in HOMA2 parameters remained when differences at baseline were controlled for using analysis of covariance ($P<0.05$). Neither HF cocoa consumption, nor regular exercise, had any significant effect on BMI, waist circumference or total body fat.

Diet and physical activity

Diet and background physical activity did not change in either group during the study period (Table 4). The apparent discrepancy between energy intake and expenditure is likely due to underreporting of total food intakes and overreporting of physical activity in this subject population.

Discussion

These results confirm previous findings of enhanced endothelial vasodilator function following acute ingestion of HF cocoa and maintenance of this effect with ongoing consumption. Furthermore, they demonstrate that this effect on FMD remains during 12 weeks of ongoing beverage consumption, which is 10 weeks longer than previously reported. They also confirm the previous finding of increased insulin sensitivity with chronic HF cocoa consumption. This is the first study to examine these effects in an overweight/obese population and to do so using a double-blind placebo-controlled study design. However, despite the fact that the regular consumption of a HF cocoa drink improved FMD and insulin resistance, these improvements were not associated with any changes in body composition. Only exercise resulted in an independent effect on body composition by reducing abdominal fat.

An increase in arterial dilatation, determined from changes in digital pulse wave amplitude following forearm occlusion has been demonstrated previously in healthy adults after consuming 851 mg cocoa flavanols per day for 5 days.[2] Recently Heiss *et al.*[3] also reported an increase in arterial dilatation, assessed by FMD, in a group of smokers following 7 days of consuming 918 mg per day of cocoa flavanols. While these studies provide clear evidence of an effect of short-term consumption of cocoa flavanols on endothelial function, the lack of placebo control prevents a definitive interpretation of the results as being directly caused by the cocoa flavanols. A previous placebo-controlled study conducted in postmenopausal hypercholesterolemic women was able to demonstrate an increase in brachial artery blood flow following 6 weeks of HF cocoa consumption (446 mg flavanols per day) but fell marginally short of showing a significant improvement in FMD,[16] potentially due to a lack of statistical power. Prior to the present study, Grassi *et al.*[17] had provided the strongest evidence for benefits of chronic consumption of cocoa-containing flavanols on FMD when it was demonstrated, using a cross-over design, that consuming 100 g of dark chocolate each day (containing around 500 mg of cocoa 'polyphenols') for 15 days resulted in significant improvements in FMD, BP and insulin sensitivity compared with consuming white choco-

Table 3 Absolute change from baseline to Week 12

	LF+Ex	*HF+Ex*	*LF−Ex*	*HF−Ex*
Number	13	13	11	12
BMI (kg/m^2)	0.2 ± 0.6	0.5 ± 0.8	0.9 ± 0.4	−0.4 ± 0.6
Waist (cm)	0 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	−0.3 ± 0.2
FMD (%)	−0.4 ± 0.77	1.5 ± 0.68	−0.3 ± 0.53	1.8 ± 0.89
SBP (mm Hg)	−0.5 ± 2.3	1.1 ± 1.6	4.2 ± 2.6	−1.9 ± 1.9
DBP (mm Hg)	−0.2 ± 1.5	−0.5 ± 1.0	2.8 ± 1.7	−1.8 ± 1.5
HR (bpm)	1.1 ± 2.1	−3.2 ± 1.2	0.1 ± 1.3	−0.1 ± 2.1
Total Chol (mmol l^{-1})	−0.56 ± 0.20	−0.42 ± 0.21	−0.28 ± 0.23	−0.16 ± 0.25
LDL Chol (mmol l^{-1})	−0.11 ± 0.08	−0.15 ± 0.09	2.02 ± 0.09	−0.08 ± 0.05
HDL Chol (mmol l^{-1})	−0.31 ± 0.12	−0.27 ± 0.22	−0.31 ± 0.15	−0.15 ± 0.24
Trigs (mmol l^{-1})	−0.17 ± 0.14	−0.02 ± 0.07	0.09 ± 0.13	0.10 ± 0.10
Glucose (mmol l^{-1})	0.23 ± 0.14	−0.03 ± 0.08	−0.12 ± 0.12	−0.23 ± 0.93
Insulin (μU ml^{-1})	0.82 ± 1.3	−3.5 ± 1.7	0.79 ± 1.34	−1.4 ± 2.7
HOMA2–IR	0.14 ± 0.17	−0.41 ± 0.22	0.08 ± 0.17	−0.21 ± 0.34
Fat oxid (g min^{-1})	0.062 ± 0.033	0.140 ± 0.042	−0.022 ± 0.024	−0.042 ± 0.043
%BF (%)	−0.46 ± 0.38	−0.42 ± 0.34	0.051 ± 0.26	0.11 ± 0.29
%ABF (%)	−0.52 ± 0.28	−1.31 ± 0.49	−0.06 ± 0.34	0.43 ± 0.32

Abbreviations: %ABF, total percentage body fat in abdominal region; %BF, total percentage body fat; BMI, body mass index; Chol, cholesterol; DBP, diastolic blood pressure; Ex, exercise program; Fat oxid, rate of fat oxidation during exercise; FMD, % change in FMD; HF, high-flavanol drink; HOMA2–IR, modified model assessment of insulin resistance; HR, heart rate; LF, low-flavanol drink; SBP, systolic blood pressure.

late containing no polyphenols. A potential limitation of this study was the lack of blinding of the treatment due to the use of dark and white chocolate. The present study design overcame this limitation by using reconstituted cocoa beverages that were well matched for taste and appearance. This also allowed a consistent dose of methylxanthine between cocoa treatments, whereas other studies have used methylxanthine-free white chocolate as a control. A further advantage of the powders used in the current study was that we were able to deliver a high-flavanol load with considerably lower total energy than chocolate. The energy per mg of flavanol in the HF cocoa drink was approximately 1.1 kJ compared to 4.02 kJ in the chocolate product used by Grassi and colleagues. This is an important consideration when long-term dietary intervention is being investigated, particularly in overweight subjects and when body composition is a primary outcome measure.

Improved endothelial function has been identified as a likely mechanism for the antihypertensive effects of cocoa flavanols and the concurrent improvements seen in FMD and BP here, as well as the effects previously reported by Grassi *et al.*,[17] provide further evidence to support this hypothesis. The magnitude of change in the present study was relatively small when compared to that reported by Grassi *et al.*, despite the higher intake and longer duration of cocoa consumption in the present study.[17] Grassi *et al.* reported a reduction of 11.9/8.5 mm Hg (systolic/diastolic) in a hypertensive population after 15 days[17] and, even in a normotensive population, they saw a reduction of 7/3 mm Hg (diastolic not significant) with the same protocol.[4] The present intervention saw a 1.6 mm Hg drop in diastolic and 1.2 mm Hg in mean arterial BP over 12 weeks. The magnitude of difference between treatments was greater than this, however, as BP tended to rise in the placebo group. The net difference from placebo treatment was still considerably lower than reported by Grassi *et al.*, but is comparable to the change seen over 18 weeks in an essential hypertensive population by Taubert *et al.*[18] with a surprisingly low dose of polyphenols/flavanols (30 mg per day). Although Taubert *et al.* controlled background dietary polyphenol consumption more tightly than in the present study, there remains an apparent discrepancy in the magnitude of response to a given level of polyphenol/flavanol consumption between these studies. Therefore, additional studies are warranted to better understand the relationship between flavanol consumption and changes in BP.

A recent review described multiple mechanisms by which reduced availability of NO, as seen in endothelial dysfunction, can impair metabolic function.[1] Among these are potential decreases in mitochondrial biogenesis and oxidative phosphorylation; activation of specific transcription factors and signaling pathways and reductions in blood flow to areas of high metabolic demand such as skeletal muscle. It is plausible therefore that improvements in muscle blood flow, and insulin sensitivity, which occur secondarily to improvements in endothelial function may be useful for the prevention or management of obesity. Supplementation with L-arginine, the primary substrate for NO, in Zucker diabetic fatty rats increased NO production by 71–85% and attenuated increases in fat mass over 10 weeks compared to those supplemented with alanine,[9] indicating a role of improved endothelial function in obesity management. More direct evidence of a potential role of cocoa in this

Table 4 Energy expenditure and intake of energy and macronutrients at weeks 0 and 12, derived from 3-day physical activity diary and weighed food diary

	Week 0	*Week 12*
Energy expenditure (kJ)		
LF+Ex	16 320 ± 575	15 534 ± 1006
HF+Ex	15 855 ± 814	15 976 ± 1058
LF−Ex	16 086 ± 1650	16 321 ± 1675
HF−Ex	16 700 ± 1203	16 258 ± 1201
Energy intake (kJ)		
LF+Ex	9587 ± 545	9139 ± 637
HF+Ex	8388 ± 420	8502 ± 611
LF−Ex	9243 ± 1136	8971 ± 1210
HF−Ex	8107 ± 463	7701 ± 501
Protein[a]		
LF+Ex	18.7 ± 1.24	18.5 ± 1.29
HF+Ex	18.9 ± 1.48	17.8 ± 1.56
LF−Ex	16.1 ± 1.73	17.5 ± 1.68
HF−Ex	19.6 ± 1.41	19.1 ± 1.28
Fat[a]		
LF+Ex	34.4 ± 2.21	34.4 ± 3.39
HF+Ex	33.4 ± 2.30	32.0 ± 3.16
LF−Ex	32.9 ± 3.13	37.2 ± 6.79
HF−Ex	32.4 ± 3.13	30.8 ± 2.98
Saturated fat[a]		
LF+Ex	14.1 ± 1.22	12.9 ± 1.52
HF+Ex	14.4 ± 1.50	13.1 ± 1.83
LF−Ex	11.7 ± 1.04	14.2 ± 2.79
HF−Ex	13.0 ± 1.61	11.9 ± 1.61
Polyunsaturated fat[a]		
LF+Ex	4.6 ± 0.53	5.0 ± 0.87
HF+Ex	4.1 ± 0.34	3.8 ± 0.47
LF−Ex	5.1 ± 0.67	6.0 ± 1.03
HF−Ex	4.8 ± 0.80	4.77 ± 0.48
Monounsaturated fat[a]		
LF+Ex	11.7 ± 0.83	11.7 ± 1.21
HF+Ex	11.9 ± 1.08	10.5 ± 1.62
LF−Ex	13.1 ± 1.70	13.7 ± 2.62
HF−Ex	11.2 ± 1.04	11.1 ± 1.19
CHO[a]		
LF+Ex	44.9 ± 3.29	44.4 ± 2.73
HF+Ex	44.7 ± 3.15	48.5 ± 3.38
LF−Ex	46.2 ± 10.4	38.9 ± 5.98
HF−Ex	41.7 ± 3.13	45.8 ± 5.80

[a]Expressed as percentage of total energy intake.

was provided by a recent Japanese study.[8] Inclusion of cocoa powder in a high-fat diet significantly blunted the increase in fat mass compared to placebo in male Wistar rats, potentially as a result of reduced gene expression for fat synthesis. Although not evaluated in this study, a potential obesity resisting effect of HF cocoa consumption would be beneficial in itself if it could be replicated in humans and warrants further investigation as energy imbalance is the underlying cause of obesity.[19] This was the first study to investigate the effects of cocoa flavanol consumption on body composition in humans but, although regular HF cocoa consumption was able to independently improve FMD and insulin sensitivity, presumably through increased NO availability, there was no effect on body composition over a 12-week period. Only regular exercise resulted in an independent, although modest, reduction in abdominal body fat, and this reduction was associated with an increase in fat oxidation during submaximal exercise.

In conclusion, this was the first study to evaluate the cardiometabolic effects of HF cocoa consumption with and without regular moderate exercise in an overweight obese population. Regular exercise for 12 weeks leads to an increased rate of fat oxidation during exercise and reduced abdominal adiposity. Although HF cocoa consumption did not directly affect body composition, nor augment the effect of exercise on body composition, it was associated with enhanced endothelial function, reductions in diastolic and mean arterial BP, and reduced insulin resistance. These observations indicate that HF cocoa consumption may be useful, particularly when combined with a program of regular modest physical activity, for reducing cardiometabolic risk factors in an obese population.

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