

The effect of polyphenol-rich dark chocolate on fasting capillary whole blood glucose, total cholesterol, blood pressure and glucocorticoids in healthy overweight and obese subjects

Suzana Almoosawi¹, Lorna Fyfe¹, Clement Ho² and Emad Al-Dujaili^{1*}

¹Department of Dietetics, Nutrition and Biological Sciences, Queen Margaret University, Queen Margaret Drive, Musselburgh EH21 6UU, UK

²Department of Clinical Biochemistry, Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh EH16 4SA, UK

(Received 31 March 2009 – Revised 21 July 2009 – Accepted 14 September 2009 – First published online 13 October 2009)

Numerous studies indicate that polyphenol-rich chocolate reduces fasting blood glucose, blood pressure (BP) and total cholesterol in healthy individuals and hypertensives with or without glucose intolerance. The aim of the present study was to investigate the effect of two doses of polyphenol-rich dark chocolate (DC) on fasting capillary whole blood glucose, total cholesterol and BP and to examine whether improvements in these parameters are associated with changes in adrenocorticoid excretion in overweight and obese individuals. The study used a randomised, single-blind, cross-over design where fourteen overweight and obese subjects were randomised to either take 20 g DC with 500 mg polyphenols then 20 g DC with 1000 mg polyphenols or vice-versa. Participants followed each diet for 2 weeks separated by a 1-week washout period. It was observed that the 500 mg polyphenol dose was equally effective in reducing fasting blood glucose levels, systolic BP (SBP) and diastolic BP (DBP) as the 1000 mg polyphenol dose suggesting that a saturation effect might occur with increasing dose of polyphenols. There was also a trend towards a reduction in urinary free cortisone levels with both groups although it did not reach statistical significance. No changes in anthropometrical measurements were seen. We suggest that more research is required to investigate the mechanism(s) by which polyphenol-rich foods influence health.

Dark chocolate: Glucose: Blood pressure: Cholesterol: Glucocorticoids: Obesity

Epidemiological studies link high polyphenol intake with reduced risk of oxidative stress-related diseases like diabetes, hypertension and CVD^(1–3). In particular, consumption of cocoa and dark chocolate (DC) has been shown to improve endothelium function, insulin sensitivity, blood pressure (BP) in healthy individuals, hypertensives with or without glucose intolerance^(4–6) and obese subjects⁽⁷⁾. Cocoa and DC are rich sources of polyphenols providing on average more polyphenols per serving than red wine, green tea or black tea⁽⁸⁾. These polyphenols confer potent antioxidant properties to cocoa and DC^(8,9) in addition to the ability to regulate NO^(4–7).

Obesity is known to be associated with insulin resistance and elevated BP⁽¹⁰⁾. One of the underlying factors linked to these cardiovascular risk factors is abnormal cortisol metabolism^(11,12). Cortisol is a counterregulatory hormone that is essential in the long-term maintenance of blood glucose⁽¹³⁾ and which could also unfavourably influence BP and lipid profile^(12–15). When present in excess, cortisol induces overproduction of reactive oxygen species^(16,17) leading to reduced endothelial NO synthase expression⁽¹⁸⁾. In obesity, particularly abdominal obesity, postprandial hypercortisolism

and enhanced peripheral metabolism of cortisol, characterised by increased urinary cortisone-to-cortisol ratio, are observed which are linked to insulin resistance and increased fasting insulin⁽¹¹⁾. Increased expression of subcutaneous adipose tissue 11 β -hydroxysteroid dehydrogenase type 1 has also been reported, which is known to impair glucose-stimulated insulin secretion⁽¹⁹⁾. Since improved NO bioavailability is the main mechanism by which DC polyphenols reduce endothelium dysfunction, insulin resistance and hypertension^(4–7), this preliminary study aimed to assess and compare the effect of DC containing two different doses of polyphenols on fasting capillary whole blood glucose levels, total cholesterol, BP, urinary free cortisol and cortisone excretion in healthy overweight and obese subjects. The other objective was to observe whether improvements in fasting blood glucose, total cholesterol and BP could be correlated with changes in urinary free cortisol or cortisone excretion. A secondary objective was to monitor Mg intake and excretion since DC is known to contain large quantities of Mg, which, in turn, could influence BP, insulin action and the metabolic syndrome^(20–22).

Abbreviations: BP, blood pressure; DBP, diastolic BP; DC, dark chocolate; FG, fasting glucose; SBP, systolic BP.

* **Corresponding author:** Emad Al-Dujaili, fax +44 131 474 0001, email ealdujaili@qmu.ac.uk

Methods

Study design

The study used a randomised, cross-over design where each subject acted as their own control. Following a 1-week run-in phase, eligible subjects were randomly assigned to one of the two polyphenol doses: 500 mg polyphenols DC or 1000 mg polyphenols DC. Participants followed each intervention for 2 weeks, after which they were crossed-over to the next intervention separated by a 1-week washout period (Fig. 1). The study included healthy non-smoker volunteers, aged 19–50 years with BMI ≥ 25 kg/m²(23), no history of diabetes, hypertension or CVD. People taking dietary supplements, BP or cholesterol-lowering drugs, or those with soya and nut allergies were excluded. Smokers were excluded to minimise confounding factors since nicotine consumption is known to enhance hypothalamic–pituitary–adrenal axis activity, hence resulting in elevated cortisol levels(24,25). Participants gave written consent, completed a lifestyle questionnaire before being screened for fasting blood glucose, total cholesterol, BP and BMI to determine their eligibility. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by Queen Margaret University Ethics Committee.

Diet

Table 1 provides a summary of the nutrient composition of the two Acticoa DC used in the present study, which were kindly supplied by Barry Callebaut (Lebbeke, Belgium). The 500 mg dose was previously shown to reduce fasting glucose (FG) levels and BP by Grassi *et al.* (4,5) and Taubert *et al.* (26). However, due to the great variation in epicatechin and catechin levels between the chocolate used in the present study and the one used by Grassi *et al.* (4,5), a higher DC dose of 1000 mg was also chosen. This 1000 mg dose was selected to provide similar quantities of polyphenol to what is consumed by the Kuna population of Panama, who are known to consume large quantities of cocoa and to have low incidence of hypertension(27). This dose will also provide

Table 1. Nutritional composition of 20 g of 500 and 1000 mg polyphenol dark chocolate (DC)

Component	500 mg DC	1000 mg DC
Polyphenols (mg)	500	1000
Epicatechin and catechin (mg)	18.99	37.98
Energy (kJ)	425.8	425.8
Fat (g)	7.34	7.34
Protein (g)	1.34	1.34
Carbohydrate (g)	7.44	7.44
Mg (mg)	33.42	33.42
Na (mg)	1.4	1.4
K (mg)	168.42	168.42

about 43.2 % of the epicatechin and catechin dose used by Grassi *et al.* (4,5). Subjects were instructed to distribute DC doses throughout the day to achieve a high steady-state concentration. They were also instructed to maintain their usual diet throughout the study but to refrain from polyphenol-rich foods and beverages that supply ≥ 15 mg/kg epicatechin and ≥ 4 mg/l epicatechin(28–30). Subjects completed a 3-d (two weekdays and one weekend) diet and physical activity(31) diary during the run-in phase and at the end of each dietary intervention. The Photographic Atlas of Food Portion Sizes was used to assist subjects in describing their portion sizes(32). Diet diaries were validated by interviewing the subjects using a validated questionnaire(33). The diet diaries were analysed and energy, fat, protein, carbohydrate and magnesium intake were estimated using Windiet software (Windiet Research, Univation Ltd, Robert Gordon University, Aberdeen, UK). Compliance with the study's protocol was assessed by direct interviewing, returning of empty chocolate foils and assessment of diet diaries.

Measurements

To measure fasting blood glucose and total cholesterol, 12-h fasting capillary whole blood samples were obtained and analysed using a calibrated Accutrend GC system (Roche diagnostics, Mannheim, Germany). Participants were instructed to consume the last DC dose 12 h before analyses(34), avoid heavy physical activity and alcohol intake 24 h before testing and consume the same diet the day before each test(30). Waist circumference, hip circumference and BMI were measured. Data on waist and hip circumference were used to calculate waist-to-hip ratio, where waist-to-hip ratio > 1.0 in men and > 0.85 in women indicate abdominal obesity(35). Both waist circumference and BMI serve as good indicators of the degree of insulin resistance in overweight and obese individuals(35) while waist-to-hip ratio serves as a predictor of hypertension(36) and hypothalamic–pituitary–adrenal axis hyperactivity, characterised by high baseline plasma cortisol and low 24-h urinary cortisol excretion in obese women(11). An automated A&D Medical UA-767 BP monitor (A&D Medical, San Jose, CA, USA) was used to measure BP according to Grassi *et al.* (5). This monitor was previously validated and was shown to achieve grade A for both systolic and diastolic BP according to the British Hypertension Society standard(37).

Urine samples were obtained for estimating 24-h urinary Mg excretion. Urinary Na and K excretion were also

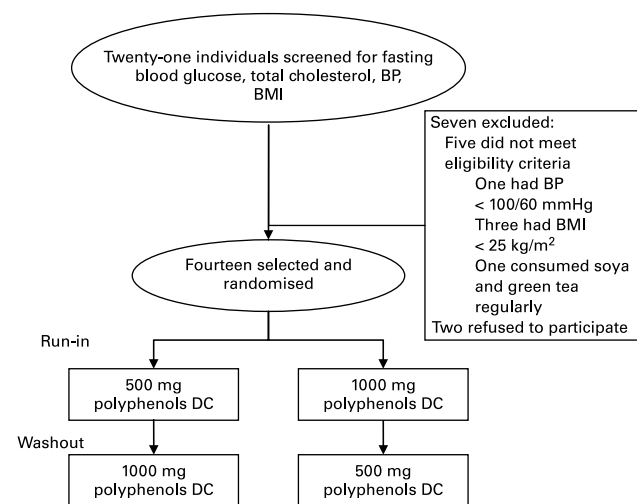


Fig. 1. Diagram showing random allocation of subjects into the different dietary interventions. BP, blood pressure; DC, dark chocolate.

monitored since they serve as direct measures of Na and K intake, which could act as confounding factors in relation to BP. The 24-h urine collections were validated by measuring creatinine excretion^(38,39). Analyses of urine Na, K and Mg concentrations were conducted using an automated platform (Olympus, Essex, UK) at the Clinical Biochemistry Laboratory, Royal Infirmary of Edinburgh, Scotland, UK. Urinary cortisol and cortisone levels were analysed in duplicates using ELISA according to the method described by Al-Dujaili & Bryant⁽⁴⁰⁾ and Al-Dujaili⁽⁴¹⁾. The data were then used to calculate urinary cortisol-to-cortisone ratio. This ratio serves as a measure of renal 11β -hydroxysteroid dehydrogenase type 2 activity⁽⁴²⁾. Monitoring the activity of this enzyme helps detect changes in peripheral metabolism of cortisol⁽⁴²⁾. All tests were carried out at baseline, before and after each intervention.

Statistical methods

All data are expressed as means and standard deviations. Mixed between–within subjects ANOVA or split-plot ANOVA was performed for multiple comparison, where time (baseline, week 1, week 2) was the within-group variable and intervention group (500 mg, 1000 mg DC) was the between-group variable and the continuous variable were FG, SBP, DBP, BMI, weight, waist circumference, hip circumference and waist-to-hip circumference. A P -value ≤ 0.05 was considered statistically significant. Within each intervention group (500, 1000 mg DC), changes in fasting blood glucose levels, SBP and DBP were analysed using repeated measures ANOVA with Bonferroni *post hoc* tests. A separate split-plot ANOVA was also performed to detect any carry-over effects between the two interventions and to ensure changes in FG, SBP and DBP following each treatment were not affected by the sequence of DC administration (1000 mg followed by 500 mg *v.* 500 mg followed by 1000 mg). Two-tailed paired sample t tests were used to assess changes between baseline and post-intervention total cholesterol, urinary free cortisol or cortisone, urinary cortisol-to-cortisone ratio and mineral excretion. Similarly, differences in response to both DC doses among the various ethnic groups were assessed using one-way between-groups ANOVA with FG, SBP and DBP as the dependent variables and ethnicity as the factor. An independent sample t test was also used to compare the response to DC polyphenols between the abdominally obese individual and the peripherally obese individuals. The relationship between fasting blood glucose levels, total cholesterol, SBP, DBP, BMI, weight, waist circumference, hip circumference, waist-to-hip circumference, urinary Mg, Na and K levels, 24-h urinary free cortisol, cortisone and cortisol-to-cortisone ratio were assessed using Pearson product-moment correlation coefficient, r . The coefficient of determination was estimated by obtaining r^2 . All statistical analyses were performed using SPSS for Windows, version 16.0.0 (SPSS Inc., Chicago, IL, USA). The sample size was calculated using G-power software version 3.0.8 (Heinrich Heine University, Dusseldorf, Germany) to detect 0.3 mmol/l reduction in FG with baseline SD = 0.5 mmol/l and post-DC SD = 0.04 mmol/l, which is similar to the reduction reported by Grassi *et al.*⁽⁶⁾.

Results

The study included fourteen healthy volunteers (eight males (five Caucasians, two Asians, one African) and six females (five Caucasians and one Hispanic)), 21–50 years old, mean age 26.4 (SD 11.5) years) with a BMI of 27.7 (SD 2.5) kg/m². Of these participants, thirteen were peripherally obese and one was abdominally obese (African).

Mixed between–within subjects ANOVA revealed a significant reduction in fasting capillary blood glucose concentrations ($P=0.002$), SBP ($P<0.0001$) and DBP ($P<0.0001$) following DC consumption. These effects were independent of the sequence of DC administration and no significant interaction between time, intervention group and sequence of DC administration was observed (FG $F(2,11) = 1.057$, $P=0.380$; SBP $F(2,11) = 0.431$, $P=0.660$; DBP $F(2,11) = 0.653$, $P=0.539$; Figs. 2 and 3). No significant differences between the effect of 1000 and 500 mg polyphenols DC on fasting capillary blood glucose ($P>0.05$) and BP ($P>0.05$) were observed indicating that both doses have a similar efficacy.

To explore the results further, a one-way repeated measures ANOVA was conducted to compare fasting blood glucose levels, SBP and DBP at baseline, week 1 and week 2 for each of the two dietary interventions. A significant effect of DC on fasting blood glucose levels ($F(2,12) = 4.305$, $P=0.039$), SBP ($F(2,12) = 12.330$, $P=0.001$) and DBP ($F(2,12) = 13.937$, $P=0.001$) was observed after consumption of 1000 mg DC. *Post hoc* comparisons using Bonferroni test indicated that mean fasting blood glucose levels and SBP at week 2 were significantly decreased after chocolate ingestion (FG 3.97 (SD 0.54) *v.* baseline 4.42 (SD 0.70) mmol/l; SBP 112.12 (SD 9.68) *v.* baseline 119.38 (SD 10.51) mmHg). Mean DBP levels were significantly lower at week 1 (74.45 (SD 7.17) mmHg) and week 2 (74.57 (SD 7.39) mmHg) compared to baseline (78.62 (SD 7.74) mmHg).

A significant effect of 500 mg DC on FG levels ($F(2,12) = 5.026$, $P=0.026$), SBP ($F(2,12) = 11.971$, $P=0.001$) and DBP ($F(2,12) = 7.709$, $P=0.007$) was also observed. *Post hoc* comparisons indicated that the mean FG levels at week 2 were significantly different from baseline (3.92 (SD 0.86) *v.* 4.42 (SD 0.30) mmol/l). Mean SBP was also reduced at the end of week 1 (114.24 (SD 9.53) mmHg)

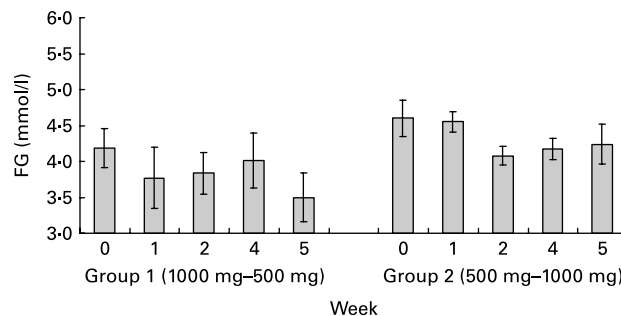


Fig. 2. Capillary fasting glucose (FG) levels at baseline (week 0), and at the end of 1 and 2 weeks of each of the polyphenols doses. Group 1 received 1000 mg polyphenols dark chocolate (weeks 1–2) followed by 500 mg polyphenols dark chocolate (weeks 4–5). Group 2 received 500 mg polyphenols dark chocolate (weeks 1–2) followed by 1000 mg polyphenols dark chocolate (weeks 4–5). Changes in FG were independent of the sequences of chocolate administration ($P>0.05$). Values are means with their standard errors represented by vertical bars.

Table 3. Results for 24-h urine collections (Mean values and standard deviations)

	1000 mg DC						500 mg DC						
	Baseline			Week 2			Baseline			Week 2			
	Mean	SD		Mean	SD		Mean	SD		Mean	SD		
Free cortisol (nmol/d)	77.33	27.09		71.16	38.90	13	86.83	44.05		78.58	47.28	14	0.620
Free cortisone (nmol/d)	54.34	26.90		45.82	17.33	13	59.64	32.56		45.80	20.34	14	0.139
Cortisol-to-cortisone ratio	1.6812	0.75		1.60	.63	13	1.68	0.72		1.77	0.62	14	0.682
Cortisone-to-cortisol ratio	0.71	0.32		0.75	0.37	13	0.71	0.31		0.63	0.21	14	0.455
Free cortisol (nmol/kg per d)	0.98	0.37		0.89	0.44	13	1.0529	0.43		0.98	0.64	14	0.731
Free cortisone (nmol/kg per d)	0.69	0.36		0.57	0.17	13	0.73	0.38		0.55	0.23	14	0.180
Creatinine (mmol/l)	12.74	5.35		11.78	4.40	13	12.83	5.15		19.29	27.37	14	0.387
Mg (mmol/l)	3.26	1.23		3.00	1.55	13	3.29	1.19		3.26	1.34	14	0.928
Na (mmol/l)	122.07	57.90		114.63	48.71	13	124.35	56.28		132.69	54.44	14	0.644
K (mmol/l)	58.20	22.12		56.14	24.08	13	61.19	24.03		67.58	22.42	14	0.377

DC, dark chocolate.

Table 4. Pearson product-moment correlations between changes in urinary glucocorticoid levels and changes in selected parameters

Correlation pair	n	r	P
Δ Urinary free cortisol (nmol/kg per 24 h)			
Δ Urinary free cortisone excretion (nmol/kg per 24 h)	27	0.599	0.001
Δ 24 h urinary Na (mmol/g creatinine)	26	0.489	0.011
Δ Physical activity (kJ)	26	-0.384	0.053
Δ Urinary free cortisone (nmol/kg per 24 h)			
Δ Cortisol-to-cortisone ratio	27	-0.662	0.000
Δ Cortisone-to-cortisol ratio	27	0.628	0.000
Δ 24 h urinary Na (mmol/g creatinine)	26	0.478	0.014

significantly. Moreover, no significant correlations were found between changes in Mg intake or excretion and the reductions in fasting blood glucose and BP seen following DC consumption. Energy expenditure, energy, macronutrient and mineral intake did not change significantly through the study period (Fig. 4).

Discussion

The present study demonstrates that polyphenol-rich DC reduces fasting blood glucose levels and BP in overweight and obese individuals. These findings are consistent with previous observations that polyphenol-rich DC intake improved insulin resistance, insulin sensitivity, FG levels and BP in healthy individuals⁽⁴⁾, hypertensives⁽⁵⁾, glucose-intolerant hypertensives⁽⁶⁾ and obese subjects⁽⁷⁾. The results are also in agreement with studies on diabetic obese mice, where reductions in blood glucose and fructosamine levels were reported following consumption of cacao liquor procyanidins⁽⁴³⁾.

Enhanced vascular function is thought to be the main mechanism by which DC polyphenols improve glucose and BP homeostasis^(4-7,23,34,44-47), although other mechanisms like decreased and delayed carbohydrate digestion and absorption might also be involved^(48,49). The present study investigated whether polyphenol-rich DC could alter cortisol metabolism and whether improvements in glucose and BP seen in obese individuals following DC consumption are linked to improved cortisol metabolism. The hypothesis was based on that cortisol plays an important role in glucose and BP homeostasis, probably through a mechanism involving increased reactive oxygen species production and decreased NO bioavailability, and that in obesity several alteration in cortisol metabolism are observed, which are, in turn, linked to increased insulin resistance and hypertension. The study demonstrates that both 500 and 1000 mg polyphenol DC decrease 24-h urinary free cortisol and cortisone levels. However, these reductions were not significant and are not associated with reductions in fasting blood glucose or BP. Such findings differ from previous findings, wherein polyphenols increased⁽⁵⁰⁻⁵³⁾ or decreased^(54,55) cortisol levels⁽⁵⁶⁾. The lack of significance could be related to a number of factors. For instance, the sample size might have not been sufficiently large to detect a significant change. In this case, using several parameters of cortisol metabolism including its measurement in urine, saliva and blood might have helped detect any such effect. Additionally, the study population consisted mainly of

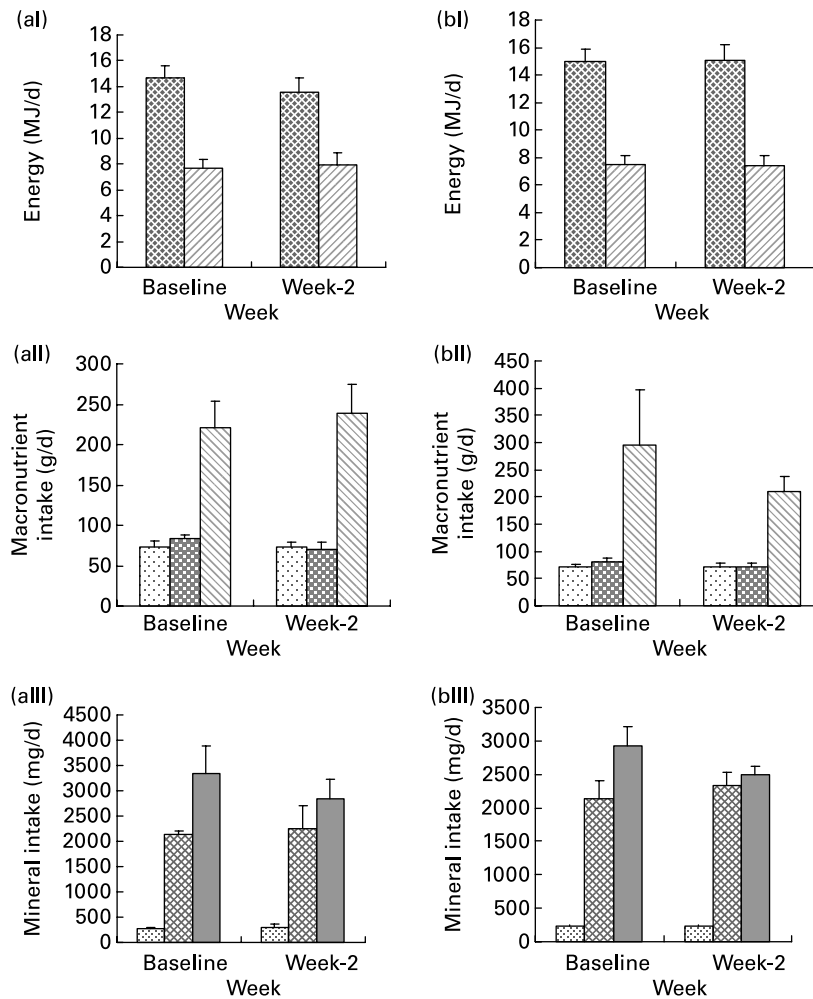


Fig. 4. Energy (I), macronutrient (II) and mineral intake (III) at baseline and at the end of each intervention: (a) 20 g dark chocolate with 1000 mg polyphenols and (b) 20 g dark chocolate with 500 mg polyphenols. Values are means with their standard errors represented by vertical bars. (□), Energy expenditure; (▨) energy intake; (▩) fat intake; (▧) protein intake; (▦) carbohydrate intake; (▥) Mg intake; (▤) Na intake; (▣) K intake.

subjects with peripheral obesity rather than those with abdominal obesity, who exhibit more prominent abnormalities in cortisol metabolism as indicated by the association between high waist circumference or waist-to-hip ratio and high urinary cortisol or cortisone-to-cortisol ratio^(11,57). In addition, differences in Na intake were not controlled for and could have acted as confounding factors⁽⁵⁸⁾. This could be observed in the association between changes in urinary free cortisol or cortisone and changes in Na excretion, and the association between changes in Na intake and changes in cortisol-to-cortisone ratio. Dietary factors have been reported to influence cortisol metabolism. High-fat low-carbohydrate diets stimulate cortisol regeneration by 11 β -hydroxysteroid dehydrogenase type 1, while reducing cortisol inactivation in liver⁽⁵⁹⁾. Na loading, on the other hand, decreases plasma cortisol levels by enhancing cortisol elimination⁽⁶⁰⁾ possibly via a mechanism involving increased hepatic blood flow⁽⁶¹⁾. The latter may explain the association between increased urinary free cortisol excretion and urinary Na levels. However, subjects did not report significant changes in Na intake during the study, which overall suggest that DC polyphenols influence glucose and BP homeostasis mainly via the NO pathway.

The present study demonstrates that DC with 500 mg polyphenols is as effective in reducing fasting blood glucose levels in overweight and obese individuals as 1000 mg polyphenol DC with a similar macronutrient composition. Furthermore, the results indicate that DC polyphenols reduce blood glucose levels after 2 weeks of commencing a polyphenol-rich DC diet. These findings are important since in relation to glucose metabolism, inconsistencies still exist regarding the treatment duration and dose required to achieve a glucose-lowering effect. For example in their pilot study, Stote *et al.*⁽⁶²⁾ failed to show any significant improvement in glucose levels, insulin resistance and insulin sensitivity following 5 d of twice daily consumption of procyanidin-rich cocoa beverage containing 22–900 mg procyanidins by insulin-resistant men and women. Similarly, Taubert *et al.*⁽³⁴⁾ failed to demonstrate any improvement in glucose or insulin levels following 18 weeks of daily ingestion of 6.3 g DC containing 30 mg polyphenols. Conversely, Davison *et al.*⁽⁷⁾ showed reduced insulin resistance following consumption of a cocoa beverage containing 902 mg flavanols twice daily for 12 weeks in overweight and obese subjects. Together, these findings suggest that a longer duration and a higher dose of

polyphenols could be required to achieve a significant reduction in glucose levels. The present study reinforces this hypothesis while demonstrating that increasing polyphenol dose does not necessarily result in further reductions in glucose and BP levels since a saturation effect may occur with increasing DC polyphenol content⁽⁶³⁾. It also highlights the need to identify the minimum polyphenol dose at which maximal health benefits could be achieved, since a reduction in the polyphenol content of chocolate implies reduced bitterness^(64,65), which could render the chocolate more palatable and acceptable to the general population. In relation to BP, 20 g DC with 500 mg polyphenols reduced SBP and DBP to a similar extent as 20 g DC with 1000 mg polyphenol. Moreover, the reduction in BP observed following the 20 g DC (500 mg polyphenols) was comparable to the reduction reported in a previous study on normotensive subjects (7 and 3 mmHg reduction in SBP and DBP, respectively)⁽⁴⁾. This might suggest that reducing the portion of DC while maintaining a similar total phenol content results in equivalent reductions in BP. This could provide several advantages since reducing the portion of DC would permit delivery of high quantity of polyphenols in a less energy-dense form, which is essential if DC is to be included as part of a healthy balanced diet.

In contrast to Fraga *et al.*⁽⁶⁶⁾ and Grassi *et al.*⁽⁵⁾, we did not observe any significant changes in total cholesterol. Such results are to be expected since our subjects had normal baseline total blood cholesterol levels as compared to Grassi *et al.*⁽⁵⁾ (baseline total cholesterol = 5.4 (SD 0.6) mmol/l). Moreover, Grassi *et al.*⁽⁵⁾ suggested that both the catechin and the fat component of DC account for its beneficial effect on total cholesterol. Similar assumptions were made in relation to stearic acid in DC^(67–69). Since in the present study a lower DC portion was used, the lack of significant change in total blood cholesterol could be related to the lower levels of linoleic, oleic and stearic acids present in this DC. There was also a lack of correlation between the reported energy intake and physical activity, which is similar to the findings of Davison *et al.*⁽⁷⁾, who argued that obese individuals might underreport energy intake and overreport physical activity.

In conclusion, the present study confirms previous reports of improved FG levels and BP following DC consumption. It also demonstrates that these effects do not appear to be mediated through changes in cortisol metabolism. Further studies are needed to identify the optimal dose of polyphenols required to improve glucose metabolism and to examine additional parameters that could be influenced by polyphenols.

Acknowledgements

We would like to thank all the volunteers who participated in the present study. We would also like to thank Barry Callebaut, Belgium, for their continuous support and for providing us with Acticoa dark chocolate. S. A. contributed to study design, data acquisition, data analysis and manuscript preparation. C. H. assisted with urinary electrolyte analysis and E. A., S. A. assisted with glucocorticoid analyses. Both E. A., S. A. and L. F. supervised the study, reviewed and edited the manuscript.

None of the authors had any personal or financial conflict of interest.

References

- Buijsse B, Feskens EJ, Kok FJ, *et al.* (2006) Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen elderly study. *Arch Intern Med* **166**, 411–417.
- McCullough ML, Chevaux K, Jackson L, *et al.* (2006) Hypertension, the Kuna, and the epidemiology of flavanols. *J Cardiovasc Pharmacol* **47**, 119–121.
- Pereira MA, Parker ED & Folsom AR (2006) Coffee consumption and risk of type 2 diabetes mellitus: an 11-year prospective study of 28812 postmenopausal women. *Arch Intern Med* **166**, 1311–1316.
- Grassi D, Lippi C, Necozione S, *et al.* (2005) Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am J Clin Nutr* **81**, 611–614.
- Grassi D, Necozione S, Lippi C, *et al.* (2005) Cocoa reduces blood pressure and Insulin resistance and improves endothelium-dependent vasodilation in hypertensive. *Hypertension* **46**, 398–405.
- Grassi D, Desideri G, Necozione S, *et al.* (2008) Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr* **138**, 1671–1676.
- Davison K, Coates AM, Buckley JD, *et al.* (2008) Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. *Int J Obes (Lond)* **32**, 1289–1296.
- Lee KW, Kim YJ, Lee HJ, *et al.* (2003) Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J Agric Food Chem* **51**, 7292–7295.
- Richelle M, Tavazzi I & Offord E (2001) Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J Agric Food Chem* **49**, 3438–3442.
- Olson TP, Schmitz KH & Leon AS (2006) Vascular structure and function in women: relationship with body mass index. *Am J Prev Med* **30**, 487–492.
- Vicennati V & Pasquali R (2000) Abnormalities of the hypothalamic-pituitary-adrenal axis in nondepressed women with abdominal obesity and relations with insulin resistance: evidence for a central and a peripheral alteration. *J Clin Endocrinol Metab* **85**, 4093–4098.
- Duclos M, Pereira PM, Barat P, *et al.* (2005) Increased cortisol bioavailability, abdominal obesity and the metabolic syndrome in obese women. *Obes Res* **13**, 1157–1166.
- Newton R (2000) Molecular mechanisms of glucocorticoid action: what is important? *Thorax* **55**, 603–613.
- Morton NM, Holmes MC, Fievet C, *et al.* (2001) Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *J Biol Chem* **276**, 41293–41300.
- Kidambi S, Kitchen JM, Grim CE, *et al.* (2007) Association of adrenal steroids with hypertension and the metabolic syndrome in blacks. *Hypertension* **49**, 704–711.
- Bjelaković G, Beninati S, Pavlović D, *et al.* (2007) Glucocorticoids and oxidative stress. *J Basic Clin Physiol Pharmacol* **18**, 115–127.
- Iuchi T, Akaike M, Mitsui T, *et al.* (2003) Glucocorticoid excess induces production in vascular endothelial cells and elicits vascular endothelial dysfunction. *Circ Res* **92**, 81–87.
- Liu Y, Mladinov D, Pietrusz JL, *et al.* (2009) Glucocorticoid response elements and 11{beta}-hydroxysteroid dehydrogenases in the regulation of endothelial nitric oxide synthase. *Cardiovasc Res* **81**, 140–147.
- Alberti L, Girola A, Gilardini L, *et al.* (2007) Type 2 diabetes and metabolic syndrome are associated with increased

- expression of 11 β -hydroxysteroid dehydrogenase in obese subjects. *Intern J Obes* **31**, e1826–e1831.
20. Meisel P (2005) Hypertension, diabetes: chocolate with a single remedy? *Hypertension* **46**, e17.
 21. Song Y, Ridker PM, Manson JE, *et al.* (2005) Magnesium intake, C-reactive protein and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* **18**, 1438–1444.
 22. Song CH, Choi WS, Oh HJ, *et al.* (2007) Associations of serum minerals with body mass index in adult women. *Eur J Clin Nutr* **61**, 682–685.
 23. Report of a World Health Organization (WHO) Consultation on obesity (2000) *Obesity: Preventing and Managing the Global Epidemic*. Geneva: WHO.
 24. Lovallo WR (2006) Cortisol secretion patterns in addiction and addiction risk. *Int J Psychophysiol* **59**, 195–202.
 25. Rohleder N & Kirschbaum C (2006) The hypothalamic-pituitary–adrenal (HPA) axis in habitual smokers. *Int J Psychophysiol* **59**, 236–243.
 26. Taubert D, Berkels R, Roesen R, *et al.* (2003) Chocolate and blood pressure in elderly individuals with isolated hypertension. *JAMA* **290**, 1029–1030.
 27. Bayard V, Chamorro F, Motta J, *et al.* (2007) Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, Stroke, diabetes mellitus, and cancer in Panama. *Int J Med Sci* **4**, 53–58.
 28. Olthof MR, Hollman PCH & Katan MB (2001) Chlorogenic acid and caffeic acid are absorbed in humans. *J Nutr* **131**, 66–71.
 29. Olthof MR, Hollman PCH, Zock PL, *et al.* (2001) Consumption of high doses of chlorogenic acid present in coffee or of black tea increases plasma homocysteine concentrations in humans. *Am J Clin Nutr* **73**, 532–538.
 30. Olthof MR, Hollman PCH, Buijsman MNCP, *et al.* (2003) Chlorogenic acid, quercetin-3-rutinoside and black tea polyphenols are extensively metabolised in humans. *J Nutr* **133**, 1806–1814.
 31. Bouchard C, Tremblay A, Leblanc C, *et al.* (1983) A method to assess energy expenditure in children and adults. *Am J Clin Nutr* **37**, 461–467.
 32. Nelson M, Atkinson M & Meyer J (2002) *A Photographic Atlas of Food Portion Sizes*. London: Food standard agency publications.
 33. Lindroos AK, Lissner L & Sjostrom L (1999) Validity and reproducibility of a self-administered dietary questionnaire in obese and non-obese subjects. *Eur J Clin Nutr* **47**, 461–481.
 34. Taubert D, Roesen R, Lehmann C, *et al.* (2007) Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide. *JAMA* **298**, 49–60.
 35. Farin HMF, Abassi F & Reaven G (2006) Body mass index and waist circumference both contribute to differences in insulin-mediated glucose disposal in nondiabetic adults. *Am J Clin Nutr* **83**, 47–51.
 36. Fuchs FD, Gus M, Moreira LB, *et al.* (2005) Anthropometric indices and the incidence of hypertension: a comparative analysis. *Obes Res* **13**, 1515–1517.
 37. Verdecchia P, Angeli F, Poeta F, *et al.* (2004) Validation of the A&D UA-774 (UA-767Plus) device for self-measurement of blood pressure. *Blood Press Monit* **9**, 225–229.
 38. Rios LY, Gonthier M, Remesy C, *et al.* (2003) Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am J Clin Nutr* **77**, 912–918.
 39. Roura E, Andres-Lacueva C, Estruch R, *et al.* (2006) Total polyphenol intake estimated by a modified Folin-Ciocalteu assay of urine. *Clin Chem* **52**, 749–752.
 40. Al-Dujaili EAS & Bryant ML (2005) Effect of meal fat content on salivary testosterone and cortisol levels in healthy female volunteers. *Endocrine Abstracts* **10**, 75.
 41. Al-Dujaili EAS (2006) Development and validation of a simple and direct ELISA method for the determination of conjugated and non-conjugated testosterone excretion in urine. *Clin Chim Acta* **364**, 172–179.
 42. Palermo M, Shackleton CH, Mantero F, *et al.* (1996) Urinary free cortisone and the assessment of 11 beta-hydroxysteroid dehydrogenase activity in man. *Clin Endocrinol (Oxf)* **45**, 605–611.
 43. Tomaru M, Takano H, Osakabe N, *et al.* (2007) Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese mice. *J Nutr* **23**, 351–355.
 44. Karim M, McCormick K & Kappagoda CT (2000) Effects of cocoa extracts on endothelium-dependent relaxation. *J Nutr* **130**, 2105S–2108S.
 45. Fisher ND, Hughes M, Gerhard-Herman M, *et al.* (2003) Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* **21**, 2281–2286.
 46. Balzer J, Rassaf T, Heiss C, *et al.* (2008) Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-blind, randomized, controlled trial. *J Am Coll Cardiol* **51**, 2141–2149.
 47. Faridi Z, Nijke VY, Dutta S, *et al.* (2008) Acute dark chocolate and cocoa ingestion and endothelium function: a randomised controlled crossover trial. *Am J Clin Nutr* **88**, 58–63.
 48. Quesada C, Bartolomé B, Nieto O, *et al.* (1996) Phenolic inhibitors of α -amylase and trypsin enzymes by extracts from pears, lentils, and cocoa. *J Food Prot* **59**, 185–192.
 49. McDougall GJ, Shpiro F, Dobson P, *et al.* (2005) Different polyphenolic components of soft fruits inhibit α -amylase and α -glucosidase. *J Agric Food Chem* **53**, 2760–2766.
 50. Song D, Lorenzo B & Reidenberg MM (1992) Inhibition of 11-beta-hydroxysteroid dehydrogenase by gossypol and bioflavonoids. *J Lab Clin Med* **120**, 792–797.
 51. Lee YS, Lorenzo BJ, Koufis T, *et al.* (1996) Grapefruit and its flavonoids inhibit 11beta-hydroxysteroid dehydrogenase. *Clin Pharmacol Ther* **59**, 62–71.
 52. Guo J & Reidenberg MM (1998) Inhibition of 11beta-hydroxysteroid dehydrogenase by bioflavonoids and their interaction with furosemide and gossypol. *J Lab Clin Med* **132**, 32–38.
 53. Sardi A, Geda C, Nericì L, *et al.* (2002) Rhabdomyolysis and arterial hypertension caused by apparent excess of mineralcorticoids: a case report. *Ann Ital Med Int* **17**, 126–129.
 54. Arion WJ, Canfield WK, Ramos FC, *et al.* (1997) Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose-6-phosphatase. *Arch Biochem Biophys* **339**, 315–322.
 55. Hemmerle H, Burger HJ, Below P, *et al.* (1997) Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J Med Chem* **40**, 137–145.
 56. Lamuela-Raventós ML & Andrés-Lacueva C (2001) More anti-oxidants in cocoa. *J Nutr* **131**, 834.
 57. Fraser R, Ingram MC, Anderson NH, *et al.* (1999) Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension* **33**, 1364–1368.
 58. Chamarthi B, Kolatkar NS, Hunt SC, *et al.* (2007) Urinary free cortisol: an intermediate phenotype and a potential genetic marker for a salt-resistant subset of essential hypertension. *J Clin Endocrinol Metab* **92**, 1340–1346.
 59. Stomson RH, Johnstone AM, Homer NZ, *et al.* (2007) Dietary macronutrient content alters cortisol metabolism independently of body weight changes in obese men. *J Clin Endocrinol Metab* **92**, 4480–4484.

60. Litchfield WR, Hunt SC, Jeunemaitre X, *et al.* (1998) Increased urinary free cortisol: a potential intermediate phenotype of essential hypertension. *Hypertension* **31**, 569–574.
61. Kerstens MN, Kleij FG, Bonnstra AH, *et al.* (2001) Salt loading affects cortisol metabolism in normotensive subjects: relationships with salt sensitivity. *J Clin Endocrinol Metab* **88**, 4180–4185.
62. Stote KS, Clevidence BA & Baer DJ (2007) Effect of cocoa and green tea consumption on glucoregulatory biomarkers in insulin resistant men and women. *FASEB J* **21**, 84717.
63. Grahame-Smith DG & Aronson JK (2002) *Oxford Textbook of Clinical Pharmacology and Drug Therapy*, 3rd ed. Oxford: Oxford University Press.
64. Luna F, Crouzillat D, Cirou L, *et al.* (2002) Chemical composition and flavor of Ecuadorian cocoa liquor. *J Agric Food Chem* **50**, 3527–3532.
65. Counet C, Ouwerx C, Rosoux D, *et al.* (2004) Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *J Agric Food Chem* **52**, 6243–6249.
66. Fraga CG, Actis-Goretta L, Ottaviani JI, *et al.* (2005) Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer player. *Clin Dev Immunol* **12**, 11–17.
67. Ding EL, Hutfless SM, Ding X, *et al.* (2006) Chocolate and prevention of cardiovascular disease: a systematic review. *Nutr Metab (Lond)* **3**, 2.
68. Ford ES, Li C, McGuire LC, *et al.* (2007) Intake of dietary magnesium and the prevalence of the metabolic syndrome among U.S adults. *Obesity (Silver Spring)* **15**, 1139–1146.
69. McKeown NM, Jacque PF, Zhang XL, *et al.* (2008) Dietary magnesium intake is related to metabolic syndrome in older Americans. *Eur J Nutr* **47**, 210–216.