

# Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons<sup>1-3</sup>

Davide Grassi, Cristina Lippi, Stefano Necozione, Giovambattista Desideri, and Claudio Ferri

## ABSTRACT

**Background:** Numerous studies indicate that flavanols may exert significant vascular protection because of their antioxidant properties and increased nitric oxide bioavailability. In turn, nitric oxide bioavailability deeply influences insulin-stimulated glucose uptake and vascular tone. Thus, flavanols may also exert positive metabolic and pressor effects.

**Objective:** The objective was to compare the effects of either dark or white chocolate bars on blood pressure and glucose and insulin responses to an oral-glucose-tolerance test in healthy subjects.

**Design:** After a 7-d cocoa-free run-in phase, 15 healthy subjects were randomly assigned to receive for 15 d either 100 g dark chocolate bars, which contained  $\approx 500$  mg polyphenols, or 90 g white chocolate bars, which presumably contained no polyphenols. Successively, subjects entered a further cocoa-free washout phase of 7 d and then were crossed over to the other condition. Oral-glucose-tolerance tests were performed at the end of each period to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI); blood pressure was measured daily.

**Results:** HOMA-IR was significantly lower after dark than after white chocolate ingestion ( $0.94 \pm 0.42$  compared with  $1.72 \pm 0.62$ ;  $P < 0.001$ ), and QUICKI was significantly higher after dark than after white chocolate ingestion ( $0.398 \pm 0.039$  compared with  $0.356 \pm 0.023$ ;  $P = 0.001$ ). Although within normal values, systolic blood pressure was lower after dark than after white chocolate ingestion ( $107.5 \pm 8.6$  compared with  $113.9 \pm 8.4$  mm Hg;  $P < 0.05$ ).

**Conclusion:** Dark, but not white, chocolate decreases blood pressure and improves insulin sensitivity in healthy persons. *Am J Clin Nutr* 2005;81:611-4.

**KEY WORDS** Insulin, insulin resistance, blood pressure, cocoa, dark chocolate

## INTRODUCTION

In vitro data suggest that cocoa and chocolate might protect the vascular endothelium by improving nitric oxide (NO) availability (1). It was recently shown in 2 different studies in healthy volunteers (2, 3) that the cocoa-related increase in NO availability is due to flavanols, ie, a subclass of flavonoids mainly represented by epicatechin and catechin and their oligomers (procyanidins) (4). In keeping with this, polyphenol-rich dark chocolate but not polyphenol-free white chocolate bars significantly reduced blood pressure in elderly patients with isolated systolic

hypertension (5). Because insulin-mediated increases in NO availability significantly improve insulin-mediated glucose uptake in healthy persons (6, 7), we hypothesized that polyphenol-rich dark chocolate was able to improve insulin sensitivity and decrease blood pressure in healthy subjects. Therefore, we compared the effects of 2 different kinds of chocolate bars, ie, either with a high or a low polyphenol content, on the glucose and insulin responses to an oral glucose challenge and on blood pressure in relatively young subjects without metabolic diseases and cardiovascular disease risk factors.

## SUBJECTS AND METHODS

### Study population

The study was conducted in 15 healthy persons (7 men and 8 women) aged  $33.9 \pm 7.6$  y ( $\bar{x} \pm$  SD) recruited from our medical staff. The study was conducted according to the World Medical Association's Declaration of Helsinki (revised in Edinburgh, October 2000).

### Experimental protocol

According to the protocol described by Taubert et al (5), after a cocoa-free run-in phase of 7 d, participants were randomly assigned to receive either 100 g dark chocolate bars containing  $\approx 500$  mg polyphenols (5) and providing 480 kcal of energy (Ritter Sport Halbbitter, Alfred Ritter GmbH & Co, Waldenbuch, Germany) or 90 g white chocolate bars over 15 d. The white chocolate bars (90 g) also provided 480 kcal and contained amounts of cocoa butter, macronutrients, fiber, electrolytes, and vitamins similar to those in the dark chocolate bars (5). At variance with the dark chocolate bars, the white chocolate bars contained skim milk powder, lactose, and butyric fat (Milka; Kraft Foods, Milan, Italy). In addition, no polyphenols are expected to be found in white chocolate (5). At the end of the above period, the subjects entered a further cocoa-free washout phase of 7 d

<sup>1</sup> From the Department of Internal Medicine and Public Health, University of L'Aquila, L'Aquila, Italy.

<sup>2</sup> Supported by the Italian Ministero della Università e della Ricerca Scientifica.

<sup>3</sup> Reprints not available. Address correspondence to C Ferri, Università di L'Aquila, Dipartimento di Medicina Interna e Sanità Pubblica, Piazzale S Tommasi n.1, 67100 Coppito, L'Aquila, Italy. E-mail: claudio.ferri@cc.univaq.it. Received June 1, 2004.

Accepted for publication September 28, 2004.



TABLE 1

General characteristics of the study population (age  $33.9 \pm 7.6$  y) at baseline and after 15 d of ingesting either dark or white chocolate bars<sup>1</sup>

	Dark chocolate		White chocolate	
	Before	After	Before	After
BMI (kg/m <sup>2</sup> )	22.6 ± 3.0	22.6 ± 3.0	22.6 ± 3.0	22.6 ± 3.0
Body weight (kg)	63.6 ± 10.3	63.6 ± 10.3	63.6 ± 10.3	63.6 ± 10.3
Total cholesterol (mmol/L)	4.6 ± 0.6	4.7 ± 0.4	4.7 ± 0.4	4.7 ± 0.4
LDL cholesterol (mmol/L)	2.8 ± 0.5	2.8 ± 0.4	2.8 ± 0.4	2.8 ± 0.4
HDL cholesterol (mmol/L)	1.5 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3
Triacylglycerols (mmol/L)	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.4	0.8 ± 0.3
Heart rate (beats/min)	67.5 ± 6.3	67.6 ± 4.5	66.8 ± 5	66.8 ± 5.1

<sup>1</sup> All values are  $\bar{x} \pm$  SD;  $n = 7$  men and 8 women. There were no significant differences between groups.

duration and then were crossed over to the other condition. During the cocoa-free periods, the participants were asked to substitute the chocolate bars for foods of similar energy and macronutrient composition. The diet during the study period was assessed by a diary of daily food intake and by measurement of body weight in accordance with Taubert et al (5). In particular, a tailored isocaloric diet equal in total energy, energy density, dietary fiber, and macronutrient composition and providing  $\approx 1400$  kcal/d, excluding the calories derived from chocolate, was given to each study participant at the beginning of the study. The subjects were carefully instructed to maintain their diet and to refrain from flavonoid-rich foods and beverages, including wine and other alcoholic beverages. In all cases, a list of these foods and beverages was given to all healthy subjects.

Each participant, after the first cocoa-free phase of 7 d and then after every 15-d cocoa phase, underwent an oral-glucose-tolerance test (OGTT) (75 g D-glucose) (8) after an overnight fast and  $\geq 12$  h from the last chocolate ingestion. Blood glucose and insulin were assessed at time 0 and then 30, 60, 90, 120, and 180 min after the glucose load. OGTT values were used for the homeostasis model assessment of insulin resistance (HOMA-IR) (9-11), the quantitative insulin sensitivity check index (QUICKI) (9), and the insulin sensitivity index (ISI) described by Matsuda and DeFronzo (12), ie, 3 well-accepted indexes of insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI and ISI) (9-12).

A routine hematochemical check—including serum electrolyte, total cholesterol, HDL-cholesterol, LDL-cholesterol, and triacylglycerols concentrations—was also performed before and after each of the above periods. In addition, blood pressure values and heart rate were also measured daily by a standard mercury sphygmomanometer and a stethoscope. In particular, blood pressure was measured in the Outpatient Unit of our University Department, always by the same physician, who was unaware of the study design, results, and purpose. On each occasion, blood pressure and heart rate were measured in a comfortable room, after the subjects had sat in a sitting position for 10 min, 4 times at 3-min intervals. The first measurement was discarded, and the average of the last 3 blood pressure and heart rate measurements was recorded.

### Statistical analysis

Continuous normally distributed data are expressed as means  $\pm$  SDs. Within each treatment group (ie, either dark or white chocolate), changes in blood pressure and metabolic indexes from baseline values were analyzed by paired Student's *t* test. For

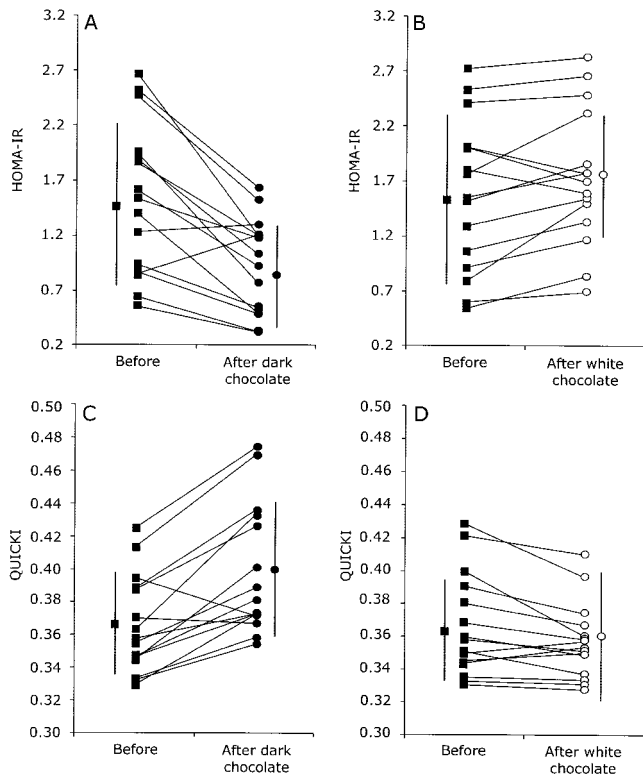
multiple comparisons, data were analyzed with a two-factor repeated-measures analysis of variance (ANOVA) with time and treatment as the 2 factors. Post hoc comparisons were performed by Tukey's honestly significant difference test. Statistical analysis and power calculation were performed with a personal computer and with SAS statistical software (version 8.12, 2000; SAS Institute Inc, Cary, NC).

### RESULTS

Baseline characteristics of the study population are shown in **Table 1**. As expected, serum lipid, glucose, and insulin concentrations were in the range of normality. Similarly, mean basal values of HOMA-IR, QUICKI, and ISI were also within the range of normality; HOMA-IR was  $< 2.77$ , QUICKI was  $> 0.331$ , and ISI was  $> 3.11$  (9-12). In keeping with this finding, no subject manifested with either impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes.

HOMA-IR was significantly lower after 15 d of dark chocolate ingestion than after 15 d of white chocolate ingestion (**Figure 1, A and B**). QUICKI was significantly higher after 15 d of dark chocolate ingestion than after 15 d of white chocolate ingestion (**Figure 1, C and D**). Moreover, ISI was found to be significantly higher after dark than after white chocolate ( $15.18 \pm 7.69$  compared with  $7.4 \pm 3.5$ ;  $P = 0.001$ ). Changes in the glucose and insulin responses to the oral glucose challenge further showed the positive effects of dark but not of white chocolate ingestion on glucose metabolism (**Figure 2**). In particular, for glucose concentrations, two-factor repeated-measures ANOVA showed a significant effect of both treatment ( $P < 0.0001$ ) and time ( $P < 0.0001$ ) as well as a significant treatment-by-time interaction ( $P = 0.0039$ ). After ANOVA, the Tukey's honestly significant difference test indicated significant differences between dark chocolate and white chocolate or baseline values at 0, 30, 60, 90, and 120 min (**Figure 2**). With regard to insulin concentrations, ANOVA again indicated a significant effect of treatment ( $P < 0.0001$ ) and time ( $P < 0.0001$ ) as well as a significant treatment-by-time interaction ( $P = 0.0191$ ). After ANOVA, the Tukey's honestly significant difference test indicated significant differences between dark chocolate and white chocolate or baseline values at 0, 30, 60, 90, 120, and 180 min (**Figure 2**).

Although within normal values (13), blood pressure showed a trend to be lower after 15 d of dark chocolate than after 15 d of white chocolate ingestion (**Figure 3**). However, only difference in systolic blood pressure after either dark or white chocolate were statistically significant ( $107.5 \pm 8.6$  compared



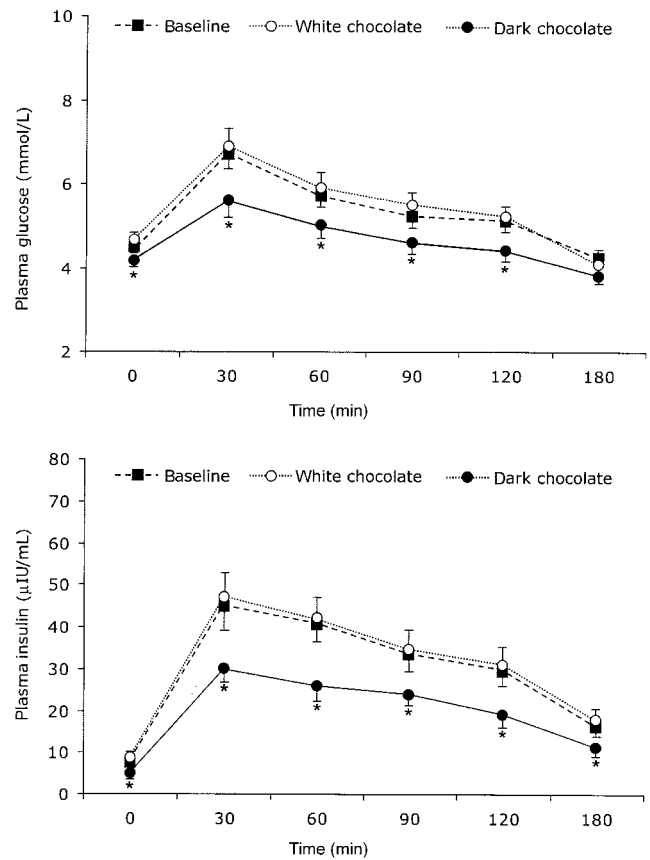
**FIGURE 1.** Effects of either dark (A and C) or white (B and D) chocolate on the homeostasis model assessment of insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) in 15 healthy subjects. No significant differences were found between the “before” values (baseline), which indicated the absence of a carryover effect. In contrast, HOMA-IR was significantly lower ( $P < 0.001$ , paired  $t$  test) and QUICKI was significantly higher ( $P = 0.001$ , paired  $t$  test) after dark than after white chocolate ingestion, which further confirmed the beneficial effect of dark chocolate on both HOMA-IR and QUICKI. In all panels, the symbols represent means, and the vertical lines represent SDs.

with  $113.9 \pm 8.4$  mm Hg, respectively;  $P < 0.05$ ). There were no influences of sex on the effects of chocolate on HOMA-IR, QUICKI, ISI, or blood pressure. No significant changes in the lipid profile were observed during the entire study period (Table 1).

## DISCUSSION

Cocoa has been claimed to protect the vascular endothelium by augmenting NO availability and thereby improving endothelium-dependent vasorelaxation (1-4). The current study expands on the above findings, showing that cocoa ingestion not only decreased blood pressure but also improved insulin sensitivity in healthy persons. Thus, although our study was not designed to provide insight about the mechanisms responsible for the observed effects, our study is the first one to show that cocoa may have favorable metabolic effects and thereby further protect against cardiovascular diseases.

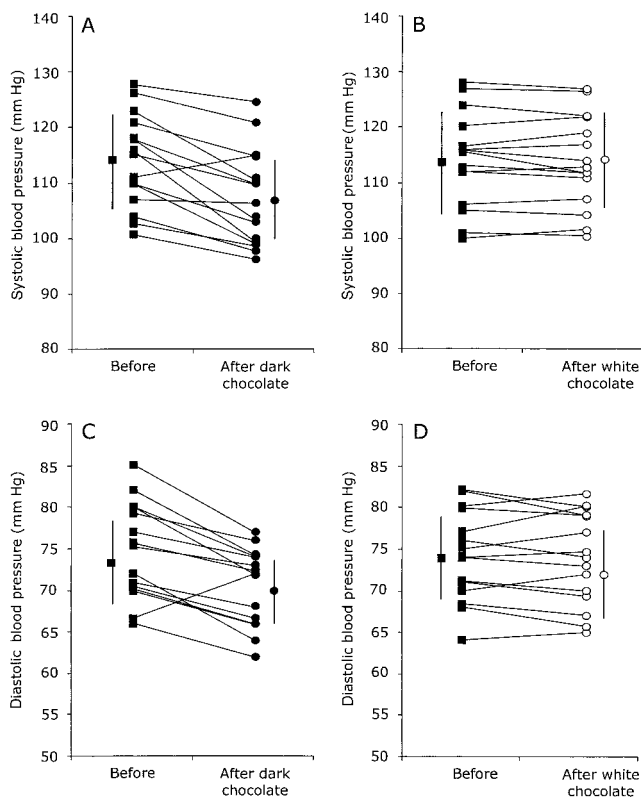
The first suggestion of the possible benefits of cocoa came from epidemiologic studies conducted in Kuna Indians, an Amerind population living in the San Blas Island chain off the Coast of Panama that is known to have an extremely low prevalence of atherosclerotic disease, hypertension, diabetes, and dyslipidemia (14, 15). The low cardiovascular mortality observed in Kuna



**FIGURE 2.** Mean ( $\pm$ SEM) plasma glucose and insulin concentrations in 15 healthy subjects in response to an oral-glucose-tolerance test (75 g D-glucose) at baseline and after ingestion of either dark or white chocolate for 15 d. \*Significantly different from white chocolate and baseline,  $P < 0.05$  (Tukey’s honestly significant difference test after a two-factor repeated-measures ANOVA found an interaction between treatment and time). The baseline data represent the first measurements; the second measurements were omitted for clarity.

Indians has been hypothesized to be consequent to high ingestion of cocoa-rich beverages (14, 15). In keeping with this, recent studies showed that flavanols, a subclass of flavonoids that is richly represented in natural cocoa beans, increase NO production by cultured human vascular endothelial cells (16) and improve endothelium-dependent vasorelaxation (NO-dependent) in finger (2) and brachial (3) arteries of healthy humans. Because insulin sensitivity is, at least in part, dependent on NO availability, ie, on insulin-stimulated NO production (6, 7), we hypothesized that dark chocolate containing polyphenols might improve insulin sensitivity *in vivo*. We showed that dark chocolate but not white chocolate bars decreased fasting insulin and glucose concentrations as well as the glucose and insulin responses to the oral glucose challenge. As a direct consequence of these changes, we also showed that dark chocolate bars simultaneously decreased the well-recognized marker of insulin resistance, HOMA-IR, and increased 2 distinct indexes of insulin sensitivity, ie, the QUICKI and the ISI.


With regard to possible study limitations, we cannot state that positive changes in insulin sensitivity induced by dark chocolate bars were due to increased NO availability. Nevertheless, the decrease in blood pressure observed after ingestion of dark chocolate bars supports this hypothesis. On the other hand, although



**FIGURE 3.** Effects of either dark chocolate (A and C) or white chocolate (B and D) on sitting systolic and diastolic blood pressure in 15 healthy subjects. No significant differences were found between the “before” values (baseline), which indicated the absence of a carryover effect. In contrast, systolic blood pressure was significantly lower ( $P < 0.05$ , paired  $t$  test) after dark than after white chocolate ingestion. No significant differences in diastolic blood pressure were observed between treatments. In all panels, the symbols represent means, and the vertical lines represent SDs.

other flavanol-related changes, such as those in prostaglandin synthesis and thromboxane production (17), have all the biological potential to induce significant modifications of insulin sensitivity, the particular study design that we have adopted minimizes study bias due to confounding factors, including type II statistical error and the order effect. Therefore, whatever the mechanism underlying the observed positive effects exerted by dark chocolate bars on insulin sensitivity, our data are consistent with the fact that increased insulin sensitivity represents a novel potential benefit derived from cocoa. Although we cannot completely exclude the contribution of other substances present in dark but not in white chocolate bars to the positive effects of dark chocolate on insulin sensitivity and blood pressure, it seems extremely likely that flavanols were responsible for the above effects.

In conclusion, the current study showed that polyphenol-rich dark chocolate but not white chocolate (which contains cocoa butter) decreases blood pressure and improves insulin sensitivity in healthy persons. These findings indicate that dark chocolate may exert a protective action on the vascular endothelium also by improving insulin sensitivity. Obviously, large scale trials are needed to confirm these protective actions of dark chocolate or

other flavanol-containing foods in populations affected by insulin-resistant conditions such as essential hypertension and obesity. In the meantime, we conclude that our findings further contribute to explain the reasons why, in his *Systema Naturae* (1735), Carl von Linnè first classified the cocoa tree as *Theobroma Cacao*, ie, “the divine food” (18). 

We thank the volunteers for their participation.

Each of the 5 authors participated in the work to take public responsibility for appropriate portions of the content. DG, CL, and GD conducted the study (from subject selection to blood samplings, including the OGTTs) and performed the statistical analysis. SN conducted the final statistical analysis with complete data review (including a rewrite of the statistical analysis and results sections). CF ideated the study, designed the study protocol, supervised the entire work, and prepared the manuscript. DG and SN prepared the figures. The authors declared that no conflict of interest existed.

## REFERENCES

1. Karim M, McCormick K, Kappagoda CT. Effects of cocoa extracts on endothelium-dependent relaxation. *J Nutr* 2000;130:2105S–8S.
2. Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* 2003;21(12):2281–6.
3. Engler MB, Engler MM, Chen CY, et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Nutr* 2004;23:197–204.
4. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr* 2002;22:19–34.
5. Taubert D, Berkels R, Roesen R, Klaus W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA* 2003;290:1029–30.
6. Konopatskaya O, Whatmore JL, Tooke JE, Shore AC. Insulin and lysophosphatidylcholine synergistically stimulate NO-dependent cGMP production in human endothelial cells. *Diabet Med* 2003;20(10):838–45.
7. Hsueh WA, Quinones MJ. Role of endothelial dysfunction in insulin resistance. *Am J Cardiol* 2003;92(suppl):10j–17j.
8. World Health Organization. Diabetes mellitus: report of a WHO Study Group. Geneva: World Health Organization, 1985.
9. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000;85:2402–10.
10. Bonora E, Kiechi S, Willeit J, et al. Prevalence of insulin resistance in metabolic disorders. The Bruneck Study. *Diabetes* 1998;47:1643–9.
11. Mannucci E, Bardini G, Ognibene A, Rotella CM. Comparison between 2 insulin sensitivity indexes in obese patients. *Diabetes Care* 2000;23(7):1042–3.
12. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462–70.
13. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 2003;21:1011–53.
14. Ferri C, Grassi G. Mediterranean diet, cocoa and cardiovascular disease: a sweeter life, a longer life, or both? *J Hypertens* 2003;21:2231–4.
15. Hollenberg NK, Martinez G, McCollough M, et al. Aging, acculturation, salt intake and hypertension in the Kuna of Panama. *Hypertension* 1997;29:171–6.
16. Leikert JF, Rathel TR, Wohlfart P, Cheyner V, Vollmar AM, Dirsch VM. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation* 2002;106:1614–7.
17. Schramm DD, Wang JF, Holt RR, et al. Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *Am J Clin Nutr* 2001;73:36–40.
18. Kennedy AJ. Cacao. *Theobroma cacao* (Sterculiaceae). In: Smartt J, Simmonds NW, eds. Evolution of crop plants. London: Longman, 1995: 472–5.