PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 7 (6): 773-781, 2008
ISSN 1680-5194
© Asian Network for Scientific Information, 2008

Short-Term Consumption of a Dark Chocolate Containing Flavanols is Followed by a Significant Decrease in Normotensive Population

Nora Abdullah Al-Faris
Department of Nutrition and Food Science, Faculty of Home Economics,
Riyadh University for Girls, Saudi Arabia

Abstract: Cardiovascular disease (CVD) is the primary chronic disease afflicting industrialized societies today. Development of atherosclerosis, thrombosis and hypertension is a multifactorial process in which endothelial dysfunction, inflammatory response, modified lipids and lipoproteins and activated platelets all play significant roles in the process. Numerous epidemiologic studies support the concept that regular consumption of foods and beverages rich in antioxidant vitamins and flavonoids is associated with a decreased risk of CVD mortality, specially hypertension. Foods that have been identified as having a high polyphenolic flavonoid content include many fruits and vegetables such as apples and onions, teas (green and black), red wines and certain chocolates. The purpose of this paper is to determine changes in systolic and diastolic blood pressure due to the intake of calculated amount of dark and white chocolate and to investigate its effects on the anthropometric measures. Eighty nine females with mean age of 21.45±19.8 years (from 18-25 years) were included in this study. The studied populations were divided into three groups; group I included thirty females who received 100 gram of dark chocolate (Galaxy, containing ≈500 mg polyphenols) every day for fifteen days, group II included thirty females who received 90 gram of white chocolate (Galaxy, containing no polyphenols) every day for fifteen days and group III included twenty nine females who were prevented from any type of chocolate for fifteen days. Along our sample population, two blood pressure readings and several anthropometric measures were recorded. After fifteen days of ingestion of dark chocolate, there was statistically significant lowering in the systolic and diastolic blood pressure ($p<0.05$) comparing with no effect to ingestion of white chocolate. There was no significant difference between groups among anthropometric measures before and after administration or avoidance of any chocolates ($p>0.05$). As expected, the serum lipid concentrations were in the range of normality with no significant differences between groups ($p>0.05$). Current dietary studies indicate that short-term administration of dark chocolate (but not white chocolate) is followed by a significant decrease in blood pressure in healthy persons. These findings indicate that dark chocolate may exert a protective action on the vascular endothelium.

Key words: Blood pressure, dark chocolate, anthropometric measures

Introduction

Dietary intake of a specific subclass of flavonoids known as flavanols has attracted increasing interest as a result of recent epidemiological (Arts *et al*., 2001; Chevaux *et al*., 2001) mechanistic (Karim *et al*., 2000) and human intervention studies suggesting potential beneficial cardiovascular effects (Schramm *et al*., 2001; Heiss *et al*., 2003). Among the wide variety of dietary flavanol sources, including apples, cranberries, purple grapes, red wine and teas, some cocoas and chocolates can be extraordinarily rich in certain types of flavanols (Fisher *et al*., 2003; Hammerstone *et al*., 2000; Liwei *et al*., 2004). Flavanols are a specific class of compounds within the much larger family of polyphenolic compounds known as flavonoids. They occur naturally in a variety of plant based foods and beverages, including cocoas, chocolates, teas, red wines, fruits, cereals, beans, spices and nuts (Hammerstone *et al*., 2000 and Liwei *et al*., 2004). The monomeric flavanols (epicatechin, catechin) and the oligomeric flavanols (procyanidins) are present in cocoas and chocolates to a varying extent. One of the primary uses of cocoa is the manufacture of chocolate. The concentration of flavanols in any chocolate depends on both the flavanol content of the cacao plant and the procedures used for transforming the cocoa into chocolate. Then, the accurate assessment of the flavanol content is pertinent to interpreting its biological effects (Heiss *et al*., 2003).

Although Grassi *et al*. (2004) indicated that 100 grams of the chocolate they used contains 500 mg polyphenols, they did not report how they determined this quantity. Nevertheless, chocolate containing 500 mg polyphenol could contain a relatively high concentration of flavanols (100-200 mg). Therefore, the interpretation that flavanols and procyanidins contained in the dark chocolate used in this study may be associated with the observed health effects is tempting but remains speculative.

As Grassi *et al*. (2004) indicated, the regulation of nitric

oxide (NO) production by the flavanols present in dark chocolate could explain its effects on both insulin sensitivity and blood pressure. This interpretation is supported by other data that have shown effects of flavanol on NO production (Duffy and Vita, 2003). However, it is uncertain how flavanols interact with the biological system to increase NO bioavailability. Insulin-mediated cell signaling could be one mechanism, because insulin can modulate several signaling molecules involved in NO-synthase regulation (Owers, 2004). A second mechanism could be an oxidant-mediated cell signaling, because flavanols can modulate oxidative stress and the cell redox state, which in turn defines NO availability and NO-synthase activity (Ackenzie *et al.*, 2004). A third mechanism could involve the renin-angiotensin system (de Cavanagh *et al.*, 2003) through the inhibition of the angiotensin-converting enzyme (Actis-Goretta *et al.*, 2003). This inhibitory effect favors NO production by preventing the induction of NADPH-oxidase activity and the resulting production of superoxide anion, which trigger NO oxidation to peroxynitrite (Cai *et al.*, 2003) and by preserving bradykinin at adequate concentrations to maintain NO-synthase activity and NO production (Prabhakar *et al.*, 1998). These potential mechanisms of NO regulation, insulin and oxidant-mediated signaling and angiotensin-converting enzyme function may be physiologically related (de Cavanagh *et al.*, 2004).

Tea and cocoa products account for the major proportion of total polyphenol intake in Western countries (Weisburger, 2002; Arts *et al.*, 1999). However, cocoa or tea are currently not implemented in cardioprotective or antihypertensive dietary advice, although both have been associated with lower incidences of cardiovascular events (Peters *et al.*, 2001; Steinberg *et al.*, 2003; Kris-Etherton and Keen, 2002). A recent cross-sectional study suggests considerable hypotensive and cardioprotective effects of cocoa (Buijsse *et al.*, 2006). Observational studies of the association between consumption of black or green tea and blood pressure yielded mixed results; some have reported a reduction of blood pressure (Stensvold *et al.*, 1992; Hodgson *et al.*, 2003; Yang *et al.*, 2004) while others found no effects (Klatsky *et al.*, 1986 and 1993). These discrepancies may be due to potential biases and confounding factors that are in particular inherent to epidemiological studies of diet and disease (Flegal, 1999).

Chocolate flavanols have demonstrated the potential to modulate cardiovascular health in at least two important ways (Wakabayashi *et al.*, 1998).

- inhibition of platelet activation; and
- improved endothelial function.

Rein *et al.* (2000) demonstrated that flavanol-rich cocoa inhibits platelet activation *ex vivo* six hours following ingestion, with significant reduction in the expression of the surface proteins glycoprotein IIB/IIIA and P-Selectin. Follow-on studies subsequently showed that flavanol-rich chocolate inhibits platelet activation *ex vivo* in humans (Holt *et al.*, 2002) suggesting that the flavanol content of chocolate-derived products has more influence on platelet function than other components in the products, such as fat or carbohydrate content.

On an acute basis, Pearson *et al.* (2002) directly compared the acute effects of flavanol-rich cocoa and aspirin with respect to inhibition of platelet function. The magnitude of the effect induced by flavanol-rich cocoa was less than that of aspirin, but still statistically significant. Existing data suggest that the primary action of flavanol-rich cocoa with respect to platelet function is to increase levels of the anti-aggregatory prostaglandin prostacyclin, decrease the levels of pro-aggregatory leukotrienes and increase levels of available nitric oxide (Holt *et al.*, 2002). More research is needed to determine whether flavanol-rich cocoa can affect other pathways important in the prevention of thrombosis.

During the mid-1990s, Hollenberg *et al.* (1997) validated the observation that the Kuna Indians of Panama do not experience an increase in hypertension as they age. This apparent protection was lost in the Kuna when they moved to the urban environment of Panama City. Subsequent dietary research revealed that the Kuna consume large quantities of flavanol-rich cocoa when living in their indigenous environment, but not when they move to the urban environment (Chevaux *et al.*, 2001). This observation, coupled with *in vitro* research demonstrating that specific cocoa flavanols could induce aortic ring relaxation *via* a nitric oxide dependent mechanism (Karim *et al.*, 2000), suggested that the frequent consumption of flavanol-rich cocoa by the Kuna could be one of the protective factors against age associated hypertension that this population enjoys. This speculation gained further support following the report of Heiss *et al.* (2003) that consumption of a single flavanol-rich cocoa beverage could transiently improve forearm brachial artery flow mediated vasodilation. Importantly, this observation correlated with increased levels of bioavailable nitric oxide measured in the blood. Neither an increase in flow-mediated vasodilatation nor an increase in bioavailable nitric oxide was observed when subjects consumed a low flavanol cocoa beverage. Most recently, Fisher *et al.* (2003) confirmed *in vivo* in human subjects the hypothesis raised by Karim *et al.* (2000) that at least part of the vascular action of cocoa flavanols is nitric oxide-dependent.

Materials and Methods

Study populations: This study included a random sample of 89 healthy adult females of Riyadh University for girls and secondary school for girls with mean age 21.45±1.98 years. The studied populations were divided into three groups; the first group (Group I) included thirty females who received 100 gram of dark chocolate

Table 1: The structure of dark and white chocolate used in our study

Description	Unit	Dark chocolate	White chocolate
Moisture	g/100g	0.44	0.44
Crude Protein	g/100g	6.30	6.30
Ether extract	g/100g	32.90	32.90
Total Carbohydrates	g/100g	58.66	59
Total Fat	g/100g	27.3	26.9
Sodium	g/100g	0.60	0.64
Potassium	g/100g	2.63	2.87
Calcium	g/100g	1.04	1.44
Magnesium	g/100g	0.30	0.25
Ash	g/100g	1.70	1.70
ME	Kcal/100g	556	556
Flavan-3-ols			
Gallic acid (GA)	mg/100g	1.89	BLD
Epigallocatechin (EGC)	mg/100g	1.08	BLD
Catechin (C)	mg/100g	3.99	BLD
Caffeine (CAF)	mg/100g	16.99	BLD
Epicatechin (EC)	mg/100g	4.01	BLD
Epigallocatechin 3-Gallate (EGCG)	mg/100g	1.40	BLD
Epicatechin 3-Gallate (ECG)	mg/100g	0.14	BLD

Abbreviation: BLD; below the limit of detection

(Galaxy) every day for fifteen days (containing ≈500 mg polyphenols and providing 480 kcal of energy), the second group (Group II) included thirty females who received 90 gram of white chocolate (Galaxy) every day for fifteen days (containing no polyphenols and providing 480 kcal and contained amounts of cocoa butter, macronutrients, fiber, electrolytes and vitamins similar to those in the dark chocolate), the third group (Group III) included twenty nine females who were prevented from any type of chocolate for fifteen days. All subjects were subjected to complete medical history and physical examination at the start of studying for diagnosis of Diabetes Mellitus (DM) and other concomitant diseases. At the beginning of the study and at the end of fifteen days and during the rest state of the subject, two blood pressure readings, ten minutes apart were taken using standard mercury sphygmomanometers with appropriate cuff sizes (KBM/SM-300). Mean of these two readings was used for analysis. Also height and weight were recorded using standard equipment and the values were rounded off to the nearest 0.5 cm and 0.2 Kg respectively (Gibson, 1990). Height was measured without shoes and the subject standing erect with the abdominal relaxed, the arms at the sides and feet together. Weight was measured with wearing minimal clothes. Hip circumference was measured at the level of greater trochanters of femur and waist circumference was measured at a point midway between the ribcage and iliac crests.

Laboratory analysis: Venous blood was drawn by vein puncture into tubes containing EDTA and plasma was obtained by immediate centrifugation at 3000g for 5 minutes at 4°C. The sample was frozen in liquid nitrogen and stored at –80°C until analysis of lipid profile. The withdrawn of blood was made twice, once at the baseline analysis and the second at the end of fifteen days.

Plasma concentrations of triglycerides, total cholesterol and high-density lipoprotein cholesterol, were determined by conventional enzymatic assays. Low-density lipoprotein cholesterol was calculated according to the Friedewald formula.

Materials

- Galaxy chocolate: Dark chocolate (Table 1 shows the structure of chocolate used in our study) and white chocolate (Lick dark chocolate in structure but without Flavan-3-ols)
- Standard mercury sphygmomanometers with appropriate cuff sizes (KBM/SM-300).
- Automatic Balance (Detcto USA)
- Graduated meter.
- HPLC system for chemistry analysis of chocolate (LC-10AT PUMP, system controller-SCL-10AVP, Wavelength 280nm, PDA detector, Column STR ODS-II 15cmx4.6mm, 5um, Model 2003 From SHIMADZU, Japan) (Fig. 1).
- Method of Analysis: by Official Method of Analysis of AOAC International, 17th Edition, 2000 (Table 2).

Statistical analysis: The data were analyzed using SPSS® version 11.0 for windows. BMI was calculated as Kg m^{-2}. Continuous variables were expressed as mean±1 SD. Statistical significance was considered at $p<0.05$ for all analysis.

Results

Table 3 summarized the characteristics of the study populations (age, anthropometric measures, education, occupational level, marriage status, concomitant diseases such as DM and family history of hypertension). The age is universal in our study (21.34±1.79 years), so there was no significant association between three groups of our study ($p>0.05$). The anthropometric measures (height, weight, BMI, waist circumference, hip circumference, waist to height ratio and waist to hip ratio) show no significant association between three groups ($p>0.05$). The occupational and education levels showed significant difference within each group ($p<0.05$)

Table 4 shows the comparison between the anthropometric measures before and after receiving dark chocolate (Group I) and white chocolate (Group II) and avoidance of receiving any chocolate (Group III). There was no significant difference between groups among anthropometric measures before and after administration or avoidance of any chocolates ($p>0.05$). Also reveals the serum lipid profile of three groups before and after administration of chocolate. As expected, the serum lipid concentrations were in the range of normality with no significant differences

Table 2: Method of Analysis; Official Method of Analysis of AOAC Internationa

Method	Test	Equipment	Mark Sign	Model No.
Moisture	AOAC 925.10	OVEN	BINDER	
Crude Protein	AOAC 978.04	KJELTEC	FOSSTECATOR	2300
Ether extract	AOAC 922.06	SOXTEC	FOSSTECATOR	2050
Fiber	AOAC 962.09	FIBERTEC	FOSSTECATOR	2010
Ash	AOAC 923.03	FURNACE	NABER THERM	
Total Carbohydrates	Calculated			

Table 3: Characteristics of the studied population

	Group I N=30	Group II N=30	Group III N=29	Total N=89	P
Age (years)	21.45±1.98	21.8±1.76	21±1.16	21.34±1.79	>0.05
Height (cm)	160±7.2	159.6±6.9	159.1±6.3	158.9±7.19	>0.05
Weight (kg)	60.6±14.9	61.5±13.7	59.8±16.8	62.67±14.1	>0.05
BMI (kg m^{-2})	19±0.4	20±0.5	21±2	22±1.5	>0.05
Waist circumference (cm)	62±6.6	67±6.8	71±10.5	88±9	>0.05
Hip circumference (cm)	63±6.6	75±7.6	73±10.6	87±11	>0.05
Waist to height ratio	0.41±0.04	0.43±0.05	0.42±0.07	0.41±0.06	>0.05
Waist to hip ratio	0.79±0.08	0.80±0.73	0.81±0.1	0.80±0.08	>0.05
Family history of hypertension (%)	7 (23.3)	9 (30)	4 (13.8)	20 (22.5)	>0.05
Diabetes Mellitus (%)	4 (13.3)	6 (20)	2 (6.9)	12 (13.5)	>0.05
Education:					
Secondary school (%)	9 (30)	5 (16.7)	5 (17.2)	19 (21.4)	
University (%)	21 (70)	25 (83.3)	24 (82.8)	70 (78.6)	<0.05
Marriage status:					
Marriage (%)	10 (33.3)	13 (43.3)	6 (20.7)	29 (32.6)	
Non-marriage (%)	20 (66.7)	17 (56.7)	23 (79.3)	60 (67.4)	>0.05
Occupational level:					
High (%)	25 (83.3)	23 (76.7)	22 (75.9)	70 (78.6)	
Low (%)	5 (16.7)	7 (23.3)	7 (24.1)	19 (21.4)	<0.05

between groups (p>0.05). The heart rate was within normal among the three groups with no significant difference.

Table 5 shows that, difference in systolic and diastolic blood pressure after ingestion of dark chocolate was statistically significant (115.9±12.6 and 73±9.9 compared with 107.5±8.6 and 67.7±9.7 mm Hg, respectively; (p<0.05). However, there was no significant difference between blood pressure parameters in group II and III (p>0.05).

Discussion

In this study, we demonstrated that intake of low habitual amounts of dark chocolate caused reductions of systolic and diastolic blood pressure in normotensive persons within normal ranges without inducing weight gain or other adverse effects. Another important finding of our study was that the decrease in systolic and diastolic blood pressure was not associated with changes in anthropometric measures. This was in agreement with the study of Taubert *et al.* (2003) who utilized a cross-over design to study the effects of polyphenol-rich and polyphenol-free chocolate on blood pressure in thirteen individuals aged between 55 and 64 years with untreated isolated systolic hypertension over a period of fourteen consecutive days. A significant reduction in blood pressure was noted 10 days after polyphenol-rich chocolate ingestion and after 14 days a mean blood pressure reduction of 5/2 mm Hg (SD 2.4/2.0) was seen. There was no change in the control group. Blood pressure returned to pre-intervention levels within two days of stopping the polyphenol-rich chocolate. Unfortunately, the flavanol composition of the chocolate products used in Taubert *et al.* (2003) study was not reported. In addition, the Zutphen study from Hertog *et al.* (1993) showed a significant inverse relationship between total flavonoid intake and coronary heart disease (CHD) mortality over a five-year follow-up period in elderly men. Hertog *et al.* (1993) also reported beneficial effects of initial high flavonoid intake on CHD mortality over a 25-year period in a total of 16 cohorts drawn from seven countries. While Fisher *et al.* (2003) revealed that the blood pressure reduction in his study was not observed in a short duration study of 27 healthy volunteers who drank a flavanol-rich cocoa (821mg per day) split into four doses per day. After four days of flavanol-rich cocoa supplementation blood pressure was measured and then measured again 90 minutes later following consumption of a single dose, with no significant change in blood pressure observed. However, other studies failed to show a significant relationship between dietary flavonoids and mortality (Rimm *et al.*, 1996; Hertog *et al.*, 1997; Woodward and Tunstall-Pedoe, 1999).

Table 4: General characteristics of the study population (age 21.34±1.79 y) at baseline and after 15 days of ingesting either dark (Group I) or white (Group II) chocolate bars or avoidance (Group III) of administration of any chocolates

	Group I N=30		Group II N=30		Group III N=29		
Parameters	Before	After	Before	After	Before	After	P
Weight (kg)	60.6±14.9	60.9±14.8	61.5±13.7	62±13.6	59.8±16.8	59.8±16.8	>0.05
BMI (kg/m^2)	19±0.4	19±0.4	20±0.5	20±0.5	21±0.2	21±0.2	>0.05
Waist circumference (cm)	62±6.6	62±6.6	67±6.8	69±6.8	71±10.5	71±10.5	>0.05
Hip circumference (cm)	63±6.6	63±6.6	75±7.6	76±7.6	73±10.6	73±10.6	>0.05
Waist to height ratio	0.41±0.04	0.41±0.04	0.43±0.05	0.44±0.05	0.42±0.07	0.42±0.07	>0.05
Waist to hip ratio	0.79±0.08	0.79±0.08	0.80±0.73	0.81±0.73	0.81±0.1	0.81±0.1	>0.05
Total cholesterol (mmol/L)	4.6±0.6	4.7±0.4	4.7±0.4	4.7±0.4	4.5±0.6	4.5±0.6	>0.05
Triacylglycerols (mmol/L)	0.7±0.3	0.7±0.4	0.7±0.4	0.8±0.3	0.6±0.3	0.6±0.3	>0.05
LDL cholesterol (mmol/L)	2.8±0.5	2.8±0.4	2.8±0.4	2.8±0.4	2.8±0.3	2.8±0.3	>0.05
HDL cholesterol (mmol/L)	1.5±0.3	1.6±0.3	1.6±0.3	1.6±0.3	1.6±0.2	1.6±0.2	>0.05
Heart rate (beats/min)	67.5±6.3	67.6±4.5	66.8±5	66.8±5.1	69.6±4.7	69.6±4.7	>0.05

Abbreviations: HDL, High-density lipoprotein. LDL, Low-density lipoprotein. BMI, Body mass index is calculated as weight in kilograms divided by height in meters squared.

Table 5: The effect of ingestion of either dark or white chocolate on the systolic (SBP) and diastolic (DBP) blood pressure, with referral to avoidance of ingestion of any type of chocolate within group III

	Group I N=30		Group II N=30		Group III N=29	
Parameters	Before	After	Before	After	Before	After
SBP (mmHg)	115.9±12.6	107.5±8.6	115.2±12.8	113.9±8.4	118±10.4	118±10
DBP (mmHg)	73±9.9	67.7±9.7	72.8±11.1	72±9.62	71.5±.6	71.5±.6
P	< 0.05		>0.05		>0.05	

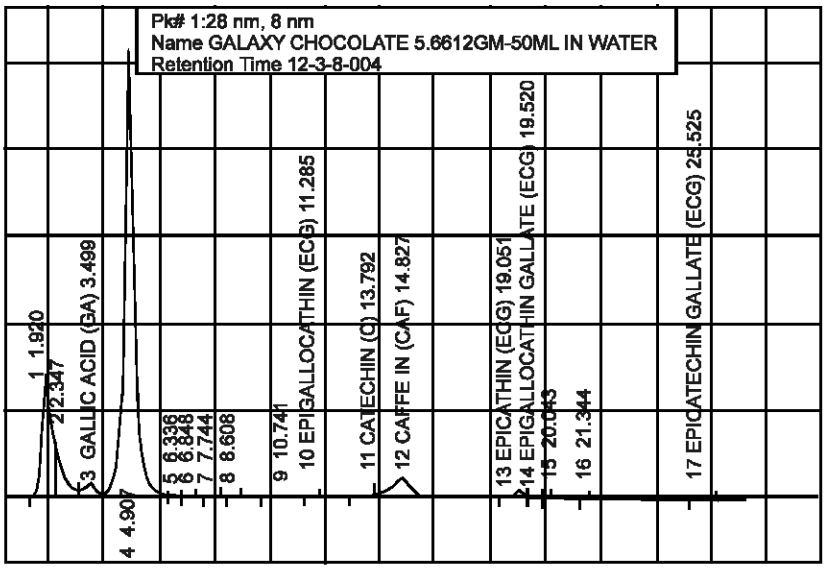

Fig. 1: High-performance liquid chromatography (HPLC) chromatogram of Galaxy dark chocolate (Lazarus *et al.* 1999)

Many studies examining specific foods rich in flavonoids, primarily tea, have suggested a significant relationship between consumption and reduced risk of myocardial infarction (Stensvold *et al.*, 1992; Sesso *et al.*, 1999). Unfortunately, with regard specifically to flavanols, there is a paucity of epidemiological data regarding any potential cardiovascular benefits (Arts *et al.*, 2001).

It is critical to note that the amount and type of flavanols in any food, including cocoa and chocolate products, can vary widely. This point must be considered when evaluating the potential bioactivity of cocoa flavanols regarding cardiovascular health. The flavanols present in a finished food product, including cocoa and chocolate, largely depends on the cultivar type, geographical origin, agricultural practices, post-harvest handling and processing of the flavanol-containing ingredient (Haslam, 1998). Thus, caution must be used when interpreting flavanol levels likely to be present in

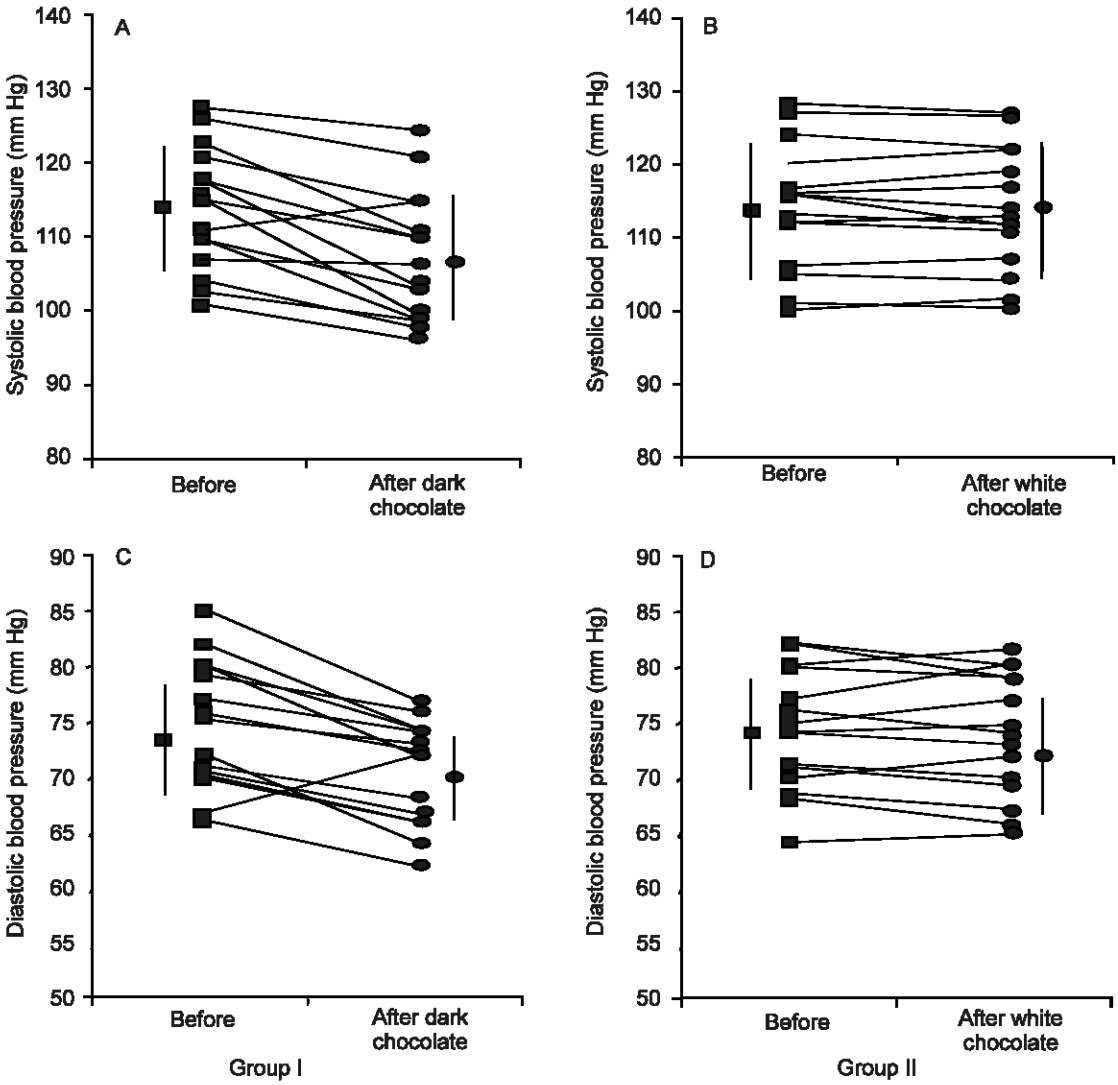

Fig. 2: Effects of either dark chocolate (A and C= Group I) or white chocolate (B and D= Group II) on sitting systolic and diastolic blood pressure in sample of our healthy subjects. Systolic and diastolic blood pressure was significantly lower ($P < 0.05$) after dark than after white chocolate ingestion. In all panels, the symbols represent means and the vertical lines represent Sds.

Although within normal values, blood pressure showed a trend to be lower after 15 days of dark chocolate than after 15 days of white chocolate ingestion. However, only difference in systolic and diastolic blood pressure after dark chocolate was statistically significant, ($P < 0.05$).

specific finished food products based on information derived from raw ingredients or generic food composition tables.

The most intriguing finding of this study is that small amounts of commercial cocoa confectionary convey a similar blood pressure-lowering potential compared with comprehensive dietary modifications (Appel *et al*., 1997, 2003) that have proven efficacy to reduce cardiovascular event rate (McCullough *et al*., 2000a, 2000b). Whereas long-term adherence to complex behavioral changes is often low and requires continuous counseling (Stevens *et al*., 2001) adoption of small amounts of flavanol-rich cocoa into the habitual diet is a dietary modification that is easy to adhere to and therefore may be a promising behavioral approach to lower blood pressure in individuals with above-optimal blood pressure. Future studies should evaluate the effects of dark chocolate in other populations and evaluate long-term outcomes.

This research should be pursued in parallel with further investigation of the potential clinical health benefits for the simple reason that compliance is the ultimate key to public health impact and cocoa-based products offer an extraordinary opportunity to successfully overcome often observed compliance issues (Robin *et al*., 2008).

Chocolate is an energy-dense food and individuals must

keep caloric intake and expenditure in mind when including it in their diet, as any food when eaten in excess will cause an increase in weight. Physical activity, diet and other lifestyle factors must be balanced carefully to avoid detrimental weight gain over time. In the context of nutrition, one must also consider that the cardiovascular benefits of flavanol-rich foods, including those chocolates that are flavanol-rich, could be offset if they were to simultaneously contribute significant levels of unhealthy fats such as certain saturated fatty acids that are known to raise blood cholesterol levels. Chocolate is rich in oleic and stearic acids, in addition to palmitic acid and several studies have demonstrated a neutral effect on blood lipids in humans following short-term consumption of cocoa butter and/or chocolate (Steinberg *et al*., 2003). As if people needed another excuse to eat chocolate, researchers have reported that a small daily intake of dark chocolate is good for his health.

Polyphenols have gained much more attention, owing to their antioxidant capacity (free radical scavenging and metal chelating) and their possible beneficial implications in human health, such as in the treatment and prevention of cancer, cardiovascular disease and other pathologies. Cocoa is rich in polyphenols particularly in catechins (flavan-3-ols) and procyanidins. Polyphenol contents of cocoa products such as dark chocolate, milk chocolate and cocoa powder have been published only recently (Hertog *et al*., 1995). However, the data vary remarkably due to the quantity of cocoa liquor used in the recipe of the cocoa products but also due to the analytical procedure employed. For example, results obtained by a colourimetric method were 5-7 times higher for the same type of product than results obtained by high performance liquid chromatography (HPLC). In general, consumers in the Northern countries consume on average more than people in the South. Thus, chocolate can be seen as a relevant source for phenolic antioxidants for some European population. However, this alone does not imply, that chocolate could be beneficial to human health. Some epidemiological evidence suggests a beneficial effect to human health by following a polyphenol-rich diet, namely rich in fruits and vegetables and to a less obvious extent an intake of tea and wine having a similar polyphenol composition as cocoa (Jan and Elke, 2000).

References

Ackenzie, G.G., F. Carrasquedo, J.M. Delfino, C.L. Keen, C.G. Fraga and P.I. Oteiza, 2004. Epicatechin, catechin and dimeric procyanidins inhibit PMA-induced NF-kappaB activation at multiple steps in Jurkat T cells. FASEB. J., 18: 167-9.

Actis-Goretta, L., J.I. Ottaviani, C.L. Keen and C.G. Fraga, 2003. Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. FEBS Lett., 555: 597-600.

Appel, L.J., T.J. Moore and E. Obarzanek, 1997. A clinical trial of the effects of dietary patterns on blood pressure: DASH Collaborative Research Group. N. Engl. J. Med., 336: 1117-1124.

Appel, L.J., C.M. Champagne and D.W. Harsha, 2003. Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. JAMA., 289: 2083-2093.

Arts, I.C., P.C. Hollman and D. Kromhout, 1999. Chocolate as a source of tea flavonoids. Lancet., 354: 488.

Arts, I.C.W., P.C.H. Hollman, E.J.M. Feskens, H.B.B. de Mesquita and D. Kromhout, 2001. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: The Zutphen elderly study. Am. J. Clin. Nutr., 74: 227-232.

Buijsse, B., E.J. Feskens, F.J. Kok and D. Kromhout, 2006. Cocoa intake, blood pressure and cardiovascular mortality: The Zutphen Elderly Study. Arch. Intern. Med., 166: 411-417.

Cai, H., K.K. Griendling and D.G. Harrison, 2003. The vascular NAD (P) H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol. Sci., 24: 471-8.

Chevaux, K.A., L. Jackson, M.E. Villar, J.A. Mundi, J.F. Commisso, G.E. Adamson, M.M. McCullough, H.H. Schmitz and N.K. Hollenberg, 2001. Proximate, mineral and procyanidin content of certain foods and beverages consumed by the Kuna Amerinds of Panama. J. Food Composition and Analysis, 14: 553-563.

de Cavanagh, E.M., B. Piotrkowski and N. Basso, 2003. Enalapril and losartan attenuate mitochondrial dysfunction in aged rats. FASEB. J., 17: 1096-8.

de Cavanagh, E.M., B. Piotrkowski and C.G. Fraga, 2004. Concerted action of the renin-angiotensin system, mitochondria and antioxidant defenses in aging. Mol. Aspects Med., 25: 27-36.

Duffy, S.J. and J.A. Vita, 2003. Effects of phenolics on vascular endothelial function. Curr. Opin. Lipidol., 14: 21-7.

Fisher, N.D.L., M. Hughes, M. Gerhard-Herman and N.K. Hollenberg, 2003. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. J. Hypertens, 21: 2,281-2,286.

Flegal, K.M., 1999. Evaluating epidemiologic evidence of the effects of food and nutrient exposures. Am. J. Clin. Nutr., 69: 1339S-1344S.

Gibson, R.S., 1990. Principles of Nutritional Assessment. New York, Oxford University Press.

Grassi, D., C. Lippi, S. Necozione, G. Desideri and C. Ferri, 2004. 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: 611-4.

Hammerstone, J.F., S.A. Lazarus and H.H. Schmitz, 2000. Procyanidin content and variation in some commonly consmed foods. J. Nutr. Supp., 130: 2,086-2,092.

Haslam, E., 1998. Polyphenols-structure and Biosynthesis. In: Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action. University Press, Cambridge, MA.

Heiss, C., A. Dejam, P. Kleinbongard, T. Schewe, H. Sies and M. Kelm, 2003. Vascular effects of cocoa rich in flavan-3-ols. JAMA., 290: 1,030-1,031.

Hertog, M.G., E.J. Feskens and P.C. Hollman, 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet, 342: 1,007-1,011.

Hertog, M.G., D. Kromhout and C. Aravanis, 1995. Flavonoid intake and long-term risk of coronary heart disease and cancer in the. Seven Countries Study. Arch. Intern. Med., 155: 381-386.

Hertog, M.G., P.M. Sweetman and A.M. Fehily, 1997. Antioxidant flavonols and ischemic heart disease in a Welsh population of men:The Caerphilly Study. Am. J. Clin. Nutr., 65: 1,489-1,494.

Hodgson, J.M., A. Devine, I.B. P uddey, S.Y. Chan, L.J. Beilin and R.L. Prince, 2003. Tea intake is inversely related to blood pressure in older women. J. Nutr., 133: 2883-2886.

Hollenberg, N.K., G. Martinez, M. McCullough, T. Meinking, D. Passan, M. Preston, A. Rivera, D. Taplin and M. Vicaria-Clement, 1997. Aging, acculturation, salt intake and hypertension in the Kuna of Panama. Hypertension, 29: 171-176.

Holt, R.R., D.D. Schramm, C.L. Keen, S.A. Lazarus and H.H. Schmitz, 2002. Chocolate consumption and platelet function. J. Am. Med. Assoc., 287: 2,212-2,213.

Karim, M., K. McCormick and C.T. Kappagoda, 2000. Effects of cocoa extracts on endothelium-dependent relaxation. J. Nutr. Suppl., pp: 2,105-2,108.

Jan, W. and A. Elke, 2000. Polyphenols in chocolate: Is there a contribution to human health? Food Res. Int., 33: 449-459.

Klatsky, A.L., G.D. Friedman and M.A. Armstrong, 1986. The relationships between alcoholic beverage use and other traits to blood pressure: A new Kaiser Permanente study. Circulation, 73: 628-636.

Klatsky, A.L., M.A. Armstrong and G.D. Friedman, 1993. Coffee, tea and mortality. Ann. Epidemiol., 3: 375-381.

Kris-Etherton, P.M. and C.L. Keen, 2002. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. Curr. Opin. Lipidol., 13: 41-49.

Lazarus, S.A., G.E. Adamson, P.C. Hammerstone and H.H. Schmitz, 1999. High-performance liquid chromatography, mass spectrometry analysis of proanthocyanidins in food and beverages. J. Agri. Food Chem., 47: 3693-3701.

Liwei, G., M.A. Kelm, J.F. Hammerstone, G. Beecher, J. Holden, D. Haytowitz, S. Gebbardt and R.L. Prior, 2004. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. J. Nutr., 134: 613-617.

McCullough, M.L., D. Feskanich and E.B. Rimm, 2000a. Adherence to the Dietary Guidelines for Americans and risk of major chronic disease in men. Am. J. Clin. Nutr., 72: 1223-1231.

McCullough, M.L., D. Feskanich and M.J. Stampfer, 2000b. Adherence to the Dietary Guidelines for Americans and risk of major chronic disease in women. Am. J. Clin. Nutr., 72: 214-1222.

Owers, J.R., 2004. Insulin resistance and hypertension. Am. J. Physiol. Heart Circ. Physiol., 286: H1597-602.

Pearson, D.A., T.G. Paglieroni, D. Rein, T. Wun, D.D. Schramm, J.F. Wang, R.R. Holt, R. Gosselin, H.H. Schmitz and C.L. Keen, 2002. The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. Thromb. Res., 106: 191-197.

Peters, U., C. Poole and L. Arab, 2001. Does tea affect cardiovascular disease? a meta-analysis. Am. J. Epidemiol., 154: 495-503.

Prabhakar, P., H.S. Thatte, R.M. Goetz, M.R. Cho, D.E. Golan and T. Michel, 1998. Receptor-regulated translocation of endothelial nitric-oxide synthase. J. Biol. Chem., 273: 27383-8.

Rein, D., T.G. Paglieroni, T. Wun, D.A. Pearson, H.H. Schmitz, R. Gosselini and C.L. Keen, 2000. Cocoa inhibits platelet activation and function. Am. J. Clin. Nutr., 72: 30-35.

Rimm, E.B., M.B. Katan and A. Ascherio, 1996. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann. Intern. Med., 125: 384-389.

Robin, R.A., C. LeaAnn, K.U. Catherine, M.E. Ellen and W.E. John, 2008. Nutrition and Disease, Daily Consumption of a Dark Chocolate Containing Flavanols and Added Sterol Esters Affects Cardiovascular Risk Factors in a Normotensive Population with Elevated Cholesterol. American Society for Nutrition. J. Nutr., 138: 725-731.

Schramm, D.D., J.F. Wang, R.R. Holt, J.L. Ensunsa, J.L. Gonsalves, S.A. Lazarus, H.H. Schmitz, J.B. German and C.L. Keen, 2001. Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. Am. J. Clin. Nutr., 73: 36-40.

Sesso, H.D., J.M. Gaziano and J.E. Buring, 1999. Coffee and tea intake and the risk of myocardial infarction. Am. J. Epidemiol., 149: 162-167.

Steinberg, F.M., M.M. Bearden and C.L. Keen, 2003. Cocoa and chocolate flavonoids: implications for cardiovascular health. J. Am. Diet Assoc., 103: 215-223.

Stensvold, I., A. Tverdal, K. Solvoll and O.P. Foss, 1992. Tea consumption: Relationship to cholesterol, blood pressure and coronary and total mortality. Prev. Med., 21: 546-553.

Stevens, V.J., E. Obarzanek and N.R. Cook, 2001. Long-term weight loss and changes in blood pressure: Results of the Trials of Hypertension Prevention, phase II. Ann. Intern. Med., 134: 1-11.

Taubert, D., R. Berkels, R. Roesen and W. Klaus, 2003. Chocolate and Blood Pressure in Elderly Individuals with Isolated Systolic Hypertension. J. Am. Med. Assoc., 290: 8: 1,029-1,030.

Wakabayashi, K., S. Kono and K. Shinchi, 1998. Habitual coffee consumption and blood pressure: A study of self-defense officials in Japan. Eur. J. Epidemiol., 14: 669-673.

Weisburger, J.H., 2002. Lifestyle, health and disease prevention: The underlying mechanisms. Eur. J. Cancer Prev., 11(suppl 2): S1-S7.

Woodward, M. and H. Tunstall-Pedoe, 1999. Coffee and tea consumption in the Scottish Heart Health Study followup: Conflicting relations with coronary risk factors, coronary disease and all cause mortality. J. Epidemiol. Community Health, 53: 481-487.

Yang, Y.C., F.H. Lu, J.S. Wu, C.H. Wu and C.J. Chang, 2004. The protective effect of habitual tea consumption on hypertension. Arch. Intern. Med., 164: 1534-1540.