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Research Article

Effects of dark chocolate Intake on Physical Functions in Japanese Subjects

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Abstract

Introcuction: This study aimed to evaluate the effects of dark chocolate consumption on physical functions (blood pressure, the fasting serum lipid profile, cognitive function, and markers of oxidative stress and inflammation) in Japanese subjects.

Methods: The clinical trial included 385 healthy Japanese subjects aged 45-69 years, who were not taking regular medication and had no history of allergy to cacao. All subjects ate 25 g of dark chocolate for 4 weeks, and 347 subjects completed the study.

Results: The backgrounds of the subjects are as follows; the average age was 55.0 ± 6.5 years, and BMI was 22.5 ± 3.2 kg/m^2. Body weight and BMI remained almost unchanged during the 4 weeks. Consumption of dark chocolate was found to decrease systolic and diastolic blood pressure significantly, increase HDL-cholesterol, and improve a marker of cognitive function (brain-derived neurotrophic factor [BDNF]). Subjects with 8-hydroxy-2'-deoxyguranosin (8-OHdG) concentration ≥ 10.52 ng/mg creatinine at baseline (the highest quartile) were found to have a reduction of 8-OHdG (a marker of oxidative stress) at the end of the study. We also observed a reduction of high-sensitivity C-reactive protein (hs-CRP) concentration after consumption of dark chocolate by subjects in the highest quartile (hs-CRP ≥ 620 μg/L) at baseline. We assessed quality of life using SF-36v2®, and the scores indicated improvement in the health-related quality of life.

Conclusion: Our study has shown that daily consumption of dark chocolate decreased blood pressure, and increased HDL-cholesterol, BDNF and the score for quality of life. It is anticipated that regular consumption of polyphenol-rich foods should lead to a decrease in the incidence of atherosclerotic disease and improvement of cognitive function.

Keywords: Dark chocolate, procyanidin, blood pressure, HDL-cholesterol, BDNF

Introduction

Cacao beans are used as an ingredient in cocoa and chocolate and are known to be rich in polyphenols, such as catechin, epicatechin, procyanidin B2, procyanidin B5, procyanidin C1, cinnamtannin A2, and other oligomeric procyanidins [1,2].

These compounds are antioxidants [3,4]. In a previous study, we showed that consumption of polyphenol-rich fractions derived from cocoa powder increased the resistance of low density lipoprotein (LDL) to oxidation, reduced LDL-cholesterol, and elevated HDL-cholesterol [5]. Marusu et al. reported that the cacao polyphenols increased the HDL-cholesterol concentlation [6]. Many epidemiological studies have shown that cacao polyphenols reduce the risk of coronary heart disease [7]. Either short-term administration of 100 g dark chocolate [8] or habitual intake of 6.3 g dark chocolate [9] decreased blood pressure. Meta-analyses of randomized, controlled trials have been performed to examine the effect of high-polyphenol chocolate on cardiovascular risk and hypertension [10-13]. In addition, Hollenberg et al. (2009) reported that the Kuna Indians of Panama, who frequently drink cacao, have low risks of cardiovascular disease and hypertension [14,15]. In addition, intake of cacao products and chocolate improved the age-related cognitive disfunction [16] and cerebral blood flow [17]. However, these reports described the results of clinical studies carried out in Europe and the United States. In Japan, the effects of dark chocolate have not been evaluated. As exemplified by the difference in glucose tolerance between Asian Indians and Caucasians, ethnicity can have significant effects on body metabolism [18]. Therefore, the aim of the present study was to examine the effects of dark chocolate on blood pressure, oxidative stress, and inflammation in Japanese.

Materials and Methods

This pre-post trial was conducted at the Healthcare Systems, Aichi Gakuin University, Gamagori City Hospital and Meiji. The study protocol was approved by the Human Ethics Committee of Gamagori City Hospital and the study was conducted in accordance with the International Ethical Guidelines and Declaration of Helsinki (UMIN000022168). We recruited subjects for public relations in Gamagori city. All subjects gave written informed consent, and the study had full ethical and regulatory approval. Subjects were screened according to the following criteria: age 45-69 years; no regular medication; no allergy to cacao. Three hundred and eighty-five subjects entered the run-in period. Fourteen were later excluded because they had consumed less than 80 per cent of the prescribed amount of chocolate, and another 24 were excluded on account of a contravention of the protocol.

The intervention lasted four weeks, at the beginning and end of which the subjects were submitted to clinical and nutritional assessment. All were instructed to consume 25 g of dark chocolate (Meiji Co. Ltd., Tokyo, Japan; containing 18 g of cacao, a total of 650 mg of polyphenols, and 150 kcal of energy). Subjects ate the chocolate freely. The nutrient and polyphenols content are shown in Table 1. All subjects were admitted at the same time into the Gamagori City Hospital. At baseline and four weeks, measurements were made of body weight, ambulatory blood pressure, the fasting serum lipid profile (triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol), a biomarker inflammation (high-sensitivity C-reactive protein, hs-CRP), and a biomarker of cognitive function (brain derived neurotrophic factor, BDNF). Urine samples were collected at baseline and after four weeks at home and used for analysis of creatinine and 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of antioxidant status.

Lipid profile, serum hs-CRP, and urinary creatinine were assayed using standard laboratory techniques (LSI Medience Corporation, Tokyo, Japan). Health-related quality of life was assessed using the validated Japanese version MOS 36-Item Short-Form Health Survey (SF-36v2®) [19,20], which includes 36 items distributed in eight dimensions: Physical Functioning, Role Physical, Bodily Pain, Vitality, Social Functioning, Role Emotional, Mental

Health, and General Health. The results are scaled between 0 and 100, with higher values representing a higher subjective quality of life.

Table 1: The nutrient content in dark chocolate

nutrient/100 g chocolate	Content
Energy (kcal)	569
Protein (g)	10.7
Fat (g)	41.1
Carbohydrate (g)	33.5
Fiber (g)	11.5
Total polyphenols*1 (g)	2.6
Total procyanidins*2 (mg)	289
Catechin (mg)	40
Epicatechin (mg)	114
Procyanidin B2 (mg)	61
Procyanidin B5 (mg)	12
Procyanidin C1 (mg)	39
Cinnamtannin A2 (mg)	24
Theobromine (mg)	93
*1Prussian Blue method (epicatechin equivalent) *2Total Procyanidins are the sum of catechin, epicatechin, procyanidin b2, procyanidin c1 and ciinamtannin A2.These substances were analyzed as epicatechin equivalent.	

Blood pressure

At baseline and four weeks, blood pressure was measured using an automated sphygmomanometer (HBP-9020, Omron, Japan) after at least 10 min in the sitting position.

Biomarkers

Quantitation of 8-OHdG in urine was measured by antibody chips (Healthcare Systems, Aichi, Japan) [21]. In brief, 8-OHdG-BSA conjugate was immobilized on the chip. The sample and the biotin-labeled anti-8-OHdG antibody were applied to the chip, and a competitive reaction occurred between the immobilized 8-OHdG-BSA and free 8-OHdG in the sample. Unbound anti-8-OHdG antibody was washed away, and alkaline phosphatase-labeled streptavidin was applied. The amount of bound antibody was determined by chemiluminescence. 8-OHdG is a widely used marker for oxidative damage to DNA. Quantitation of BDNF in serum was performed in the same manner as 8-OHdG, except that the competitive reaction was replaced by a sandwich reaction. BDNF plays a role in the survival, growth and maintenance of neurons.

Safety assessment

The following variables were measured in the blood samples collected at baseline and four weeks: serum total protein, albumin, glucose, uric acid, urea nitrogen, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, sodium potassium, chloride, and calcium. All were assayed using standard laboratory techniques (LSI Medience Corporation).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22 (IBM, Tokyo, Japan). Data were expressed as mean ± SD (Standard Deviation). We compared data obtained at baseline with those obtained at four weeks. Two readings for hs-CRP at baseline and one at four weeks were below the detection limit (<40 μg/L) and seven at baseline and six at four weeks were above the limit (>5000 μg/L). Therefore, three readings below the limit were converted to 20 μg/L and 13 above the limit were converted to 5000 μg/L. The

Wilcoxson signed rank test was used for variables not having a normal distribution. We also performed two-way repeated measures analysis of variance (ANOVA) to determine differences in blood pressure between the groups (normal blood pressure or hypertension) and over time. When there are significant interactions between groups and time, multiple comparisons are performed by unpaired *t*-tests with groups, and by paired *t*-tests with time. In all analyses, P<0.05 was considered to be significant.

Results

Three hundred and forty-seven (male 123, female 224) participants completed the study, and were included in the final analysis. Their average age was 55.0 ± 6.5 years, and BMI was 22.5 ± 3.2 kg/m^2.

Body weight and BMI remained almost unchanged during the four weeks (Table 2). The mean of systolic and diastolic blood pressure was significantly lower at four weeks than at baseline, respectively (Table 2). When we compared subjects with normal blood pressure and those with hypertension (diastolic ≥ 140 mmHg or systolic ≥ 90 mmHg), the induced changes differed significantly between the groups (Table 3) (P<0.001).

Table 2: Changes in characteristics of subjects during dark chocolate intake

Item	All subjects (n=347*)		
	Baseline	4 weeks	p-value[a]
Body weight (Kg)	58.44 ± 11.06	58.46 ± 10.92	0.601
BMI	22.54 ± 3.23	22.56 ± 3.20	0.601
Blood Pressure (mmHg)			
Systolic	125.30 ± 16.39	122.68 ± 16.32	<0.001
Diastolic	78.76 ± 12.90	76.88 ± 12.55	<0.001
Pulse	72.75 ± 10.09	72.59 ± 10.02	0.554
Cholesterol (mmol/L)			
Total	212.69 ± 31.38	213.61 ± 31.13	0.279
HDL	67.86 ± 16.23	69.66 ± 16.39	<0.001
LDL	131.14 ± 29.28	131.59 ± 29.30	0.676
Triglyceride (mmol/L)	88.63 ± 57.61	87.03 ± 47.15	0.688
hs-CRP (μg/L)	0.058 ± 0.090	0.058 ± 0.082	0.134
8-OHdG ng/mg creatinine	7.77 ± 6.22	7.77 ± 4.15	0.154
BDNF (ng/mL)	6.07 ± 3.13	7.39 ± 5.87	0.005

Mean ± SD, SD: Standard Deviation;
*Insulin:n=346, Hs-CRP: n=345, 8-OHdG: n=344;
[a]Wilcoxon signed rank test

Table 3: Startified analyses of blood pressure

Clinical test item	systolic BP ≧ 140 or diastolic BP ≧ 90 (n=82*)		systolic BP<140 & diastolic BP<90 (n=265)		p-value[a]		
	Baseline	4 weeks	Baseline	4 weeks	Time[b]	Group[c]	Time × Group
systolic blood pressure (mmHg)	145.60 ± 12.47	139.74 ± 13.99	119.02 ± 11.69	117 ± 13.05	<0.001[d]	<0.001[e]	0.001
diastolic blood pressure (mmHg)	94.65 ± 9.37	89.99 ± 10.49	73.85 ± 9.40	72.8 ± 10.14	<0.001[f]	0.001[e]	0.001

Mean±SD, SD: Standard Deviation;

[a]Two-way repeated measure ANOVA;

[b]Baseline vs 4 weeks; multiple comparisons are performed by paired *t*-tests and calculation of Bonferroni-adjusted p-values with p<0.05;

[c]systolic BP ≧ 140 or diastolic BP ≧ 90 vs systolic BP<140 & diastolic BP<90; multiple comparisons are performed by unpaired *t*-tests and calculation of p-values with p<0.05 Bonferroni-adjusted;

[d]p=0.009 (systolic BP<140 & diastolic BP<90); p<0.001 (systolic BP ≧ 140 or diastolic BP ≧ 90);

[e]p<0.001 at Baseline and 4weeks, respectively;

[f]p=0.052 (systolic BP<140 & diastolic BP<90); p<0.001 (systolic BP ≧ 140 or diastolic BP ≧ 90)

Serum total-cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride concentrations at baseline and four weeks are summarized in Table 2. HDL-cholesterol increased during the four-week period. The concentrations of 8-OHdG in urine, and of hs-CRP and BDNF in serum at baseline and four weeks are shown in Table 2. The concentration of BDNF increased significantly, but those of 8-OHdG and hs-CRP did not change significantly. Stratified analysis of 8-OHdG ≥ 10.52 ng/mg creatinine, which concentration is the highest quartile of 8-OHdG concentration at baseline in all subjects, showed that 8-OHdG concentrations at four weeks were significantly lower than at baseline (Table 4). Stratified analysis of hs-CRP concentrations ≥ 620 μg/L, which concentration is the highest quartile of hs-CRP concentration at baseline in all subjects, showed that hs-CRP at four weeks was significantly lower than at baseline (Table 4).

Table 4: Startified analyses of hs-CRP and 8-OHdG

	Baseline	4 weeks	p-value[a]
hs-CRP mg/dL	0.158 ± 0.134	0.119 ± 0.111	0.002
8-OHdG ng/mg creatinine	16.17 ± 6.48	9.57 ± 4.67	<0.001
Mean ± SD, SD: Standard Deviation; * 8-OHdG: n=86; [a]Wilcoxon signed rank test			

The scores of SF-36v2 are shown in Table 5. The scores for bodily pain, general health perceptions, vitality, role emotional, mental health and mental component score were significantly higher at four weeks than at baseline.

Table 5: Results of SF36-V2

	Baseline	4 weeks	p-value[a]
Physical functioning	52.53 ± 5.77	52.67 ± 6.65	0.194
Role physical	53.18 ± 5.62	53.28 ± 6.38	0.607
Bodily pain (BP)	52.4 ± 8.51	54.56 ± 8.35	<0.001
Social functioning	52.82 ± 7.53	52.89 ± 7.80	0.849
General health perceptions	52.67 ± 8.71	53.53 ± 8.18	0.032
Vitality	52.92 ± 7.48	54.14 ± 6.46	0.001
Role emotional	52.21 ± 7.26	54.71 ± 5.79	<0.001
Mental health	52.69 ± 8.02	53.96 ± 7.75	<0.001
Physical component summary	52.06 ± 6.80	51.92 ± 7.42	0.851
Mental component score	52.2 ± 8.39	53.31 ± 8.35	0.001
Role social component score	52.01 ± 7.62	53.01 ± 7.23	0.071
[a]Wilcoxon signed rank test			

Discussion

We found that consumption of 25 g of dark chocolate daily for four weeks reduced systolic and diastolic blood pressures in Japanese subjects. This result is consistent with the evidence from meta-analyses of randomized trials that eating dark chocolate lowers blood pressure [10-13]. It has been suggested that the improvement of blood pressure by chocolate is owing to its content of polyphenols. In previous studies, 100 g dark chocolate daily for two weeks [8] and 6.3 g of dark chocolate daily for 18 weeks [9] reduced blood pressure. One hundred g of dark chocolate contains 500 mg procyanidins (from monomer to pentamer) and 6.3 g of dark chocolate contains 30 mg procyanidins. Twenty-five g of dark chocolate in the present study contained 75 mg cacao procyanidins (from monomer to tetramer). So, improvement in blood pressure is estimated if subjects obtained a certain amount or more with the amount procyanidins for the intake and duration.

The present study recorded a 3 mmHg reduction in systolic blood pressure and a 2 mmHg reduction in diastolic. It has been estimated that a 3-mmHg reduction in systolic blood pressure would reduce the relative risk of stroke mortality by eight per cent [22]. In hypertensive subjects (diastolic blood pressure ≥ 140 mmHg or systolic blood pressure ≥ 90) there was a 5.9 mmHg reduction in systolic blood pressure and a 4.7 mmHg reduction in diastolic.

Our study also showed that HDL-cholesterol concentration was increased by consumption of dark chocolate. Other studies have provided evidence that the increase in plasma HDL-cholesterol produced by dark chocolate is owing to the presence of cacao polyphenols [6]. Furthermore, our previous study found that consumption of 26 g cocoa enriched with polyphenols for 12 weeks decreased LDL-cholesterol and LDL-oxidation, and increased HDL-cholesterol [5]. The content of procyanidins in 26 g cocoa is 164.5 mg. The present study observed an increase in HDL-cholesterol but did not observe a reduction of LDL-cholesterol or LDL oxidation. This may be owing to the fact that the amount of procyanidins consumed was smaller, and the duration of the study shorter, than in the previous study. We also observed a reduction of 8-OHdG concentration ≥ 10.52 ng/mg creatinine which concentration is the highest quartile of 8-OHdG concentration at baseline in all subjects. Urinary 8-OHdG concentration has been reported to be correlated with atherosclerotic plaques in humans [23]. Oxidative stress in the body was reduced, and reduction of the oxidative modification of LDL inferred. Subjects with hs-CRP concentration ≥ 620 μg/L which concentration is the highest quartile of hs-CRP concentration at baseline in all subjects were found to have a reduction of hs-CRP, which is known to be a risk factor for future coronary heart disease [24].

These combined data showing an increase in HDL-cholesterol, a reduction of hs-CRP, and a reduction of 8-OHdG suggest that chocolate consumption can contribute to the prevention of atherosclerosis and coronary heart disease.

We assessed quality of life using the SF-36 v2. The scores for bodily pain, general health perceptions, vitality, role emotional, mental health, and mental component were significantly increased by consumption of chocolate. Others have found that consumption of cocoa of high polyphenol content reduced mental fatigue [25] and chronic fatigue [26]. Thus, intake of dark chocolate may improve the quality of life. Additionally, we observed an increase in the concentration of BDNF, a member of the neutrophic factor family that plays a key role in regulating the survival, growth and maintenance of neurons [27]. A decrease in BDNF concentration in the brain and plasma has been observed in patients with depression [28] and in those with Alzheimer's disease [29], while an epidemiologic study has provided evidence of a positive association between polyphenol intake and cognitive function [30]. Recently, several reports found that consumption of cacao products with high polyphenol content improved cognitive function [25,31-33]. Moreover, flavonoid-rich cocoa was found to increase cerebral blood flow measured by transcranial doppler in healthy elderly humans [34]. The blood flow relates to endothelial NO synthesis [35]. Ingestion of cocoa with enriched cacao polyphenols was associated with acute elevations of circulating NO species [36]. An increase in plasma NO was also observed after consumption of cocoa with enriched cacao polyphenols for four weeks [37]. Epicatechin, the principal component of cacao polyphenol, elevated NO in endothelial cells [38]. A previous study defined the relation between the consumption of procyanidins and the increase of BDNF [39]. Thus, the intake of diets rich in polyphenols may increase the synthesis of NO in endothelial cells, lower blood pressure, increase BDNF in plasma and brain, and improve cognitive function.

Our study found that consumption of dark chocolate had no effect on BMI or body weight. Although several plasma biochemical analytes at four weeks were significantly higher than at baseline, they nevertheless remained within the normal range.

In conclusion, our study has shown that daily consumption of dark chocolate decreased blood pressure, and increased HDL-cholesterol, BDNF and the score for quality of life. It is anticipated that regular consumption of polyphenol-rich foods, such as cocoa, dark chocolate, fruits and vegetables, should lead to a decrease in the incidence of atherosclerotic disease and improvement of cognitive function.

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