Effect of Dark Chocolate on Nitric Oxide Serum Levels and Blood Pressure in Prehypertension Subjects

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ABSTRACT

Aim: *to investigate the effect of consumption of dark chocolate 30 g/day for fifteen days on Nitric oxide (NO) serum levels and blood pressure in male and female employees with prehypertension.*

Methods: *the study was a parallel randomized clinical trial. A total of thirty-two subjects was divided into two groups using block randomization. Sixteen subjects received 30 g/day dark chocolate and dietary counseling (treatment group) and the other 16 subjects received white chocolate 25 g/day and dietary counseling (control group) for fifteen days. Data collected in this study consisted of age, physical activity, body massa index, intake of energy, intake of sodium, and intake of polyphenol, NO serum levels and blood pressure. The measurement of NO serum levels was done in pre- and after- treatment, while blood pressure was assessed in pre- , during- and after- treatment. Statistical analysis was performed using independent t-test for normal distribution data and Mann-Whitney test for not normal distribution data, with the level of significancy of 5%.*

Results: *after 15 days treatment, NO serum level between treatment and control groups were significantly different 7.70±3.84 vs 1.92(-0.79±17.78) (p=0.001). Both groups had decreased systolic and diastolic blood pressure. Systolic blood pressure was different significantly between groups after treatment 120.64±8.47 vs 131.19±7.45 (p=0.001), while diastolic blood pressure was not significant 74.14±6.30 vs 77.44±10.29 (p=0.308).*

Conclusion: *in prehypertension subjects, dark chocolate 30 g/day increased NOx serum levels and decreased systolic blood pressure after 15 days of treatment.*

Key words: *dark chocolate, polyphenol, nitric oxide serum levels, prehypertension.*

INTRODUCTION

Prehypertension is usually defined as a systolic blood pressure between 120-139 mm Hg or diastolic blood pressure between 80-89 mm Hg.[1,2] The mean blood pressure in Indonesian people at 25-34 years old is 124.7/79.9 mm Hg and there is a positive correlation between age and blood pressure.[3] Prehypertension patients have two folds risk to suffer from coronary heart disease or stroke compared with normotensive patients. Risk factors for prehypertension are obesity, age, high intake of energy and sodium, low physical activity and alcohol intake.[4]

Vascular disorders in prehypertension patients are influenced by, among others, decrease in synthesis and bioavailability of nitric oxide (NO) leading to endothelial dysfunction.[5] Nitric oxide is a vasodilator produced in endothelium cells to maintain vascular tone. Synthesis and bioavailability of NO were influenced by arginine, Nitric Oxide Synthase (NOS) and superoxide anion.[6] NO has a fast half life, therefore in clinical analysis the NO metabolite (NOx) is used as a marker of NOS activity and NO production.[7]

Polyphenols are abundant in human diet. One class of polyphenols, flavanols, based on clinical and epidemiology studies showed to have protection effect on cardiovascular system.[8] Flavanols are able to increase antioxidant plasma, decrease oxidation products and activate endothelial Nitric Oxide Synthase (eNOS) which will increase synthesis and bioavaibility of NO that in turn will restore endothelial function.[9]

Dark chocolate is a major source of flavanols which have the highest antioxidant level compared with other food source based on Oxygen Radical Absorbance Capacity (ORAC) measurement.[10] Previous studies had shown that dark chocolate intake reduces blood pressure significantly.

Grassi et al studied the effects of 100 g/day (88 mg flavanol) dark chocolate in hypertensive subjects for fifteen days, which showed significant decrease of systolic and diastolic blood pressure.[11] Taubert et al studied the effects of dark chocolate 6.3 g/day compared with white chocolate 5.6 g/day for 18 weeks in prehypertension and stage 1 hypertension subjects. The results showed a significant decrease in systolic and diastolic blood pressure in subjects who received dark chocolate.[12] This study was a parallel randomized clinical trial, comparing two groups, i.e, a treatment group who received dark chocolate 30 g/day which contained 70% cocoa, and dietary counseling while the control group who received white chocolate 25 g/day which contained equal calories i.e., 150 ccal and dietary counseling for fifteen days on NOx serum levels and blood pressure in male and female employees with prehypertension.

This study was conducted to investigate the effect of dark chocolate 30 g/day for fifteen days on Nitric oxide (NO) serum levels and blood pressure in male and female employees with prehypertension.

METHODS

Subjects

The study was a parallel randomized clinical trial. Male and female employees of a dental faculty and a private company recruited and written informed consents obtained. Thirty-two subjects, were divided into two groups using block randomization, i.e., sixteen subjects in the treatment group and the other sixteen in the control group. The inclusion criteria were male and female aged 25-44 years old, prehypertension (systolic 120-139 mmHg and or diastolic 80-89 mmHg), and had a body mass index (BMI) range 18.5-24.9 kg/m^2. The exclusion criteria were persons with hypertension history, smokers, red wine consumption, or alcohol consumption, pregnant or breast feeding women, have had menopause, used antihypertension drugs and supplementations of vitamin C, vitamin E or other antioxidant suplements. This study was conducted after being approved by the Ethics Committee of Faculty of Medicine of University of Indonesia.

Study Measurements

Body weight and height were measured to determine the BMI. Food intake data were obtained using 2x24 hours food record to determine energy, sodium, and polyphenol intakes. The individual energy requirement was calculated using Harris-Benedict Equation. Polyphenol intakes were the sum of polyphenol content in food consumed in a day and was calculated manually by using the Table of Polyphenol-containing Food Sources. Mean polyphenol intake was compared with the recommended daily intake which is 500-1000 mg.[13,14]

Subjects were asked to fast overnight for 10-12 hours before withdrawing blood samples. Ten millilittres blood sample was withdrawn to measure NOx serum levels in pre- and after- treatment. The measurement of NOx serum levels which is the sum of nitrite and nitrate levels as the metabolite of NO, used the colorimetric Cayman method.[15]

Blood pressure was measured in pre treatment, 8th day of treatment and after treatment. The measurement of blood pressure used Bionet monitor device. The subjects took five minute rest prior the blood pressure measurements. The blood pressure values taken as an average of two readings with five minutes interval between readings. In this study, the third measurement was taken using standard sphygmomanometer as confirmation.

Statistical Analysis

All statistical calculations were performed with Statistical Package for Social Science (SPSS version 11,5) software. The normality of data distribution was assessed by using Kolmogorov-Smirnov test. Differences in mean values were assessed by unpaired t-test for the normal distributed data and Mann Whitney test for the not normal data. The values of $p<0.05$ were applied to indicate statistical significance.

RESULTS

The characteristics of two groups at baseline were not significantly different, except in physical activity index, in which the treatment group had higher physical activity index compared with the control group. The characteristics of the two groups were closely matched at baseline (**Table 1**).

Mean intakes of energy and sodium between both groups were not significantly different and were lower than that of Indonesian Recommended Dietary Allowance 2004. Polyphenol intake of the treatment group in first and second weeks were significantly higher than those of the control group (**Table 2**).

The NOx serum levels in treatment group increased significantly, on the contrary, there was significant decrease in control group. The changes of NO serum levels after treatment, between treatment, and control groups were significantly different (**Table 3**).

Table 1. The baseline data of subject's characteristic

Variable	Treatment	Control
Sex	1 (1-2)#	1 (1-2)#
- Male	10 (71.43%)	10 (62.5%)
- Female	4 (28.57%)	6 (37.5%)
Age (years)	36 ± 7.42	33.44 ± 6.08
- 25-34	6 (42.86%)	8 (50%)
- 35-44	8 (57.14%)	8 (50%)
Education level	2 (1-3)#	2.5 (1-3)#
- Low	1 (7.14%)	1 (6.25%)
- Middle	7 (50%)	7 (43.75%)
- High	6 (42.8%)	8 (50%)
Physical activity (MET-h/wk)	31.79 ± 16.81	19.12 ± 8.5
- Low	4 (28.57%)	8 (50%)
- Sufficient	4 (28.57%)	8 (50%)
- Average	6 (42.86%)	-
Body mass index (kg/m^2)	23.5 (17.10-24.9)#	23.6 (17.22-24.3)#
- Normal	6 (42.86%)	7 (43.75%)
- Overweight	8 (57.14%)	9 (56.25%)
Systolic blood pressure (mmHg)	128.64 ± 5.89	128.12 ± 7.72
Diastolic blood pressure (mmHg)	80.5 ± 6.3	77.75 ± 5.40

= median (minimum - maximum)

Table 2. The average intakes of energy, sodium and polyphenol using food record 2 x 24 hours before treatment, first and second week of treatment period

Variable	Treatment	Control	p
Energy (kcal)			
- Pre-treatment	2111.57±238.25	2157.86±595.66	0.9[tt]
- First week	1511.86±66.25[a]	1973.14±225.14	0.073[tt]
- Second week	1759.14±232.36[a]	2160.71±742.21	0.292[tt]
Sodium (mg/day)			
- Pre-treatment	2271.57±244.06	2152.14±256.59	0.391[tt]
- First week	2130.86±182.73[a]	2110.57±230.62	0.858[tt]
- Second week	2079.71±176.77[a]	2010.29±185.99	0.488[tt]
Polyphenol (mg/day)			
- Pre-treatment	128.86±73.32	136.14±86.25	0.868[tt]
- First week	316.43±70.46[a]	141.43±88.14	0.002*[tt]
- Second week	369.14±91.61[a]	172.29±136.80	0.01*[tt]

a=14 subjects; *=significant; tt=unpaired t-test

Table 3. NOx serum levels before and after treatment

Variable	Treatment	Control	p
NOx serum levels (mmol/L)			
Pre treatment	4.98 ± 3.07	4.13 (0.37-41.95)	0.868[m]
After treatment	7.70 ± 3.84[a]	1.92 (-0.79-17.78)	0.001*[m]
Changes (Δ)	2.72 ± 2.22	-2.36 (-24.17-0.14)#	0.000*[m]
p	0.001*[t]	0.001*[w]	

a=14 subjects; *=significant; m=mann whitney; w=wilcoxon; t=paired t-test

Both groups had significant decrease systolic and diastolic blood pressures compared with baseline measurement. After treatmnent there was significant decrease in systolic between groups (p=0,001), while diastolic blood pressure was not significant (p=0,308) (**Table 4**).

Table 4. Systolic and diastolic blood pressure

Variable	Treatment	Control	p
Systolic (mmHg)			
- Pre-treatment	128.64±5.89	128.12±7.72	0.837[tt]
- 8th day	122.71±10.36[a]	126.44±8.02	0.287[tt]
- After treatment	120.64±8.47[a]	131.19±7.45	0.001*[t]t
- change (Δ)	-8.00±5.67	-3.06±4.39	0.000*[t]t
- p	0.000*[t]	0.432[t]	
Diastolic (mmHg)			
- Pre-treatment	80.5+6.3	77.75+5.40	0.215[tt]
- 8th day	74.07+8.38[a]	76.19+8.85	0.507[tt]
- After treatment	74.14+6.30[a]	77.44+10.29	0.308[tt]
- change (Δ)	-6.36+6.15	-0.31+7.24	0.021*[tt]
- p	0.009*[t]	0.283[t]	

a=14 subjects; *=significant; t=paired t-test; tt=unpaired t-test

There was significantly strong negative correlation between changes of NOx serum levels and the changes of systolic blood pressure, while there was significantly moderate negative correlation for the changes of diastolic blood pressure (**Table 5**).

Table 5. The correlation between the changes of NOx serum levels and the changes of blood pressure

	Δ NOx serum levels	
	r[$]	p
Δ Systolic blood pressure	-0,640	0,000*
Δ Diastolic blood pressure	-0,377	0,04*

* = significant; r = coeficient correlation with correlation test Rank Spearman

DISCUSSION

Twenty subjects in this study were male (66.67%) and ten were female (33.33%). The prevalence of prehypertension in male is higher than in female. In males, the production of aldosterone hormones from progresterone will increase sodium absorption in the kidney.[3,16] The mean age of subjects was 36±7.42 years old, while the mean blood pressure in Indonesian people at 25-34 years old was 124.7/79.9 mm Hg and will rise along with aging process.[3] Age correlates with decrease of elasticity of vascular walls which in turn increases peripheral resistance.[1]

Both groups have equal educational levels, which was considered to have correlation with chocolate eating habits and compliance to the study protocol.

The number of overweight subjects was higher than subjects with normal BMI. In obese people, the risk to increase blood pressure was 2-6 times higher compared with normal BMI. Excess of body weight of more than 20% from the ideal body weight will increase prehypertension risk twice more than that of the non obese.[4] Physical activity index was low because all subjects had sedentary activity. Hu et al reported that low physical activity increased risk for hypertension.[17] Theoretically, in low physical activity index there is a lack of pulsatile blood flow.[5]

Although the average energy intake of subjects was lower than RDA 2004, it was still in the normal range of Individual Energy Requirement (80-120%) which caused overweight in most subjects due to low activity.[1] Sodium intake analysis was difficult because of the difficulty to get accurate information on sodium intake in daily meals.[18] The average sodium intake was lower than RDA 2004 (1500-2400 mg/day).[19] Sodium intake and blood pressure have positive correlation, by which the high intake of sodium will decrease kidney function to excrete sodium and furthermore leading to increased plasma volume and cardiac output.[4] The analysis of polyphenol was done manually using a list of polyphenol content in food sources.[20] The limitations of this method posed a difficulty in determining polyphenol intake, it could underestimate or overestimate. During the treatment period, the polyphenol intake in the treatment group was higher than in the control group, the difference was sugessted because of polyphenol content in dark chocolate.

NOx serum levels in all subjects were low. Fortuńo et al proposed that NOx serum levels decreased in hypertension subjects because of the lack of arginine as substrate for NO synthesis.[21,22] After 15 days of treatment, NOx serum levels between treatment and control groups was significantly different (p=0.001). After treatment period, NOx serum levels in the treatment group was higher (p=0.001), while it was lower in control group (p=0.001) compared to the baseline. This was suitable with Fisher et al who proposed that polyphenol increase regulation of eNOS transcription to increase NO synthesis.[23] Heiss et al also found that flavanol in chocolate has a capability to increase bioactivity of NO.[24] It is not clear how flavanol interacts in the biological system to increase NO bioavailability. Some theories explained that flavanol could modify oxidative stress and induce NO availability as well as activate NO synthase. Dark chocolate inhibits activity of NADPH-oxidase to produce superoxide anion which will oxidize NO to release peroxinitrite.[25]

Both groups had decrease systolic and diastolic blood pressure which might be caused by dietary counseling. Izzo et al stated that dietary counseling was able to decrease blood pressure.[16] Systolic and diastolic blood pressure decreased significantly in the treatment group, which was assumed to be caused by polyphenol content in dark chocolate. Ghosh et al stated that vascular effect of polyphenol was able to decrease blood pressure.[26] In this study, the systolic blood pressure was decreased significantly between groups after treatment (p=0,001), while diastolic blood pressure was not significant (p=0,308). This was relevant with studies by Grassi et al[27], Fraga et al[25] and Vlachopoulos[28] et al which involved hypertensive subjects but not supported by the other study of Grassi et al[11] which involved diabetic hypertensive subjects and Taubert et al[12] which involved prehypertensive and hypertensive subjects. This study used a small dose of dark chocolate and more short treatment period compared with the studies of Grassi et al[27] and Taubert et al[12] which might cause the different result of this study. The Ambulatory Blood Pressure Monitoring (ABPM) used in Grassi et al[11] was more accurate than the patient monitor used in this study which also might influence the findings in this study.

Vasodilatative effects of cocoa was obtained by its capability to supress arginase enzyme activity which will lose in the competition with NO endothelial synthase. This in turn will increase the production of NO.[25] The other mechanism of cocoa on vasodilatation is associated with increased epicatechin levels in plasma which will give a signal to release vasoactive components from endothelial cells, i.e., NO and prostacyclin.[9] This process will also decrease ratio leukotriene-prostacyclin to cause vasodilatative effect.[25]

There was significantly strong negative correlation between the change of NOx serum levels and the change of systolic blood pressure, while there was significant moderate negative correlation with the change of diastolic blood pressure. Hall et al confirmed that increase NOx serum levels will increase vasodilatation, which in turn will decrease blood pressure.[29]

The limitation of this study was difficulty in blinding between dark chocolate and white chocolate though it was minimalized by placing the chocolate in the box with the same shape and colour. The limitation of nutrisurvey 2005 programme which was used to analyze the nutrient intakes did not have enough database on local foods.

CONCLUSION

In prehypertension subjects, dark chocolate with 70% cocoa as much as 30 g per day during fifteen days increased NOx serum levels, decreased systolic blood pressure significantly but did not significantly decrease diastolic blood pressure.

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