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Protective Effect of Dark Chocolate on Cardiovascular Disease Factors and Body Composition in Type 2 Diabetes: A Parallel, Randomized, Clinical Trial

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Abstract

Background: Diabetes leads to complications such as cardiovascular diseases. There are limited data about the effect of dark chocolate on cardiovascular function in patients with diabetes.
Objectives: The current study aimed at determining the effect of dark chocolate on cardiovascular health and body composition among people with diabetes.
Methods: The current parallel, randomized, clinical trial was conducted on 44 patients with diabetes (Ahvaz, Iran). They were randomly assigned into the intervention (n = 21, 30 g dark chocolate daily for 8 weeks) and the control groups (n = 23). At the beginning and end of the intervention period, fasting blood samples were collected to measure nitric oxide (NO) and angiotensin II. Also, anthropometric measurement, body composition analyses, and blood pressure were compared between the 2 groups before and after the intervention.
Results: A significant reduction in systolic (-6.9 $\pm$ 7.3 vs. 0.3 $\pm$ 1.9; P = 0.001) and diastolic blood pressure (-5.8 $\pm$ 6.7 vs. 0.5 $\pm$ 3.9; P = 0.001), waist circumference (WC) (-0.7 $\pm$ 1.0 vs. 0.1 $\pm$ 1.2; P = 0.007), and significant increase in soft lean mass (P = 0.045) was observed in the intervention group. There were no significant changes in NO levels, but a trend close to significance for angiotensin II (P = 0.052) at end of the intervention between the 2 groups.
Conclusions: The current study findings showed that dark chocolate consumption in patients with diabetes might improve their WC, body composition, and blood pressure, but had no effect on NO in this dosage.

Keywords: Chocolate, Diabetes Mellitus, Blood Pressure, Body Composition, Nitric Oxide, Angiotensin

1. Background

The prevalence of type 2 diabetes is rising rapidly worldwide (1). The world health organization (WHO) reported that the number of persons with diabetes increased from 108 million in 1980 to 422 million in 2014 (2). In 2005, two million Iranian adults were affected and the incidence is predicted to increase to 5.1 million in 2025 (3, 4).

Diabetes mellitus results in different kinds of complications such as cardiovascular disease (CVD) (5). Persons with diabetes have 2 - 4 folds increase of CVD compared to the ones without diabetes; CVD is the leading cause of death in such patients (6, 7). The most important risk factor of CVD is hypertension (8). Persons with diabetes and hypertension face other complications such as retinopathy and neuropathy, more than the ones without hypertension (9).

Nitric oxide (NO) is a physiological vasodilator that can modulate blood pressure and is known as an anti-atherosclerotic molecule. Production and bioavailability of NO are reduced in type 2 diabetes (10, 11).

Because of the social and financial burden imposed on the healthcare system by diabetes and its complications, it is interesting for researchers to find an effective management protocol for it. The use of alternative treatments such as functional foods to manage diabetes and its complications is imperative. One of the most important functional foods is cocoa as well as cocoa products such as chocolate. Flavanols are the effective component of chocolate. Epidemiological investigations showed that consumption of flavanols was associated with reduction in CVD mortality and morbidity (12-14) and the incidence of diabetes (15).

However, there are limited data about the way that chocolate can improve CVD in patients with diabetes. Earlier studies indicated improvement in lipid profile, blood pressure, and glycemic control in persons with diabetes (16, 17). Haghighat et al., showed that 25 g dark chocolate

can improve blood pressure and lipid profile (18), but most previous studies investigated the effect of dark chocolate on endothelial function and the cardiovascular system in healthy cases and the ones with hypertension (19-22). In addition, these studies mostly involved short-term administration of dark chocolate. Because of the limited information about the effect of dark chocolate on cardiovascular health in patients with type 2 diabetes, the current study aimed at evaluating the effect of 8 weeks of dark chocolate consumption on blood pressure, levels of NO, angiotensin II, and body composition in patients with type 2 diabetes. The current study considered the effect of dark chocolate on body composition for the first time.

2. Objectives

The current study aimed at investigating the effect of dark chocolate consumption on blood pressure and some related factors in patients with diabetes to decrease diabetes complications.

3. Methods

The current study was a single-blind, parallel, randomized, clinical trial approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (ajums.REC.1393.407). The trial was registered by the Iranian registry of clinical trials at http://www.irct.ir under the code number: IRCT2015022116123N5. All participants were selected from patients referred to Golestan hospital affiliated to Ahvaz Jundishapur University of Medical Sciences, from March to June 2015. The sample size was calculated based on the suggested formula for a parallel, randomized, clinical trial: the type 1 error (α) was considered 1% and type 2 error (β) 5%. Twenty-two subjects were determined in each of the intervention and control groups with 10% predicted dropouts; 50 patients were altogether enrolled in both groups of the trial.

Inclusion criteria were the diagnosis of type 2 diabetes based on the American Diabetes Association guidelines; one of the following criteria was considered as diabetes: Fasting plasma glucose (FPG) $\geq$ 126 mg/dL, oral glucose tolerance test $\geq$ 200 mg/dL; age range of 30 to 60 years, body mass index (BMI) range of 18.5 to 35 kg/m^2, using metformin or glibenclamide as medications with a stable dosage for at least 3 months prior to the study, and no longer consumption of antioxidant supplements. The exclusion criteria were as follows: Treatment with insulin, smoking, alcohol consumption, pregnancy or breastfeeding, history of hepatitis, renal or lung failure as well as CVD and kidney stones. One hundred and eighty-seven patients were primarily selected out of which 50 were enrolled in the study according to the inclusion and exclusion criteria. They were divided into 2 groups (n = 25) by stratified randomization based on BMI. The flowchart of the study is shown in Figure 1. After obtaining the written consent forms from all subjects, they were advised to use therapeutic lifestyle changes (TLC) guidelines in food pattern (total fat 30% to 35%, monounsaturated fatty acids (MUFA) 20%, polyunsaturated fatty acids (PUFA) 10%, protein 15% , saturated fatty acid (SFA) less than 7%, and cholesterol less than 200 mg) for 8 weeks. Twenty-one subjects in the intervention group and 23 subjects in the control group completed the study. The intervention group was instructed to consume 30 g of 84% dark chocolate (Parmida, Kian Chocolate Kimia company, Tehran, Iran) daily for 8 weeks; (chocolate composition per 100 g: total fat: 42.1 g, protein: 11.9 g, saturated fatty acids: 6.6 g, and carbohydrate: 35.2 g). The current study was a single-blind and a third party provided chocolate for the patients. Chocolate was packed into a plastic zip lock package for daily use and 7 packages were provided for each subject for 1 week and every week they got them. Compliance was monitored weekly by counting the remaining chocolates. If compliance was less than 80% and participants started insulin injection or changed the dosage and type of medication throughout the study, they were excluded.

3.1. Anthropometric Assessment

All measurements were made by a trained observer. Weight (in kilograms) was measured to the nearest 0.1 kg by a calibrated scale (Seca GmBH and Co. KG., Hamburg, Germany) in the morning after 12 hours fasting, waste expelled from the body, without shoes and heavy clothing. Height (in centimeters) was measured with bare feet while the subject standing upright and looking straight ahead. BMI was calculated as body weight in kilograms divided by height squared in meters. Waist circumference (WC) in centimeters was measured with plastic tapes calibrated mid-way between the lowest rib and the iliac crest.

After weight measurement, body composition was assessed using the bioelectrical impedance analysis (ioi 353, Jawon Medical Co., Ltd., Korea), while subjects were asked to drink 2 glasses of water 1 hour before measurement and not to do activities or use food and caffeine 2 hours before the test. Body fat and soft lean mass in kilograms were assessed using this method.

3.2. Biochemical Measurement

Fasting blood samples were obtained from all participants before intervention and after 8 weeks. Sera were separated and kept at -70°C until the saturation of samples. Then, NO and angiotensin II were measured using

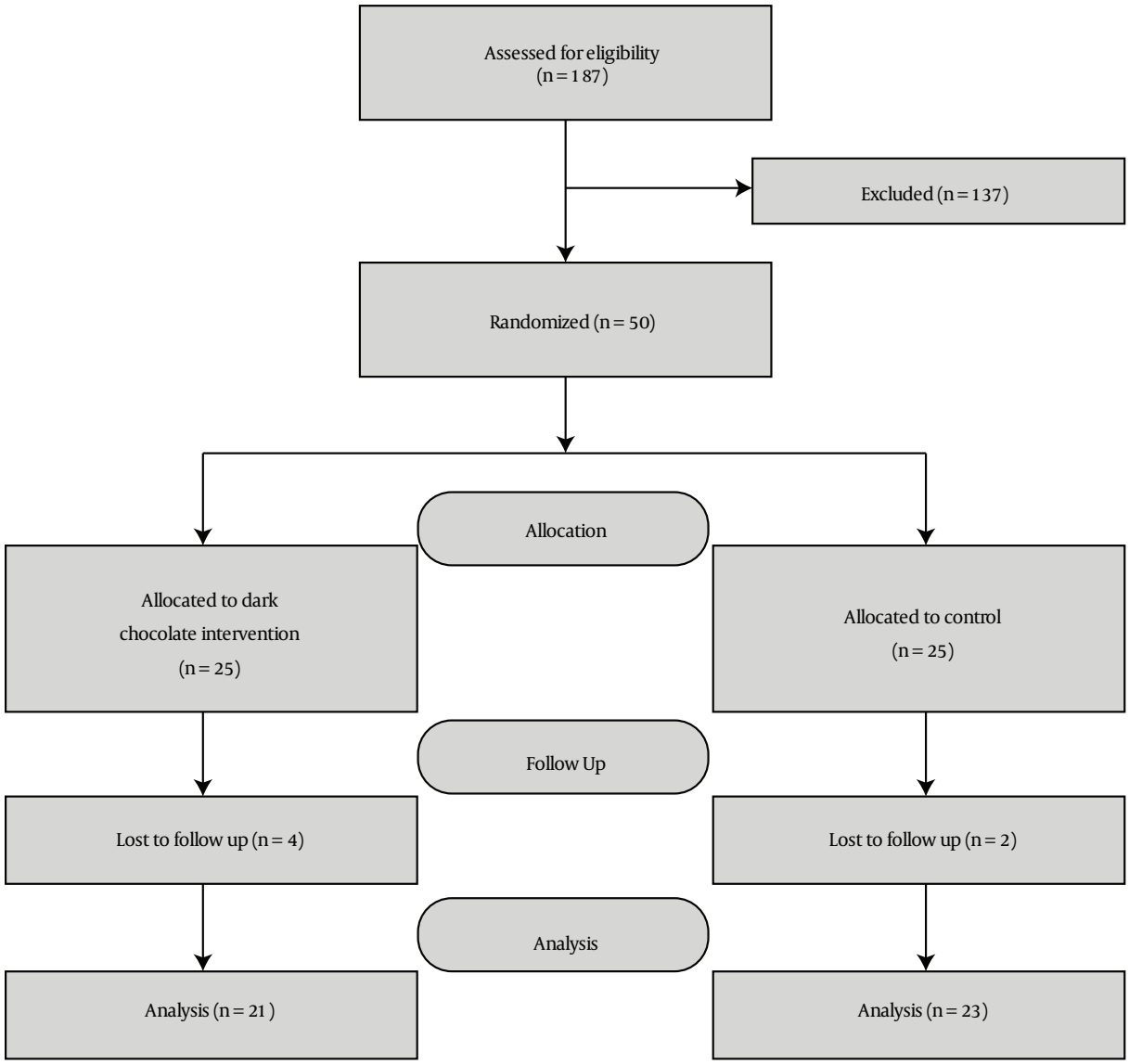

Figure 1. Flowchart of the Study

enzyme-linked immunosorbent assay (ELISA) technique (Hangzhou Eastbiopharm Co., Ltd., China).

3.3. Blood Pressure Assessment

Subjects rested for 10 minutes, and then, their blood pressures (systolic and diastolic blood pressure in mmHg) were measured by a standard mercury sphygmomanometer (ALPK 2, Japan), while they were in sitting position.

3.4. Data Analysis

All data were analyzed using SPSS (statistical package for social sciences) version 16.0. The mean of the quantitative variables were compared between the 2 groups using the independent samples t test, and the 2-paired t test was used to compare the means of variables in each group, before and after the intervention. Normality was tested using the Kolmogorov- Smirnov test; if data distribution was abnormal, nonparametric (the Mann-Whitney and Wilcoxon) tests were used.

4. Results

General characteristics of patients are shown in Table 1. Baseline characteristics of the participants did not dif-

fer between the intervention and control groups. Less than half of the subjects were male.

Table 1. General Characteristics of the Study Participants at Baseline

Variable	Intervention Group[a] (n = 21)	Control Group[b] (n = 23)	P[c]
Age (year)[d]	50.6 ± 7.5	50.7 ± 7.9	0.9
Gender, n (%)			0.39
Male	7 (33.3)	10 (43.5)	
Female	14 (66.7)	13 (56.5)	
Duration of diabetes (year)[d]	4.1 ± 1.32	3.8 ± 1.31	0.46

[a]Intervention group received 30 g of 84% dark chocolate and therapeutic life-change guideline.
[b]Control group received only therapeutic life-change guideline.
[c]Independent samples t test or Chi-square test.
[d]Values are expressed as means ± SD.

The effect of intervention on anthropometric measurements is presented in Table 2. Mean weight and BMI of subjects in the 2 groups decreased, however, the difference of before and after intervention between the groups was insignificant. Consumption of dark chocolate for 8 weeks resulted in significant reduction in WC. Also, soft lean mass increased significantly (Table 2).

Table 3 shows changes of blood pressure, NO, and angiotensin II after 8 weeks. Compared to the baseline, dark chocolate caused significant reduction in systolic and diastolic blood pressure, but results did not show significant changes in NO. However, angiotensin II showed a trend close to significance (P = 0.052, Table 3).

Table 2. Effect of Dark Chocolate on Anthropometric Measurements of Participants in the Study Groups

Variable	Intervention[a] Percentiles 25th, 75th	Control[b] Percentiles 25th, 75th	P Value[c]
Weight (kg)			
Before	61.2, 80.3	67.9, 82	0.549
After	60.75, 80.45	66.5, 81.8	0.63
Change	-1.5, 0.15	-1.5, 0	0.897
P value[d]	0.033	0.014	
BMI (kg/m^2)			
Before	25, 30.65	24.5, 31	0.672
After	24.85, 30.05	24.5, 30.2	0.707
Change	-0.07, 0	-0.6, 0	0.532
P value[d]	0.021	0.025	
Waist circumference (cm)			
Before	90, 105	91, 101	0.403
After	89, 104.75	91, 101	0.589
Change	-1.5, 0	0, 0	0.007
P value[d]	0.035	0.334	
Body fat (kg)			
Before	18.9, 29.35	19.1, 27.6	0.63
After	18.45, 28.3	18.1, 26.2	0.769
Change	-1.35, -0.35	-0.9, 0	0.099
P value[d]	0.004	0.027	
SLM (kg)			
Before	38.05, 47.55	38.3, 53.3	0.366
After	38.4, 47.45	38.3, 53	0.445
Change	-0.2, 0.95	-0.5, 0.4	0.045
P value[d]	0.053	0.481	

Abbreviations: BF, body fat; BMI, body mass index; SLM, soft lean mass.
[a]Intervention group received 30 g of 84% dark chocolate and therapeutic life-change guideline.
[b]Control group received only therapeutic life-change guideline.
[c]Intergroup comparison by the Mann-Whitney test.
[d]Intragroup comparison by the Wilcoxon test.

5. Discussion

The current study was conducted on patients with diabetes and investigated the effect of daily consumption of dark chocolate for 8 weeks on blood pressure, NO, angiotensin II blood levels, and anthropometric measurements. Significant decreases in systolic and diastolic blood pressures were observed following the consumption of dark chocolate in persons with diabetes. Despite the expectation, dark chocolate consumption could not affect NO significantly in the current study, but a slight increase in NO was observed in the intervention group, compared with the control group. Also, consumption of dark chocolate for 8 weeks led to a borderline significant change in angiotensin II levels.

The most common macrovascular complication in diabetes is CVD; it is the first cause of death among patients with diabetes (7, 23, 24). Different studies revealed that dietary antioxidants, especially flavonoids, have protective effects on chronic diseases such as CVD and diabetes. The major sources of antioxidants are fruits, vegetables, tea, wine, and chocolate. Chocolate is the richest source of flavonoids (catechin, epicatechin, and procyanidins). Several studies showed the positive effect of chocolate on the prevention of CVD in healthy people and on the improvement of diabetes (25, 26).

Hypertension is a risk factor for CVD. Endothelial dysfunction is caused by high blood pressure and abnormal

Table 3. Effect of Dark Chocolate on Blood Pressure, Nitric Oxide, and Angiotensin II

Variable	Intervention[a]	Control[b]	P Value[c]
	Percentiles	Percentiles	
	25th, 75th	25th, 75th	
SBP (mmHg)			
Before	115, 130	110, 130	0.299
After	110, 120	110, 140	0.249
Change	-10, 0	0, 10	0.001
P value[d]	0.001	0.142	
DBP (mmHg)			
Before	70, 80	70, 80	0.518
After	70, 80	70, 85	0.10
Change	-10, 0	0, 0	0.001
P value[d]	0.005	1.00	
Nitric oxide (μM/L)			
Before	68, 140	73, 159	0.664
After	80, 136	84, 132	0.787
Change	-4, 18	-38, 14	0.222
P value[d]	0.177	0.590	
Angiotensin II (ng/L)			
Before	14, 68	12, 59	0.869
After	14, 98	27, 116	0.417
Change	-7, 15	-3, 38	0.052
P value[d]	0.338	0.012	

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.
[a] Intervention group received 30 g of 84% dark chocolate and therapeutic life-change guideline.
[b] Control group received only therapeutic life-change guideline.
[c] Intergroup comparison by the Mann-Whitney test.
[d] Intragroup comparison by the Wilcoxon test.

endothelium function is associated with progression of CVD (27). Insulin resistance and endothelial dysfunction are common in type 2 diabetes. In addition, endothelial NO production is stimulated by insulin, which is probably decreased in persons with diabetes (28).

Contradictory effects of dark chocolate on blood pressure were shown by earlier studies. Similarly, Rostami et al., showed a significant reduction in systolic and diastolic blood pressures in patients with diabetes and hypertension (17). Other studies indicated that dark chocolate consumption improves hypertension in subjects with hypertension (26, 29). In contrast to the current study results, some of the previous studies did not find any significant reduction in blood pressure following the consumption of dark chocolate (16, 21).

To the best of the authors' knowledge, is the current study was the first one on the effects of dark chocolate on NO and angiotensin II in patients with diabetes. Sudarma et al., found that consumption of 30 g chocolate for 15 days caused a significant increase of NO in subjects with prehypertension, which was consistent with the current study results (22). Similar to the current study, Persson et al., did not find any significant change in NO levels, but found a significant reduction in angiotensin converting enzyme activity in healthy volunteers (30).

The antihypertensive effect of dark chocolate in the current study could be explained by some mechanisms that showed this effect on healthy people or people with CVD. The first mechanism is that endothelial function and NO secretion are improved by cocoa consumption (31). This mechanism may be due to an increase in endothelial nitric oxide synthase (eNOS) activity that catalyzes the production of NO from L-arginine (10). NO results in the relaxation of vascular smooth muscle cells (32). The second mechanism is explained by angiotensin converting enzyme. An increase in NO production and eNOS activity can reduce blood pressure in small amounts (33), but the main mechanism to decrease blood pressure is inhibition of angiotensin converting enzyme (34). Also, cocoa antihypertensive effect is induced by stearic acid or theobromine, 2 cocoa chemical compounds (35). The other mechanism is the protective effect of dark chocolate as an antioxidant that decreases inactivation of NO by free radicals (36). The last mechanism is that dark chocolate consumption can improve insulin sensitivity and β-cell function, and diminish insulin resistance; these properties are caused by the antioxidant effect of dark chocolate (37), and new insight can explain increasing the adiponectin secretion and improvement of hypertension by catechins in dark chocolate (37, 38).

The current parallel, clinical trial showed that dark chocolate consumption reduced waist circumference significantly, as a risk factor for CVD, although no significant changes in BMI or body weight were observed. Another finding was the significant changes in body composition and increase in soft lean mass. Several studies showed different results. Similar to the current study, Di Renzo et al., found significant reduction in WC (39) in contrast to Nogueira et al. (40).

The mechanism of how dark chocolate can influence body composition and WC can be explained by increasing the adiponectin secretion (41). Body fat distribution was affected by adiponectin concentration (42).

The limitation of the study was that the control group did not receive placebo; therefore, the study was single blinded; it means that the researchers did not know who got dark chocolate. The strong point of the study was using TLC guidelines both in the intervention and control groups

and studying the effect of dark chocolate as a functional food along with these guidelines.

In conclusion, it seems that consumption of 30 g dark chocolate for 8 weeks in patients with diabetes might improve hypertension, WC, and body composition. Therefore, this functional food could be administrated along with TLC guidelines in patients with diabetes. Further studies are required to determine the appropriate dosage for such patients.

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Footnotes

Authors' Contribution: Nina Ayoobi: data collection, study concept and design, and drafting of the manuscript; Sima Jafarirad: study supervision, study concept and design, and critical revision of manuscript for important intellectual content; Mohammad Hossein Haghighizadeh: analysis and interpretation of data; Alireza Jahanshahi: administrative technical and material support.

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