

Dark Chocolate Effect on Serum Adiponectin, Biochemical and Inflammatory Parameters in Diabetic Patients: A Randomized Clinical Trial

Abstract

Background: An appropriate snack for patients with diabetes mellitus should be considered to help them in their treatment due to their hard administrative diet. This study was conducted to evaluate the effect of dark chocolate on inflammatory markers, serum adiponectin, and certain biochemical factors in patients with type 2 diabetes (T2D). **Methods:** This study was a randomized parallel clinical trial. Thirty grams of 84% dark chocolate, along with therapeutic lifestyle changes (TLCs) guidelines, were administered to patients with T2D. Control group received only TLC guidelines. The intervention period was 8 weeks. Twenty-one subjects in dark chocolate and 23 subjects in control group completed the study. Fasting blood samples were collected before and after the intervention period and inflammatory markers, biochemical factors, and adiponectin levels were assessed. **Results:** Fasting blood sugar, hemoglobin A1C, low-density lipoprotein and triglyceride levels declined significantly in the dark chocolate group and this decrease was significant between the intervention and control groups. Tumor necrosis factor-alpha, interleukin-6, and high sensitive C-reactive protein were significantly decreased in the dark chocolate group. Adiponectin levels were not significantly different between the two groups. **Conclusions:** In this study subjects who received dark chocolate along with TLC guidelines had lower levels of inflammatory markers such as hs-CRP, TNF- α , and IL-6, compared with the subjects who were devoid of dark chocolate and followed only the TLC guidelines. Other studies should be conducted to evaluate the most effective and administrative dosage of dark chocolate as a snack along with the common treatment of diabetes.

Keywords: Adiponectin, chocolate, diabetes mellitus type 2, inflammation mediators

Introduction

Diabetes mellitus is a chronic disease, generally prevalent in both developed and developing countries. The World Health Organization published that the diabetes prevalence in adults aged above 18 years has increased from 4.7% in 1980 to 8.5% in 2014, and in 2012, mortality was seen in about 1.5 million people.^[1,2] Between Iranians, the diabetes prevalence was 7.7% in 2005^[3] and its incidence is estimated to rise from 2 million adults in 2005 to 5.1 million in 2025.^[4]

Diabetes may lead to several complications such as nephropathy, retinopathy, and neuropathy.^[5] It can damage the blood vessels and heart, causing cardiovascular disease (CVD). The CVD risk is 2–4-fold higher in diabetic patients than the nondiabetic patients.^[6,7] CVD is a leading cause of mortality worldwide, and approximately 50% of the diabetic patients die from CVD.^[8]

Risk of developing type 2 diabetes (T2D) and CVD has been predicted by inflammatory markers such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and high sensitive C-reactive protein (hs-CRP).^[9] The activation of immune system and low grade of inflammation occurs during T2D.^[10,11]

Adiponectin is known as an antiatherogenic protein that could reduce the accumulation of lipids in macrophages also prevents macrophage-to-foam cell formation.^[12,13] This compound can modulate insulin sensitivity and stimulates insulin secretion.^[14,15] Alternatively, it decreases the insulin resistance and diabetic occurrence.^[16,17]

Certain approaches to prevent T2D and its complications include healthy lifestyle, healthy eating habits, regular physical activity, and nonsmoking.^[2] Healthy eating habits indicate selecting nutritious foods that prevent chronic diseases or delay their progress.

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Cocoa is an ingredient that is used in many foods or snacks, for example, chocolate. Dark chocolate is one of the cocoa products containing several health-enhancing compounds. Hence, cocoa and its products could be considered as functional foods. The key ingredients of cocoa include powerful antioxidants such as catechin, epicatechin, and procyanidins.^[18] Some studies that were conducted on patients with CVD reported the healthy effect of dark chocolate on lipid profile, vascular endothelial function, blood pressure, and inflammation.^[19-21] To the best of our knowledge, no research is available revealing the effect of dark chocolate on adiponectin levels in diabetic patients; moreover, limited studies have reported exact effects of dark chocolate on the inflammatory factors in diabetic patients. Furthermore, the effect of dark chocolate, along with therapeutic lifestyle changes (TLCs) guidelines, needs to be studied. Intake of some delicious snacks may help the diabetic patients deal with their harsh diabetic diets. Hence, this study was designed to evaluate the effect of dark chocolate consumption on adiponectin and some inflammatory factor levels in diabetic patients who received TLC guidelines. Biochemical parameters such as lipid profiles, fasting blood sugar, and insulin levels were also measured.

Methods

Ethics

This study was conducted after being approved by the Ethics Committee of Ahvaz Jundishapur of Medical Sciences (ID: ajums. REC.1392.157). Participants were informed about the research status, and only those who approved and verified the consent form, were included. The trial has been registered in the Iranian Registry of Clinical Trials at <http://www.irct.ir> with the following identification: RCT2014010716123N1.

Patients' enrollment

This study was conducted on 50 T2D patients. All patients were recruited from Golestan Hospital of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Sample size was calculated based on hs-CRP levels in a related previous study^[22] and following formula:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta/2})^2 2(S^2)}{(d)^2}$$

with 95% confidence, and a power of 80%. The values of “d” and “S” were equal to 0.25 and 0.3, respectively. Sample size was determined 22 subjects in each intervention and control group. Ten percent prediction dropouts were considered and totally 50 subjects were enrolled in the trial.

Randomization

This study was a parallel randomized clinical trial. Researchers were blinded to the patient's identity. A third

party recorded the patients belonging to the dark chocolate or control groups, and the researchers were uninformed about these data till the end of the study. The inclusion criteria were as follows: 1–5-year history of diabetes (based on the American Diabetes Association criteria),^[23] age range of 30–60 years, body mass index ranging between 18.5 and 35, glibenclamide or metformin as medication with stable dosage for at least 3 months before the commencement of the study, and avoiding consumption of antioxidant supplements. The exclusion criteria were as follows: sensitivity to dark chocolate, treatment with insulin, pregnancy or lactation, not signing the informed consent form, smoking or alcohol consumption, drinking green tea, individuals with a history of hepatic, renal, lung, and CVDs and those with kidney stones. The flowchart of the study is presented in Figure 1.

Intervention

Before the intervention, all participants were informed to use the TLC guidelines then they were randomly divided into two groups by simple random allocation. One group received 30 g (84%) dark chocolate (Parmida, Kian Chocolate Kimia Company, Tehran, Iran), which comprised five pieces of chocolate, each of them was 6 g and packed separately, enhancing multiple consumption in a day. These chocolates were preserved in a plastic zip-lock packet for 1 day consumption and seven such packets were provided to each subject for 1 week consumption. It was asked subjects to consume chocolates between two meals. The compositions of dark chocolate were as follows: total fat 42.1%, protein 11.9%, and carbohydrate 35.2%. The period of intervention was 8 weeks and compliance was monitored weekly by counting the remaining chocolates. Subjects with weekly compliance less than 80% were excluded from the study.

Dietary and physical assessment

Dietary intakes were determined using three days food recall (two work days and one holiday) before and after the intervention. Nutritionist 4 (First Data Bank, San Bruno, CA, USA) was used to calculate the nutrient intakes. Subjects completed a standard brief form of International Physical Activity Questionnaire at the beginning and end of study. Physical activity levels were expressed as MET-hour/day.

Biochemical measurement

At baseline and after intervention, fasting blood samples were collected and serum was isolated and preserved at -70°C until the last sample was collected. Biochemical parameters such as fasting blood sugar (FBS), uric acid, lipid profiles (total cholesterol, triglycerides, low-density lipoprotein-cholesterol [LDL-C], high-density lipoprotein-cholesterol [HDL-C]) and hemoglobin A1C (Hb A1C) were measured using an auto-analyzer (Hitachi 911, Japan). Three inflammatory markers such as hs-CRP, IL-6, and TNF- α (AviBion, Vantaa, Finland) serum

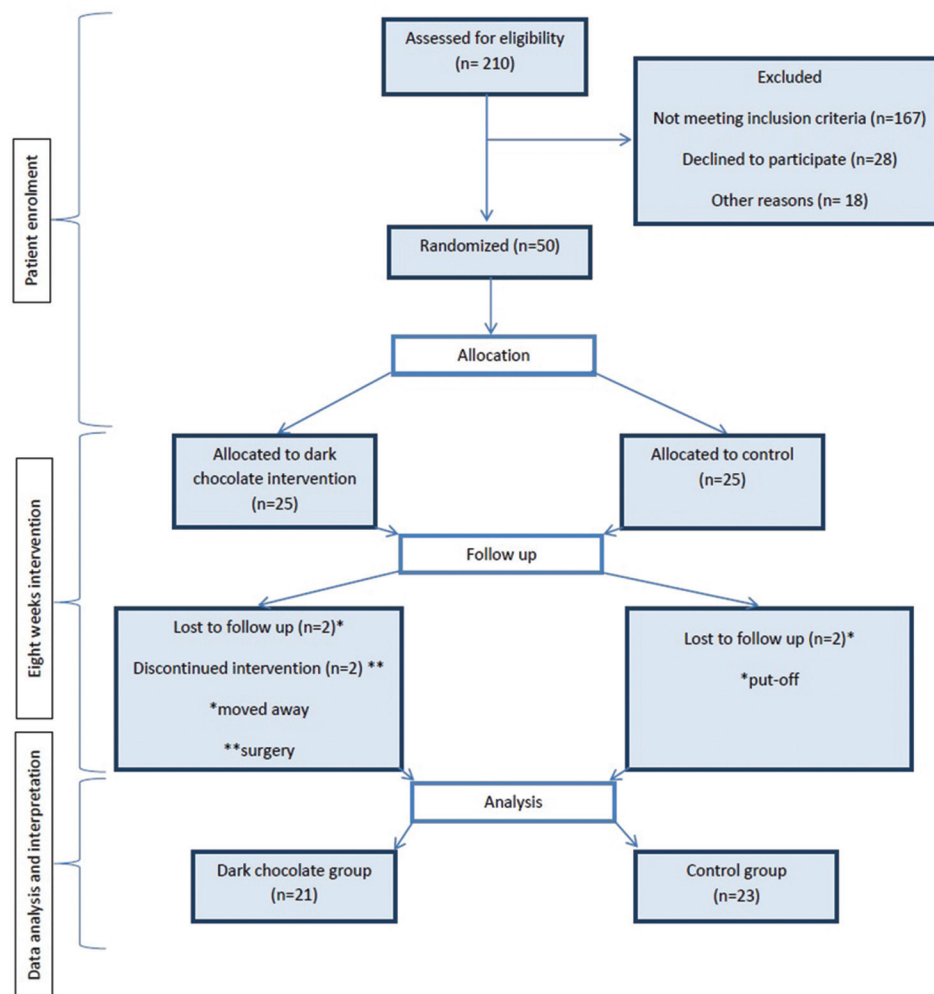


Figure 1: The summarized protocol of the study

insulin and adiponectin (Boster Biological Technology, Pleasanton CA, USA) were measured by the enzyme-linked immunosorbent assay method.

Data analysis

All data were analyzed by SPSS version 17.0 (SPSS Inc. SPSS Statistics for Windows, Chicago, 2008). Quantitative variables were described as mean \pm standard deviation (SD). Independent sample *t*-test was used to compare the mean of variables between two groups and paired sample *t*-test was used to compare the mean of variables before and after intervention in each group. Kolmogorov–Smirnov test was used to analysis normality of data distribution. Nonparametric analysis was used in case of abnormal distribution. To eliminate the effect of covariates, analysis of covariance (ANCOVA) was used.

Results

At the end of study, data of 21 patients in the dark chocolate group and 23 in the control group were analyzed. The mean age of patients was 52.3 ± 6.4 years and no significant

difference was observed between the two groups. About 67.3% subjects were women and there was no difference between two groups for numbers of women and men. In addition, there was no differences between two groups for anthropometric measurements, and blood pressure before the intervention period of the study.^[24] The mean \pm SD of MET hour/day before and after the study was 32.73 ± 1.8 , 32.8 ± 1.7 , 33.25 ± 1.7 , and 33.15 ± 1.6 in dark chocolate and control group, respectively, there was no significant difference for activity level between two groups.

Dietary food analysis was presented in Table 1. Significant difference was observed for the carbohydrate and zinc intake after the intervention period and for Vitamin A intake, before the intervention, between the two groups. The effect of these confounders was removed via ANCOVA.

Biochemical parameters data are presented in Table 2. Changes in FBS, Hb A1C, cholesterol, and triglyceride levels, before and after intervention were significant among the dark chocolate group; moreover, these changes (except cholesterol) were significant between the two groups. These

Table 1: The comparison of energy and nutrient intake between dark chocolate and control groups

Nutrients intake	Mean±SD		P*
	Dark chocolate	Control	
Energy (kcal/day)			
Before	1970.2±317.5	2022.00±316.2	0.591
After	1933.00±319.5	2020.35±296.7	0.352
P**	0.086	0.925	
Carbohydrate (g/day)			
Before	290.5±40.5	300.0±40.8	0.442
After	271.3±44.2	297.3±35.1	0.035
P**	<0.001	0.313	
Fat (g/day)			
Before	51.0±13.6	54.8±12.3	0.329
After	57.3±12.7	52.1±12.5	0.184
P**	<0.001	0.19	
Protein (g/day)			
Before	93.8±13.8	95.2±15.0	0.750
After	87.2±16.9	96.4±15.7	0.069
P**	0.018	0.351	
Fiber (g/day)			
Before	20.9±1.7	21.0±2.4	0.816
After	20.7±1.9	20.9±2.6	0.813
P**	0.272	0.583	
Vitamin A (RE/day)			
Before	2455.5±565.6	2895.7±562.4	0.013
After	3100.6±496.7	2895.3±596.3	0.224
P**	<0.001	0.997	
Vitamin E (mg/day)			
Before	9.3±1.8	8.9±2.1	0.455
After	8.2±1.8	8.2±1.8	0.319
P**	0.009	0.674	
Potassium (g/day)			
Before	4536.1±611.2	4559.7±698.0	0.906
After	5702.8±638.1	4669.1±814.8	0.445
P**	0.431	0.280	
Magnesium (mg/day)			
Before	393.9±82.9	425.5±89.9	0.234
After	425.9±98.2	431.6±91.0	0.845
P**	0.01	0.363	
Zinc (mg/day)			
Before	12.3±4.13	13.7±3.9	0.233
After	10.4±2.8	13.2±3.6	0.006
P**	0.850	0.262	

*Independent sample *t*-test, **Paired *t*-test. SD=Standard deviation

markers were decreased in dark chocolate group [Table 2]. LDL-C levels were decreased and HDL-C was increased among the dark chocolate group, and comparing these changes between the two groups revealed significant difference. No significant difference was observed for insulin level changes between the two groups.

Inflammatory factors such as TNF- α and IL-6 indicated significant decreases in the dark chocolate group, and changes in these inflammatory marker levels revealed significant differences between the dark chocolate and

control groups. Although the changes in the levels of hs-CRP were not significant in the dark chocolate group, the level of this parameter was increased in the control group after the intervention period and a significant difference was observed between the two groups. Adiponectin levels revealed no significant difference between the two dark chocolate and control groups [Table 2].

Discussion

This study was conducted on patients with T2D mellitus and reported that subjects who received dark chocolate along with TLC guidelines had lower levels of inflammatory markers such as hs-CRP, TNF- α , and IL-6, compared with the subjects who were devoid of dark chocolate and followed only the TLC guidelines. It seems the decreased level of TNF- α and IL-6 was remarkable. In a previous study, healthy controls consumed cocoa supplement, however, no effect was observed on IL-6, TNF- α , hs-CRP, and P-selectin. It seems normal inflammatory markers among healthy controls, have led to findings of this study.^[25] Other study on diabetic patients that received cocoa powder revealed lower level of inflammatory markers,^[22] moreover, a previous study indicated that dark chocolate led to lower levels of hs-CRP in diabetic patients.^[26] Dark chocolate contains more cocoa and more polyphenols than other chocolates, so has a potential antioxidant effect on the inflammatory factors. This property could lead to lower levels of plasma leukotrienes and leukotriene-prostacyclin ratio. This process could explain the effect of cocoa polyphenols on enzymes, which involve the degradation or synthesis of eicosanoids.^[27] Cocoa polyphenols also could inhibit nuclear factor kappa B (NF- κ B) activation. NF- κ B is a transcription factor that regulates genes responsible for the immune system.^[28]

In this study, dark chocolate led to lower levels of FBS, HbA1C, LDL-C, and triglyceride and higher level of HDL-C, when compared with control. However, these changes had not high rates but were considerable for significant changes compared to control group. Another study that was conducted on diabetic patients which used cocoa powder confirmed lower levels of total cholesterol, LDL-C, and triglyceride and higher level of HDL-C.^[22] In another study where diabetic subjects consumed 25 g dark chocolate daily, only triglyceride was decreased and no differences were found between the dark chocolate and control groups for cholesterol, LDL-C, and HDL-C.^[26] This study used milk chocolates for control group that contain cocoa butter (so have some properties of cocoa). Furthermore, the amount of dark chocolate in intervention group were lower than our study (25 g/day vs. 30 g/day), maybe for these reasons they found no effect on these lipid profiles. The effect of cocoa and dark chocolate on the lipid profiles in subjects with CVDs was revealed^[29,30] and this effect was similar to that observed in the diabetic patients.

Dark chocolate consumption had no effect on the adiponectin levels. To the best of our knowledge, no

Table 2: Effect of dark chocolate on serum adiponectin and biochemical and inflammatory markers of participants in two dark chocolate and control groups

	Dark chocolate*				Control**				P [¶]
	Before	After	Change	P [§]	Before	After	Change	P [§]	
		Mean±SD				Mean±SD			
FBS (mg/dL)	139.38±35.2	123.57±29.5	-15.0±11.6	<0.001	152.13±47.4	167.2±67.9	15.3±38.19	0.07	<0.001
HbA1C (%)	7.1±0.93	6.8±0.83	-0.2±0.33	0.009	7.6±1.18	7.8±1.38	0.19±0.47	0.06	0.001
Cholesterol (mg/dL)	186.14±38.1	169.19±28.4	-16.95±25	0.01	180.56±33.2	178.56±36.3	-2±30.6	0.62	0.08
LDL-C (mg/dL)	112.57±32.3	96.9±25.6	-15.6±21	0.004	100.4±31.2	106.5±28.7	6.5±17.4	0.01	0.001
HDL-C (mg/dL)	40.5±7.6	42.28±7.5	1.7±3.8	0.05	41.2±8.9	37.6±7.8	-3.6±3.5	<0.001	<0.001
Triglyceride (mg/dL)	174.3±58.6	140.7±7.1	-33.6±37	0.001	179.5±77.3	179.8±78.4	0.3±34.4	0.96	0.015
Uric acid (mg/dL)	4.9±1.1	4.7±0.78	-0.2±0.59	0.14	4.5±1.07	4.7±1.06	0.18±0.68	0.21	0.057
Insulin (Iu/μml)	7.2±8.08	7.5±10.9	0.27±12.5	0.92	7.3±7.8	9.8±11.3	2.5±14.05	0.4	0.58
hs-CRP (mg/L)	4.2±1.27	3.9±2.1	-0.35±2.3	0.499	3.2±1.34	4.21±1.6	1±0.86	<0.001	<0.001
TNF-α (pg/ml)	34.33±21.8	23.42±12.1	-10.9±15.7	0.005	24.6±10.2	25.65±9.51	1.04±12.17	0.68	0.04
IL-6 (pg/ml)	6.61±3.78	4.04±3.23	-2.57±2.9	0.01	4.08±2.17	10.3±30.06	6.30±29.94	0.34	0.003
Adiponectin (μg/ml)	18.66±50.2	23.04±17.07	4.38±14.9	0.19	30.91±16.91	32.2±18	1.30±17.5	0.72	0.6

*Dark chocolate group received 30 g 84% dark chocolate and therapeutic life-change guideline, **Control group received only therapeutic life-change guideline, §Comparison with-in group by Wilcoxon test, ¶Comparison between-groups by Mann-Whitney test. FBS=Fasting blood sugar, HbA1C=Hemoglobin A1C, LDL-C=Low density lipoprotein-cholesterol, HDL-C=High density lipoprotein-cholesterol, hs-CRP=High sensitive-C reactive protein, TNF-α=Tumor necrosis factor-α, IL-6=Interleukin-6, SD=Standard deviation

studies explored the effect of cocoa or dark chocolate on the adiponectin levels in diabetic patients. The only animal study that was conducted on obese male mice revealed that cocoa powder supplementation could increase the levels of adiponectin.^[31] Adiponectin is an adipocytokine and its level is negatively correlated with the body fat percentage.^[32] Although the body fat percentage decreased in the dark chocolate group, this decline was also observed in the control group, due to TLC guidelines.^[24] This may have led to no differences for the adiponectin levels between the dark chocolate and control groups.

In this study, control group did not give placebo, so it was the limitation of the study, however, the researchers did not know which subjects belong to each of dark chocolate and control groups until the end of the study. The TLCs guidelines were used in the dark chocolate and control groups for homogeneity of groups and ethical aims; this was considered as an advantage of the study.

Conclusions

Dark chocolate has some beneficial effects on the inflammatory markers and lipid profiles that act as the potential factors for CVD incidence; therefore, further studies are needed to elucidate the different dosage and the effect of long-term consumption of dark chocolate in diabetic patients to find an administrative guideline of this functional food for T2D and type 1 diabetes.

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Conflicts of interest

There are no conflicts of interest.

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