

## The Effect of Dark Chocolate Consumption on Lipid Profile in Patients with Metabolic Syndrome: A Randomized Clinical Trial

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### Abstract

**Objective:** Patients with metabolic syndrome are prone to cardiovascular disease and diabetes. Regarding the importance of nutritional factors in management of metabolic syndrome, this study was designed to evaluate the effects of dark chocolate consumption on serum lipid profile in patients with metabolic syndrome.

**Materials and Methods:** In this randomized clinical trial, 114 patients with metabolic syndrome, aged 30 to 60 years, without heart, renal and hepatic diseases were recruited. The eligible patients were randomly allocated to receive either dark chocolate (76% purity), 20 or 40 grams daily for two months. Total cholesterol, high-density lipoprotein (HDL), triglycerides (TG) and fasting blood glucose (FBG) were measured with enzymatic methods before and after intervention. A low-density lipoprotein (LDL) level was calculated by the Friedewald formula. Data were analyzed using SPSS version 16.0 and ANOVA test  $P < 0.05$  was considered significant. Dietary intake was measured with NUT4 software at the beginning and the end of the study.

**Results:** There were no significant changes in dietary intakes of patients during the intervention in three groups. No significant differences were seen in mean concentration of lipid profile before and after intervention in groups ( $P > 0.05$ ).

**Conclusion:** Consumption of 20-40g/day dark chocolate with 76% purity for 2 months doesn't change the lipid profile of patients with metabolic syndrome.

**Keywords:** Metabolic syndrome, Dark chocolate, Lipid profile.

### Introduction

Metabolic syndrome is a cluster of metabolic and medical disorders that increases the risk of type 2 diabetes mellitus and cardiovascular disease (CVD) (1-3). nowadays different definitions are used to diagnosis of metabolic syndrome (2). The

main components of Metabolic Syndrome include obesity, glucose or insulin disturbance, dyslipidemia and hypertension (4).

According to all studies, metabolic syndrome prevalence has raised over the past few years.

Thus it can be considered as tsunami of chronic disease (4-6).

Recently, many studies have been conducted on the relationship between diet and metabolic syndrome. They believed high intake of fruits and vegetables rich in flavonoids could clarify the profitable effects of these foods on metabolic syndrome (7).

Human and animal studies showed that flavonoid-rich foods or drinks decrease triglyceride (TG), Total cholesterol (TC), Low-density lipoprotein cholesterol (LDL) and increase high-density lipoprotein cholesterol (HDL) (7,8).

Cocoa products contain more flavonoids in each serving compared with other foods (9). However, some studies showed positive effects of cocoa on blood lipid levels (reduced LDL and TG and increased HDL), some others have shown no effectiveness (10-12). Other elements of cocoa also could be responsible for these beneficial effects; so more researches are needed to achieve a unique result. Besides, a number of studies on association between dark chocolate consumption and cardio metabolic disorders have been conducted in developed countries (13), but further studies in different populations and geographic locations are needed. This study was designed to evaluate the effects of dark chocolate consumption on lipid profile in patients with metabolic syndrome.

## Materials and Methods

**Participants and study design:** This randomized clinical trial was conducted in Yazd Diabetic Research Center of Shahid Sadoughi University of Medical Sciences, Yazd, Iran in 2013 to 2014. Inclusion criteria included 1) having metabolic syndrome with definition of NCEP, national cholesterol education program (*Adult treatment* panel III or ATP III), 2) aged 30 to 60 years, 3) No pregnancy or lactation, 4) No smoking or using any tobacco production, 5) using 80% of given oil. Exclusion criteria were 1) any Change in physical activity, diet or

medications, 2) taking antioxidant supplements 3) history of cardiovascular, hepatic and renal disease. One hundred and fourteen patients with metabolic syndrome who met inclusion and exclusion criteria were recruited. Participants were randomly assigned to three groups using random numbers generated by computer. Eligible patients were randomly allocated to receive either dark chocolate (76% purity, Aidin Company, Tabriz, Iran) 20 and 40 grams daily for two months in a parallel design and no chocolate intake group.

They were asked to keep their usual diet, medication and physical activity throughout the study. The consumption of dark chocolate was monitored every 15-day via phone call.

**Ethical considerations:** This study was approved by the Ethical Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Also, the study was registered at Iranian website of clinical trials with code IRCT2013022812122N2 ([www.irct.ir](http://www.irct.ir)). Also, informed written consent was obtained from all participants.

**Measurements:** A dietitian met with each participant at first of the study to explain purpose and method of the study. Twenty four hour dietary recall was recorded in the beginning and the end of study. Blood samples was obtained after 12-hour of fasting period. TC, HDL-C, and TG levels determined by enzymatic methods (PARS AZMOON, Tehran, Iran) and LDL cholesterol levels was calculated by the Friedewald formula ( $LDL=TC-HDL-TG/5.0$  (mg/dl)).

Measurement of height was done using a tape measure in standing position without wearing shoes while shoulders were relaxed. Weight was measured to the nearest 100 g using a digital scale in light clothing (Seca, Hamburg, Germany). BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the smallest circumference, and hip circumference was measured at the maximum level using an un-stretched tape measure. To avoid subjective

error, all measurements were made by the same person.

The amount of macronutrients such as vitamins, fiber, electrolytes, and polyphenols of dark chocolate are shown in Table 1. Polyphenols content of dark chocolates was estimated by spectrophotometry procedure in biochemistry laboratory of Shahid Sadoughi University of Medical Sciences.

### Statistical analysis

Data were analyzed using SPSS version 16.0. To ensure the normal distribution of data, Kolmogorov–Smirnov test was performed. Data were presented as means  $\pm$  SD.

Comparison of variable was analyzed by paired t- test and ANOVA test. For multiple comparisons, Post hoc comparisons were performed by LSD's honestly significant difference test.

### Results

Nightly three of 114 participants completed the study and 21 were excluded. Mean ( $\pm$ SD) age, weight, BMI, and waist circumference of participants were  $51.38\pm 6.95$  years,  $77.34\pm 12.86$  kg,  $28.65\pm 4.4$  kg/m<sup>2</sup>, and  $103.69\pm 15.21$ cm, respectively. Baseline characteristics of the participants based on three groups are shown in Table 2.

Significant difference was only observed in baseline weight and BMI, but No significant differences were seen in other baseline variables.

Dietary intakes of energy and macronutrients were estimated at the beginning and end of the study; no significant changes were observed during the intervention.

There was no significant difference in changes of anthropometric between groups (Table 3).

**Table 1. The amount of Energy, Macronutrients and Minerals of dark chocolate (Aidin Company, Tabriz, Iran)**

Items	Amount in 100g
Protein	8.5 g
Carbohydrate	46.7 g
Fat	31.8 g
Fiber	1.06 g
Vitamin B1	0.007mg
Vitamin A	1.2 mg
Vitamin E	5.6 mg
Sodium	44.8 mg
Calcium (mg)	345.9 mg
Phosphorus	0.05 mg
Iron	9.04 mg
Polyphenols	12.3 mg

Mean concentration of total cholesterol, TG, LDL and HDL and the ratio of LDL to HDL are shown in Table 4. According to these findings, There were no significant differences in these measurements before and after the intervention in groups ( $P > 0.05$ ). Furthermore no significant differences were seen in mean changes of TG, total cholesterol, LDL and HDL concentrations and the ratio of LDL to HDL between groups ( $P > 0.05$ ).

### Discussion

Present study demonstrated that 2 months consumption of 20 and 40 g dark chocolate didn't have significant effect on lipid and lipoprotein profiles. Kris-Etherton et al. recently conducted a study on young men who had consumed 10 oz chocolate with their daily food. They received 37% of energy from fat. Eighty percent of this amount was provided by chocolate fat. Although chocolate diet contains amounts of saturated fatty acids, but no cholesterol change was reported compared with their usual diet (14). Another study showed that daily intake of 1.6 oz milk chocolate instead of carbohydrate rich snacks had no negative effect on LDL levels (15).

**Table 2. Baseline characteristics of patients in three groups**

Variables	Control	20g dark chocolate	40g dark chocolate	P-value
Age (year)	52.78 $\pm$ 6.8*	49.62 $\pm$ 6.8	51.77 $\pm$ 7.04	N.S**
Weight (kg)	75.32 $\pm$ 12.75	74.68 $\pm$ 13.11	82.13 $\pm$ 11.72	0.04
High (cm)	164.49 $\pm$ 8.03	164.87 $\pm$ 11.53	164 $\pm$ 9.29	0.93
BMI (kg/m <sup>2</sup> )	28.01 $\pm$ 4.76	27.48 $\pm$ 4.14	30.53 $\pm$ 3.76	0.01
Waist circumferences (cm)	102.18 $\pm$ 22.95	100.81 $\pm$ 9.80	108.44 $\pm$ 7.51	0.11
Hip circumferences (cm)	110.95 $\pm$ 18.71	105.87 $\pm$ 9.56	111.72 $\pm$ 10.49	0.18

\* Mean  $\pm$  Standard Deviation, \*\*: Not Significant

**Table 3. Changes of anthropometric measures between groups through the intervention**

Changes	Control	20g dark chocolate	40g dark chocolate	P-value
Weight (year)	0.4821± 2.07	0.22±3.57	0.4±2.25	0.93
BMI (kg/m <sup>2</sup> )	0.14±0.78	0.1±1.19	0.13±0.84	0.99
Waist circumference (cm)	2.5±20.8	2.7±3.96	1.03±4.44	0.22
Hip circumference (cm)	-1.76±13.53	1.96±5.04	1.39±5.96	0.85

**Table 4. Biochemical parameters Changes in three study groups**

Variables		40g dark chocolate	20g dark chocolate	Control	P-value**
TC (mg/dL)	Before	190.45±37.03*	191.19±44.46	182.57±41.58	0.68
	After	187.31±43.25	183.52±39.02	182±35.55	0.87
	Change	-1.69±29.52	-6.96±28.55	-6±44.79	0.84
	P-value***	0.72	0.18	0.72	
TG (mg/dL)	Before	235.69±113.5	213.37±128.34	194.64±88.77	0.36
	After	230.9±127.44	206.16±133.07	165.59±65.22	0.08
	Change	-1.57±77.88	-6.35±93.09	-34.96±93.99	0.3
	P-value	0.91	0.71	0.053	
LDL (mg/dL)	Before	111.48±36.8	112.6±0.68	102.45±25.25	0.47
	After	104.2±35.88	101.38±36.8	107.45±26.53	0.77
	Change	-4.2±28.75	-8.07±26.67	2.24±27.86	0.37
	P-value	0.66	0.12	0.6	
HDL (mg/dL)	Before	49.39±11.02	49.16±10.3	49.69±13.63	0.98
	After	49.21±12.62	49±12.63	49.24±11.1	0.99
	Change	-0.86±8.41	-0.03±7.61	-1.21±11.31	0.87
	P-value	0.57	0.98	0.59	
LDL/HDL (mg/dL)	Before	2.27±0.83	2.27±0.85	2.16±0.65	0.81
	After	2.16±0.73	2.88±4.11	2.33±0.72	0.5
	Change	0.14±0.47	0.66±4.6	-0.02±0.62	0.55
	P-value	0.87	0.39	0.16	

\*:Mean ± SD \*\*: One way ANOVA \*\*\*: Paired t-test

Gradually Muniyappa et al.(16), Balzer et al. (17) and Mathur et al. (8) conduct studies on healthy people, hypertensive and diabetic patients. Dark chocolate consumption did not cause significant changes on lipid profile. The results of these studies are consistent with our study. Grassi et al. in two different studies on patients with primary hypertension (10) and impaired glucose tolerance (8) showed more reduction in LDL and total cholesterol in the group received dark chocolate than those received white chocolate. Fraga et al. also obtained the same results in a study with healthy subjects. Wan et al. (12), Vinson et al. (8) and Baba et al. (18) observed increased HDL levels after consumption of dark chocolate and cocoa in their studies which is not in line with our results. Since polyphenolic content of dark chocolate used in our study was low compared to other studies, we can charge no significant change in total cholesterol to its fatty acids content. So

differences in results may also because of the amount of consumed chocolate and its flavonoid content (13). Although most of these studies have been conducted in developed countries, but our study is the first study on the effect of dark chocolate consumption on lipid profile in patient with metabolic syndrome in the Middle East. So this difference in results may also be because of different populations and different geographic locations and so further investigations must be done in future. According to results of the present study, consumption of 20-40g/day dark chocolate with 76% purity cannot improve the lipid profile levels of patients with metabolic syndrome.

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## References

1. Grundy SM. Does a diagnosis of metabolic syndrome have value in clinical practice? *AJCN*. 2006;83(6):1248-51.
2. de Rooij SR, Painter RC, Holleman F, Bossuyt PM, Roseboom TJ. The metabolic syndrome in adults prenatally exposed to the Dutch famine. *AJCN*. 2007;86(4):1219-24.
3. Day C. Metabolic syndrome, or What you will: definitions and epidemiology. *Diabetes and Vascular Disease Research*. 2007;4(1):32-8.
4. Steiner M. Genetic determinants of the metabolic syndrome: uniwiien; 2008.
5. Ford ES. The metabolic syndrome and mortality from cardiovascular disease and all-causes: findings from the National Health and Nutrition Examination Survey II Mortality Study. *Atherosclerosis*. 2004;173(2):307-12.
6. Bremer AA, Mietus-Snyder M, Lustig RH. Toward a unifying hypothesis of metabolic syndrome. *Pediatrics*. 2012;129(3):557-70.
7. Galleano M, Calabro V, Prince PD, Litterio MC, Piotrkowski B, Vazquez-Prieto MA, et al. Flavonoids and metabolic syndrome. *Ann N Y Acad Sci*. 2012;1259(1):87-94.
8. Faghihzadeh F, Esmailzadeh A. Impact of Cacao Consumption on Cardiovascular Risk Factors: Review of Current Evidence. *I.U.M.S*. November 2010. 111(28): 591-605.
9. Hutfless SM, Ding X, Girotra S, Ding EL. Chocolate and prevention of cardiovascular disease: a systematic review. 2006.
10. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, et al. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension*. 2005;46(2):398-405.
11. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, et al. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr*. 2008;138(9):1671-6.
12. Fraga CG, Actis-Goretta L, Ottaviani JJ, Carrasquedo F, Lotito SB, Lazarus S, et al. Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *J Immunol Res*. 2005;12(1):11-7.
13. Buitrago-Lopez A, Sanderson J, Johnson L, Warnakula S, Wood A, Di Angelantonio E, et al. Chocolate consumption and cardiometabolic disorders: systematic review and meta-analysis. *BMJ*. 2011;343.
14. Kris-Etherton P, Derr J, Mitchell DC, Mustad VA, Russell ME, McDonnell ET, et al. The role of fatty acid saturation on plasma lipids, lipoproteins, and apolipoproteins: I. Effects of whole food diets high in cocoa butter, olive oil, soybean oil, dairy butter, and milk chocolate on the plasma lipids of young men. *Metabolism*. 1993;42(1):121-9.
15. Kris-Etherton PM, Derr JA, Mustad VA, Seligson F, Pearson TA. Effects of a milk chocolate bar per day substituted for a high-carbohydrate snack in young men on an NCEP/AHA Step 1 Diet. *AJCN*. 1994;60(6):1037-42.
16. Muniyappa R, Hall G, Kolodziej TL, Karne RJ, Crandon SK, Quon MJ. Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension. *AJCN*. 2008;88(6):1685-96.
17. Balzer J, Rassaf T, Heiss C, Kleinbongard P, Lauer T, Merx M, et al. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients: a double-masked, randomized, controlled trial. *Am. J. Cardiol*. 2008;51(22):2141-9.
18. Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, et al. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. *AJCN*. 2007;85(3):709-17.