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The effect of polyphenol-rich dark chocolate on serum lipids in healthy subjects

O efeito do chocolate amargo rico em polifenóis nos lipídios séricos em indivíduos saudáveis

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ABSTRACT

Objective
The present study aims to investigate the effects of consuming dark chocolate on the serum lipid profile of healthy adults.

Methods
The study was conducted over 4 weeks with a total of 37 subjects, including control (n=20) and intervention (n=17) groups. While the intervention group consumed 36g/day of dark chocolate (400 mg flavanol/day), the control group received no intervention. At the beginning and end of the study, some anthropometric measurements, blood pressure and biochemical parameters (low-density lipoprotein, high-density lipoprotein and total cholesterol, triglycerides, haemoglobin A1c and C-reactive protein, fasting blood glucose) were measured and 3-day food and physical activity records were taken every 15 days during the study period.

Results
After four weeks, body weight and body mass index decreased in the intervention group (p<0.05). Low-density lipoprotein and total cholesterol also decreased in the intervention group (-8.16mg/dl and -10mg/dl, respectively; p<0.05), and no change was observed in high-density lipoprotein cholesterol (p>0.05). While an increase in fasting blood glucose was observed (p<0.05), there was no difference in hemoglobin A1c and C-reactive protein levels (p>0.05). Similarly, there was no change in systolic or diastolic blood pressure in either group (p>0.05).

Conclusion
In conclusion, the consumption of 36g/day (400mg/day flavanol) for 4 weeks in healthy individuals can reduce low-density lipoprotein and total cholesterol without causing weight gain. Thus, cocoa consumption as a dietary intervention has a possible role in reducing the risk of cardiovascular disease as an age-related lifestyle disease. Long-term studies with larger samples are needed.

Keywords: Blood Glucose. Blood Pressure. Chocolate. Cholesterol. Cocoa.

RESUMO

Objetivo

O presente estudo tem como objetivo examinar os efeitos do consumo de chocolate amargo no perfil lipídico sérico de indivíduos adultos saudáveis.

Métodos

O estudo foi realizado com 37 indivíduos no total, incluindo os grupos controle (n=20) e intervenção (n=17), por 4 semanas. Enquanto o grupo de intervenção consumiu 36g/dia de chocolate amargo (400mg de flavanol/dia), o grupo controle não recebeu nenhuma intervenção. No início e no final do estudo, algumas medidas antropométricas, pressão arterial e parâmetros bioquímicos do sangue (lipoproteína de baixa densidade, lipoproteína de alta densidade e colesterol total, triglicerídeos, hemoglobina A1c (HbA1c) e C- proteína reativa, glicemia de jejum) foram medidos, e durante o período de estudo o consumo alimentar de 3 dias e os registros de atividade física foram feitos a cada 15 dias.

Resultados

Ao final de quatro semanas, os níveis de peso corporal e índice de massa corporal do grupo de intervenção diminuíram (p<0,05). Além disso, LDL e colesterol total diminuíram no grupo de intervenção (respectivamente; -8,16mg/dl, -10mg/dl; p<0,05) e nenhuma alteração determinada no colesterol HDL (p>0,05). Enquanto um aumento é observado na glicemia evidente (p<0,05), não há diferença nos níveis de HbA1c e PCR (p>0,05). Da mesma forma, nenhuma alteração foi encontrada na pressão arterial sistólica e diastólica em ambos os grupos (p>005).

Conclusão

Concluiu-se que, o consumo de 36g/dia (400mg/dia de flavanol) por 4 semanas em indivíduos saudáveis pode reduzir o LDL e o colesterol total sem causar ganho de peso. Assim, o consumo de cacau como uma intervenção dietética tem um possível papel para diminuir o risco de doença cardiovascular como uma doença relacionada ao estilo de vida relacionada à idade. Estudos de longo prazo com amostras maiores são necessários.

Palavras-chave: *Glicemia. Pressão Arterial. Chocolate. Colesterol. Cacau.*

INTRODUCTION

Cocoa products are widely consumed around the world for their taste, richness in polyphenols and antioxidant activity. The antioxidant activity of cocoa is generally associated with flavanols, a subclass of polyphenols [1]. The polyphenol content of chocolate, one of the most commonly consumed cocoa products, varies. Chocolate with a high cocoa content is considered to be rich in polyphenolic compounds. Accordingly, the flavanol content of dark chocolate with a higher cocoa content (≥60% cocoa) is higher than the flavanol content of milk and white chocolate [2,3]. In addition to its flavanol content, cocoa also contains health-promoting components such as theobromine, magnesium, iron, potassium, copper and insoluble dietary fiber [1,4]. Cocoa has been reported to have beneficial effects on endothelial dysfunction, insulin resistance, high blood lipids, elevated blood pressure, and increased inflammation due to its rich antioxidant and biologically active components [5]. In a study of 100 healthy adults, consumption of a cocoa drink containing 900 mg of flavanols per day for 1 month had a beneficial effect on blood pressure and blood lipid profile and reduced the 10-year risk of Cardiovascular Disease (CVD) [6]. Another similar study reported that a cocoa flavonoid-based dietary intervention in healthy male subjects lowered blood pressure and improved atherosclerosis in elderly individuals and that dietary cocoa flavanols have important effects in protecting cardiovascular health [7]. In line with this finding, the potential role of cocoa products high in flavanols in reducing and treating cardiovascular disease risk remains relevant [8]. The European Food Safety Authority recommends a daily intake of 200mg of flavanols for the general population due to the beneficial effects of cocoa flavanols on blood pressure and endothelium-dependent function. It is stated that this amount can be achieved with an additional 2.5g/day of high-flavanol cocoa powder or 10g/day of high-flavanol dark chocolate, in addition to a

balanced diet [9]. The potential beneficial effects of dark chocolate on vascular health are notable for their simplicity, affordability and public acceptance. Studies in healthy individuals are important to determine the effect of dark chocolate on the risk of cardiovascular disease, which is an age-related lifestyle disease. This study aims to evaluate the effect of dark chocolate consumption on serum lipid parameters in healthy individuals.

METHODS

Participants

The study was conducted on healthy volunteers aged 19-50 working at the Samsun Provincial Gendarmerie Command. As a result of the power analysis, 60 individuals volunteered for the study. The inclusion criteria of study individuals were as follows: age between 19-50, BMI<30kg/m^2, no diagnosed chronic or acute disease, not allergic to cocoa products, not in the process of body weight loss, non-smokers and not consuming alcohol, not taking medication or vitamin/mineral supplements, not doing heavy physical activity, frequency of chocolate/cocoa product consumption below 1 serving 3-4 times per week for the last 1 month, not pregnant or lactating for female individuals. Individuals who had been diagnosed with a disease based on biochemical parameters or who had started taking medication/supplements, whose Systolic Blood Pressure (SBP) was higher than 140mmHg and/or whose Diastolic Blood Pressure (DBP) was higher than 90mmHg, who had been diagnosed with Coronavirus Disease 2019 (COVID-19) or who had been in contact with COVID-19 and who consumed cocoa/chocolate products during the study, who did not consume the intervention product regularly and who did not wish to continue participating were excluded from the study (Figure 1). A total of 37 volunteers (female: 4, male: 33) completed the study. The study was approved by the Ethics Committee for Scientific Research and Publication of the Eastern Mediterranean University on December 17, 2020 and was numbered 2020-08. All participants were asked to sign an informed consent form in accordance with the Declaration of Helsinki. The Clinical Trial ID number for the current study is: NCT05290012.

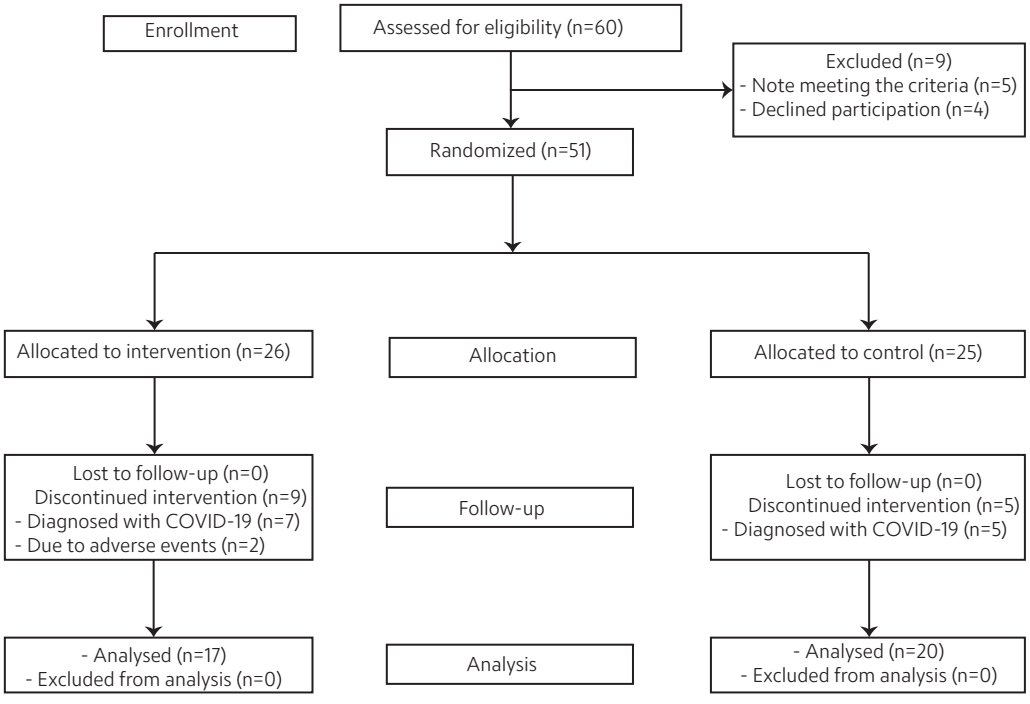

Figure 1 – Flowchart of the subject for the study. Samsun, Turkey, 2021.

Study Design

The study was conducted as a randomized controlled trial. Individuals who met the inclusion criteria were randomly divided into two groups, a control group and an intervention group, which were as similar as possible in terms of body weight, age, gender and body mass index. During the study period, it was ensured that both groups did not consume products containing cocoa/chocolate outside the scope of the study. The intervention group consumed 36 g/day of dark chocolate (400mg/day of flavanols) in addition to their daily diet, while the control group had no intervention. Chocolate was distributed to the intervention group on a weekly basis and was packaged according to the amount to be consumed daily. To monitor individual compliance, food consumption and physical activity were recorded for 3 consecutive days from the start of the study, once every 15 days, including 1 day on the weekend, 2 days on weekdays. Average daily energy and nutrient intakes were calculated using EBISpro for Windows, Stuttgart, Germany; Turkish version (BeBiS 8.2). Factorial calculations of total energy expenditure for a population group were used according to World Health Organization 1985. Physical activity status was then determined by asking individuals how much time they spent sleeping, resting, sitting, working while seated, etc. by using factorial calculations during the day. From the data obtained, individual Basal Metabolic Rate (BMR), Total Energy Expenditure (TEE), and individual Physical Activity Level (PAL=TEE/BMR) were calculated. As a summary, PAL was measured from the average 24-hour TEE and BMR (PAL=TEE/BMR). To determine the energy expenditure of the participants, PAL was multiplied by BMR to give the actual energy requirement [10]. At the beginning and at the end of the study, biochemical blood values, including serum lipids and glucose, blood pressure and anthropometric measurements of the individuals were taken.

Characteristics of Chocolate Used in the Research

The chocolate was weighed and packaged at 36g per day using a digital food scale. Participants were asked to consume the packaged dark chocolate product twice a day (as a morning snack and afternoon snack). In addition, each pack was labelled with usage instructions. According to the analysis and information provided by the manufacturer, it was stated that there were 1111mg flavanols in 100 grams of chocolate. Since the daily amount consumed by the participants was 36 grams, the daily flavanol intake was also calculated. Accordingly, participants consumed 400mg of flavanols per day by consuming 36 grams of chocolate per day. The nutritional content of the chocolate used in the study is shown in Table 1.

Table 1 – Nutritional content of the research chocolate.

Research Chocolate	100 grams	36 grams
Energy (kj/kcal)	2,475 / 597	891/214.9
Carbohydrates (g)	32.6	11.7
Protein (g)	8.9	3.2
Fat (g)	45.9	16.5
Saturated Fat (g)	29.2	10.5
Fiber (g)	9	3.2
Sugar (g)	12.1	4.4
Flavanol (mg)	1,111.1	400

Anthropometric Measurements and Blood Pressure

Body composition was measured and the Basal Metabolic Rate (BMR) was calculated using bioelectrical impedance analysis with the TANITA BC-601. Prior to measurement, subjects were warned not to exercise for the previous 24 hours, not to consume caffeinated beverages for at least 4 hours, to be fasting for at least 3 hours, to avoid excessive fluid intake, and female subjects were asked if they were menstruating [11]. Before the body analysis, all participants were asked to remove metal jewellery, buckles, watches, belts, etc., as well as shoes and socks. Participants were then placed in a suitable position to step barefoot on the metal detectors of the machine and their measurements were taken [12].

Waist and hip circumferences were measured using a non-stretch tape measure. The waist circumference was measured from the midpoint between the iliac crest and the lowest rib bone, while the hip circumference was measured by turning the tape around the widest part. The waist-hip ratio is calculated by mathematically dividing the waist circumference by the hip circumference [11]. Participants' height measurements were taken with their bare feet against the wall using a non-stretchable tape measure.

Blood pressure was measured using a Nimo LD-520 digital sphygmomanometer. After resting for 10 minutes, blood pressure measurements were repeated three times at two-minute intervals with the feet on the floor in a seated position, with the left arm supported at heart level, and the systolic and diastolic blood pressures were recorded by averaging the results [13].

Biochemical Blood Parameters

Blood samples taken from participants at baseline and at the end of the study were analysed at the Family Health Centres' central laboratory by general practitioners. Participants were warned to fast for at least 10-12 hours and to avoid drinking water in the morning before testing. Regarding the blood samples, fasting blood glucose, HbA1c, total cholesterol, LDL cholesterol, HDL cholesterol and C-Reactive Protein (CRP) levels were checked, and the total cholesterol/HDL and LDL/HDL ratios were mathematically calculated.

Statistical Analysis

Statistical evaluation of the data in the study was carried out using the Statistical Package for Social Science, version 26 (IBM Corp. Released 2019, IBM®SPSS®). To determine the methods to be used in testing the research hypotheses, the Shapiro-Wilk test was used to determine whether the data set fit the normal distribution, and it was determined that it did not have a normal distribution. The Mann-Whitney U test was used for between-group comparisons of pre-test and post-test anthropometric measurements, biochemical measurements, blood pressure measurements, energy and macronutrient intakes, energy expenditure, Physical Activity Level (PAL) and BMR measurements of the intervention and control groups, and the Wilcoxon test was used for within-group comparisons.

RESULTS

Thirty-seven individuals, 20 (2 females, 18 males) from the control group and 17 (2 females, 15 males) from the intervention group, completed the study. The mean age of the control group was 31.4±5.43 years and that of the intervention group was 32.7±8.59 years (p>0.05).

The changes in anthropometric measurements over the 4 weeks are shown in Table 2. There was no statistically significant change in body fat percentage, waist and hip circumference measurements in either group during the study ($p>0.05$). At the end of the study, there was a decrease in muscle mass in the control group, while a decrease in body weight and Body Mass Index (BMI) was observed in the intervention group ($p<0.05$). Corresponding to this change in body weight, the BMR level also decreased in the intervention group. There was no statistically significant change in PAL and energy expenditure ($p>0.05$).

Table 2 – Comparison of pre-intervention and post-intervention anthropometric and energy expenditure, Physical Activity Level and Basal Metabolic Rate of the intervention and control groups. Samsun, Turkey, 2021.

Group	Pre-Intervention				Post-Intervention				Z	p^3
	$\bar{\chi}$	s	Z	p^1	$\bar{\chi}$	s	Z	p^2		
Body Weight (kg)										
Intervention (n:17)	79.1	12.83	-0.274	0.80	78.4	12.38	-0.472	0.64	-2.018	0.04*
Control (n:20)	81.1	12.64			80.8	11.55			-0.542	0.59
Height (cm)										
Intervention (n:17)	177.4	5.41	-0.519	0.60	177.4	5,41	-0.519	0.60	0.000	1.00
Control (n:20)	176.0	6.36			176.0	6.36			0.000	1.00
BMI (kg/m²)										
Intervention (n:17)	25.1	3.08	-1.021	0.31	24.8	2.96	-1.281	0.21	-1.994	0.05*
Control (n:20)	26.1	3.37			26.0	3.04			-0.464	0.64
Body Fat Percentage (%)										
Intervention (n:17)	20.3	4.48	-0.716	0.47	20.1	4.64	-1.280	0.20	-0.402	0.69
Control (n:20)	21.3	4.32			22.1	3.87			-1.631	0.10
Body Muscle Mass (kg)										
Intervention (n:17)	59.7	9.64	-0.244	0.81	60.5	9.87	-0.472	0.64	-0.095	0.93
Control (n:20)	60.7	9.23			59.8	8.49			-2.017	0.04*
Waist Circumference (cm)										
Intervention (n:17)	89.5	11.97	-1.084	0.28	88.5	10.67	-1.297	0.20	-1.390	0.17
Control (n:20)	93.2	10.89			93.2	11.32			-0.073	0.94
Hip Circumference (cm)										
Intervention (n:17)	104.8	5.44	-0.687	0.49	104.2	5.82	-0.794	0.43	-1.021	0.31
Control (n:20)	106.2	5.21			106.2	5.32			-0.249	0.80
Waist/ Hip Ratio										
Intervention (n:17)	0.9	0.09	-0.611	0.54	0.8	0.08	-0.886	0.38	-0.738	0.46
Control (n:20)	0.9	0.07			0.9	0.08			-0.063	0.95
Energy Expenditure (kcal/day)										
Intervention (n:17)	2.758.8	490.42	-0.396	0.69	2.725.7	501.77	-0.152	0.88	-0.450	0.65
Control (n:20)	2.738.3	408.92			2.708.9	358.97			-0.933	0.35
PAL										
Intervention (n:17)	1.5	0.17	-0.320	0.75	1.51	0.18	-0.076	0.94	-0.057	0.96
Control (n:20)	1.5	0.14			1.48	0.13			-0.349	0.73
BMR (kcal/day)										
Intervention (n:17)	1.812.5	222.81	-0.305	0.76	1.803.0	217.50	-0.457	0.65	-2.036	0.04*
Control (n:20)	1.836.4	207.19			1.827.9	191.93			-1.083	0.28

Note: *$p<0.05$ significant. p^1: Comparison of pre-intervention results between groups, p^2: Comparison of post-intervention results between groups, p^3: Comparison of intra-group pre-intervention and post-intervention results. BMI: Body Mass Index; BMR: Basal Metabolic Rate; PAL: Physical Activity Levels.

Table 3 shows the amounts of energy and macronutrients consumed during the study. There was no difference between the amount of energy consumed at baseline and at the end of the study, and this energy was derived from the ratio of carbohydrate, protein and fat in both the control and intervention groups ($p>0.05$). The PAL of the two groups were similar and were considered

equivalent to mild activity (around 1.5 PAL). Consumption of saturated fat and fiber increased in the intervention group compared to baseline (p<0.05). At the end of the study, there was no difference in cholesterol levels in either group compared to baseline (p>0.05).

Table 3 – Comparison of pre-intervention and post-intervention energy and macronutrient intake of the intervention and control groups. Samsun, Turkey, 2021.

Group	Pre-Intervention				Post-Intervention				Z	p^3
	$\bar{\chi}$	s	Z	p^1	$\bar{\chi}$	s	Z	p^2		
Energy (kcal)						—				
Intervention (n:17)	2140.9	376.41	-0.152	0.88	2.143.7	368.40	-0.030	0.98	-0.544	0.59
Control (n:20)	2104.1	337.60			2.112.0	380.23			-0.261	0.79
Protein (g)										
Intervention (n:17)	86.1	19.55	-1.097	0.27	80.9	13.99	-0.518	0.60	-1.065	0.29
Control (n:20)	78.3	20.21			83.9	18.65			-1.344	0.18
Protein (%)										
Intervention (n:17)	16.7	3.12	-1.505	0.13	15.4	1.77	-1.731	0.08	-1.507	0.13
Control (n:20)	15.2	2.81			16.3	1.65			-1.576	0.12
Fat (g)										
Intervention (n:17)	104.2	13.88	-1.051	0.29	110.8	22.18	-1.631	0.10	-1.302	0.19
Control (n:20)	100.6	20.65			99.7	17.90			-0.747	0.46
Fat (%)										
Intervention (n:17)	43.9	5.45	-0.794	0.43	46.4	9.51	-2.031	0.04*	-1.447	0.15
Control (n:20)	42.7	6.52			42.2	4.81			-0.492	0.62
Saturated fatty acid (g)										
Intervention (n:17)	34.9	6.21	-2.301	0.02*	42.3	11.55	-2.804	0.01*	-2.296	0.02*
Control (n:20)	29.6	9.71			31.8	7.67			-1.661	0.10
Cholesterol (mg)										
Intervention (n:17)	361.5	129.80	-1.036	0.30	310.9	130.86	-0.213	0.83	-1.160	0.25
Control (n:20)	315.7	102.73			329.1	132.16			-0.523	0.60
Carbohydrate (g)										
Intervention (n:17)	211.1	65.33	-0.701	0.48	206.7	73.46	-0.640	0.52	-0.355	0.72
Control (n:20)	218.0	52.14			214.8	52.30			-0.112	0.91
Carbohydrate (%)										
Intervention (n:17)	39.6	6.28	-1.133	0.26	38.4	9.52	-1.587	0.11	-0.571	0.57
Control (n:20)	42.2	6.81			41.4	4.73			-0.565	0.57
Fiber (g)										
Intervention (n:17)	21.5	6.81	-1.951	0.05	23.9	6.65	-0.701	0.48	-2.012	0.04*
Control (n:20)	24.1	4.75			23.2	7.20			-1.494	0.14

Note: *p<0.05 significant. p^1: Comparison of pre-intervention results between groups, p^2: Comparison of post-intervention results between groups, p^3: Comparison of intra-group pre-intervention and post-intervention results.

The changes in the biochemical blood parameters and blood pressure of the study subjects are shown in Table 4. At the end of the study, there was no difference in systolic or diastolic blood pressure between the intervention and control groups compared to baseline (p>0.05). In the intervention group, total cholesterol and LDL cholesterol decreased by 5.6% and 7.4% respectively, while fasting glucose increased by 5.4% compared to baseline (p<0.05). There was no difference in HDL cholesterol, CRP or HbA1c levels (p>0.05). There was no difference in the levels of fasting blood glucose, total cholesterol, LDL cholesterol, HDL cholesterol, LDL: HDL, total cholesterol: HDL, CRP or HbA1c measured at the end of the study in participants in the control group (p>0.05). There was a 6.2%, 6.6% and 4.4% reduction in triglyceride, LDL: HDL and total cholesterol: HDL levels respectively in the intervention group, while a 5% increase in triglyceride levels was observed in the control group compared to baseline, but the difference was not statistically significant in either group (p>0.05).

Table 4 – Comparison of pre-intervention and post-intervention blood parameters and blood pressure measurements of the intervention and control groups. Samsun, Turkey, 2021.

Group	Pre-Intervention				Post-Intervention				Z	p^3
	$\bar{\chi}$	s	Z	p^1	$\bar{\chi}$	s	Z	p^2		
Fasting Blood Glucose (mg/dl)										
Intervention (n:17)	90.9	5.65	-0.397	0.69	95.9	8.42	-1,405	0.16	-2,089	0.04*
Control (n:20)	90.2	6.06			92.7	9.14			-1,027	0.30
Total Cholesterol (mg/dl)										
Intervention (n:17)	178.2	16.85	-2,240	0.03*	168.1	18.43	-0.030	0.98	-2,296	0.02*
Control (n:20)	163.7	21.50			164.9	25.12			-0.411	0.68
LDL Cholesterol (mg/dl)										
Intervention (n:17)	110.7	19.35	-1,935	0.05	102.5	20.27	-0.792	0.43	-2,154	0.03*
Control (n:20)	93.8	23.68			94.3	25.75			-0.560	0.58
HDL Cholesterol (mg/dl)										
Intervention (n:17)	47.1	6.87	-1,006	0.32	46.4	6.04	-0.564	0.57	-0.640	0.52
Control (n:20)	47.1	13.74			46.7	10.73			-0.141	0.89
Triglyceride (mg/dl)										
Intervention (n:17)	102.2	32.75	-0.839	0.40	95.9	36.13	-1,815	0.07	-1,065	0.29
Control (n:20)	113.8	43.16			119.6	47.41			-0.523	0.60
LDL:HDL										
Intervention (n:17)	2.4	0.63	-1,051	0.29	2.3	0.59	-0.274	0.78	-1,913	0.06
Control (n:20)	2.1	0.94			2.2	0.85			-0.691	0.49
Total Cholesterol: HDL										
Intervention (n:17)	3.9	0.72	-0.503	0.62	3.7	0.65	-0.427	0.67	-1,734	0.08
Control (n:20)	3.7	1.03			4.0	1.77			-0.672	0.50
CRP (mg/l)										
Intervention (n:17)	0.2	0.16	-1,190	0.23	0.1	0.17	-0.641	0.52	-1,508	0.13
Control (n:20)	0.1	0.13			0.1	0.10			-1,131	0.26
HbA1c (%)										
Intervention (n:17)	5.1	0.35	-0.506	0.61	5.1	0.39	-0.509	0.61	-0.635	0.53
Control (n:20)	5.0	0.37			5.0	0.23			-0.631	0.53
Systolic Blood Pressure (mmHg)										
Intervention (n:17)	122.4	10.35	-0.885	0.38	120.8	9.83	-1,655	0.10	-1,062	0.29
Control (n:20)	126.7	7.60			126.2	7.71			-0.505	0.61
Diastolic Blood Pressure (mmHg)										
Intervention (n:17)	78.1	6.34	-0.702	0.48	77.2	5.39	-0.504	0.61	-0.783	0.43
Control (n:20)	76.8	6.58			78.1	7.23			-1,106	0.27

Note: *$p<0.05$ significant. p^1: Comparison of pre-intervention results between groups, p^2: Comparison of post-intervention results between groups, p^3: Comparison of intra-group pre-intervention and post-intervention results. CRP: C-Reactive Protein; HbA1c: Hemoglobin A1c; HDL: High Density-lipoprotein Cholesterol; LDL: Low Density-Lipoprotein Cholesterol.

DISCUSSION

The antioxidant profile of cocoa and the nutrients it contains may reduce the risk of cardiovascular disease. The association of cocoa with cardiovascular disease is generally linked to the modification of the blood lipid profile by the bioactive compounds in the composition of cocoa [14]. In this study, the consumption of dark chocolate leads to a significant reduction of 5.6% in total cholesterol and 7.4% in LDL cholesterol, while the reduction in triglycerides is not statistically significant. However, there was no effect on HDL cholesterol (Table 4). In a 6-month study of 84 young and healthy subjects, total cholesterol, LDL cholesterol and triglyceride levels were reduced by 9.1%, 22.4% and 32.9%, respectively, after consumption of dark chocolate (2 g/day, 70% cocoa) compared with baseline [15]. In a meta-analysis of ten clinical trials, dark chocolate/cocoa consumption was associated with a reduction in both LDL and total cholesterol (-5.90mg/dl and -6.23mg/dl, respectively; $p<0.05$). The same study also reported a statistically insignificant reduction in triglyceride and

HDL levels (-5.06mg/dl, -0.76mg/dl; p>0.05) [16]. Regarding HDL and triglyceride levels, studies have shown that the decrease is greater when the study duration is >4 weeks and the BMI of the participants is above >30kg/m^2 [17,18]. Thus, the duration (4 weeks) and small sample size of this study may be among the reasons for the lack of significant effects on HDL and triglycerides.

Cocoa flavanols have been suggested to improve blood lipid profile in a dose-dependent manner [19]. A 4-week study was conducted with healthy subjects to evaluate the effect of cocoa with different flavanol content on blood lipid profile. It was found that consumption of 220mg/day of cocoa flavanols increased HDL cholesterol by 3.37mg/dl compared to baseline, while decreasing total cholesterol by 12.37mg/dl, triglycerides by 3.81mg/dl, and LDL cholesterol by 14.98mg/dl [20]. A meta-analysis of cocoa flavanols conducted by Lin et al. [21] found that <200mg/day of flavanol and ≥200 and <600mg/day of flavanol had significant effects on increasing HDL cholesterol levels, while >600mg/day of flavanol only decreased triglyceride levels. A recent review of seven randomized controlled trials (an updated review) reported that cocoa-based products containing 0.3-480mg of flavanol monomer generally had a beneficial effect on blood lipid profiles [19]. The moderate range of cocoa flavanols from dark chocolate used in this study (400mg/day) supports the positive results on blood lipid profiles. In general, the optimal dose of cocoa flavanols for blood lipid profiles is not yet known and there are conflicting results; further studies are needed to clarify this situation. Another meta-analysis study by Tokede et al. [16] found that cocoa products containing <500mg/day of flavanols were associated with a decrease in LDL cholesterol of -7.82mg/dl, and the same study showed that this decrease may be greater with higher polyphenol intakes (≥500mg/day of flavanol). The fact that the cocoa flavanol in the dark chocolate used in this study was <500mg/day supports the beneficial effects on the blood lipid profile, but as stated in the above study, further reductions would have been expected if a higher polyphenol content had been used.

Cocoa butter, a fat derived from cocoa plants and found primarily in dark chocolate [22], contains an average of 33% oleic acid (cis-18:1 monounsaturated), 25% palmitic acid (16:0 saturated), and 33% stearic acid [23]. Chocolate consumption has often been hypothesized to reduce the risk of cardiovascular disease due to chocolate's high levels of stearic acid and antioxidant flavonoids. While chocolate has sometimes been criticized for its saturated fat content, mostly in the form of long-chain stearic acid, chocolate has also been praised for its antioxidant potential. Saturated fat has long been thought to contribute to atherosclerosis and thus be detrimental to CVD risk. However, stearic acid has been suggested to be a non-atherogenic form of dietary saturated fat [24]. This has been confirmed in a number of studies and in a meta-analysis of 60 controlled feeding trials, which concluded that stearic acid does not lower HDL or increase LDL or total cholesterol [25,26]. In conclusion, given that the vast majority of studies show that stearic acid has beneficial or neutral effects on blood pressure and coagulation parameters, it seems unlikely that stearic acid intake would adversely affect CVD risk via these risk factors [21-26].

A cohort study by Kiriyama et al. [27] found that weight loss improves the blood lipid profile in non-obese individuals, with a reduction in blood lipids being expected in direct proportion to weight loss. A systematic review and meta-analysis of 83 studies found that a 5-10% reduction in body weight proportionally reduced LDL cholesterol by 16.4mg/dl, total cholesterol by 25.9mg/dl and triglycerides by 6.5mg/dl [28]. In this study, there was a 0.9% reduction in body weight in the intervention group compared to baseline, and a 5.6% and 7.4% reduction in total and LDL cholesterol, respectively. There was no statistically significant change in body weight or cholesterol levels in the control group. A meta-analysis study found that the consumption of dark chocolate >30g/day for 4-8 weeks was associated with a reduction in BMI. There was a non-linear decrease in

waist circumference with consumption of 40-60g/day [29]. Therefore, it can be assumed that dark chocolate consumption has beneficial effects on the blood lipid profile independent of body weight control. In addition, some studies have reported beneficial effects of cocoa products on adipose tissue [30-32]. Although a detailed analysis of adiponectin levels and other markers was not performed in this study, it is predicted that cocoa polyphenols have beneficial effects on white adipose tissue.

One study found that cocoa butter had no effect on postprandial plasma CRP levels [33]. In contrast, another study reported that three types of cocoa powder containing 180mg, 400 mg and 900 mg flavanols reduced CRP levels with a linear dose-response compared to a drink containing 30 mg flavanol [34]. In general, the anti-inflammatory effect of cocoa is related to the dose and dietary matrix of cocoa flavanols. It has been noted that the bioavailability of flavanols is higher when cocoa powder is used instead of chocolate and does not contain milk protein. In addition, the BMI, health status and degree of inflammation of the people in the study may also influence the results [35]. The reason why there was no significant change in CRP levels in this study may be related to the limited study period, the small sample size, the food matrix of chocolate and the flavanol dose.

Dark chocolate helps stabilise blood glucose levels by slowing the digestion and absorption of carbohydrates [36]. In a study of diabetics and hypertensives, consumption of dark chocolate (25 g/day) significantly reduced HbA1c and fasting glucose levels after 8 weeks compared with white chocolate [37]. In an 8-week study with elderly subjects, blood glucose levels were reduced by 11% and 9.4%, respectively, in subjects consuming cocoa powder containing high flavanols (993mg/day) and medium flavanols (520mg/day) [38]. The results of this study contradict the above studies. The reason for this is that in similar studies, the sample was usually made up of people at cardiovascular risk, while the control group was given chocolate with a high sugar content, such as milk/white chocolate, and the intervention group was given sugar-free, low-fat cocoa powder, which may have indirectly led to a reduction in fasting blood glucose levels. In addition, the effect of a high-sugar product such as milk chocolate on blood glucose depends on the timing of the meal. In a 4-week study on the timing of chocolate consumption by Hernández-González et al. [36], consumption of milk chocolate (100g/day) in the morning (within 1 hour of waking up) resulted in a 4.4% decrease in fasting blood glucose, whereas consumption (100g/day) in the evening/night (1 hour before going to bed) showed a significant increase of 4.9%. The people in this study consumed dark chocolate at lunchtime and in the afternoon. In this case, it can be assumed that there is a possibility that blood glucose levels have increased in relation to the time of meal consumption. On the other hand, it can be predicted that the flavanols in the dark chocolate used in this study were not in a sufficient amount to prevent the effect of fat and sugar content on blood glucose levels.

It is stated that cocoa components generally increase Nitric Oxide (NO) production and support vascular vasodilation, which has a beneficial effect on blood pressure [39]. At the end of this study, although a decrease in dark chocolate consumption and SBP and DBP was observed in the intervention group, it was not considered statistically significant (Table 4). Similar results have been reported in other studies [8,40,41]. However, the magnitude of the effect on blood pressure (-1.59mmHg for SBP; -0.88mmHg for DBP) is generally smaller when comparing these studies with the present data. This may be related to the duration of the study, the composition of healthy subjects, and the nutrient matrix. Studies comparing cocoa and white/milk chocolate have shown that the effect of cocoa on blood pressure is greater than that of white/milk chocolate [42,43]. This may be due to the high sugar content of milk and white chocolate and has been linked to the fact that milk protein reduces the absorption of epicatechin, one of the cocoa flavanols, by binding to it and negatively affecting its antihypertensive effect [44,45]. On the contrary, results suggest that

there is no interaction between milk protein and epicatechin [46,47]. The fact that the study included a control group that did not consume chocolate may be related to the small effect size. However, the lack of information on the amount of epicatechin is one of the limitations of the study and is insufficient to explain the reason for this situation. In addition, the fact that blood pressure is a dynamic measure and responds to many physical or emotional stimuli is a factor that can suppress the detection of differences in measurement results [8].

The study has several limitations. Firstly, the vast majority of people who took part in the study were men. Therefore, the results of the study can only apply to male individuals and cannot be generalized to female ones. In addition, the sample size was small and the study period of 4 weeks may not be long enough for the relevant parameters to change. In addition, the nutritional content of chocolate was evaluated using only the nutritional information on the label, and there was no information on micronutrients or other components, such as epicatechin and procyanidin, which may influence the study results. In addition, the amount of flavanols ingested from foods other than dark chocolate is not known, and individuals' flavanol intake was not controlled or measured in this way. Therefore, it may not be sufficient to attribute changes in total cholesterol, LDL cholesterol and fasting blood glucose levels to cocoa flavanols alone.

CONCLUSION

In healthy individuals, consumption of 36 g/day (400mg flavanol) for 4 weeks has a beneficial effect on serum lipid profile while, contrary to expectations, it increases fasting blood glucose. Thus, cocoa consumption as a dietary intervention has a possible role in reducing the risk of cardiovascular disease as an age-related lifestyle disease. To determine the effect of cocoa on cardiovascular health, it is important to focus on the mechanisms of action, the nutritional components associated with this effect and the determination of optimal doses of these components. The effects of cocoa products on cardiovascular health vary depending on the sample size, the amount of flavanols and other bioactive components they contain. In this direction, future research should be planned, taking into account the general characteristics of the population and the nutritional components of cocoa products, and should have a longer-term and larger samples.

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