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Impact of Consumption of Dark Chocolate Enriched with Blackberry and β-Glucan on Cardiometabolic Markers in Overweight Individuals

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Abstract

Objective: To evaluate the effect of the consumption of 70% cocoa dark chocolate, enriched with dehydrated blackberry (*Rubus ulmifolius*) and β-glucan, administered together with regular and low-fat diets, on anthropometric and biochemical parameters and dietary intake in overweight or obese individuals. **Methods:** A Randomized Complete Block design with 2 × 2 factorial arrangement was used, comparing four treatments during nine weeks, a Phase III case-control observational clinical study, with a population of 16 students of both sexes aged 19-23 years. Anthropometric measurements (weight, height, waist-hip index, BMI, blood pressure, percentage of total and visceral fat), biochemical parameters (capillary glucose and fat profile: total cholesterol, HDL, LDL, and triglycerides), and intake record by 24-hour recall were evaluated. **Results:** No significant changes were observed in BMI or blood pressure. Women's waist-to-hip ratio decreased significantly in treatment 2 ($P < 0.05$). Triglycerides were reduced considerably in treatment 3 ($P < 0.02$). In the low-fat diet, calorie ($P < 0.022$), carbohydrate ($P < 0.018$), protein ($P < 0.049$), and sodium ($P < 0.03$) intakes showed significant reductions. **Conclusions:** Consumption of dark chocolate enriched with blackberry and β-glucans, in the context of a low-fat diet, improved cardiometabolic markers and reduced triglyceride levels and calorie, carbohydrate, protein, and sodium intake. There were no statistically significant differences in body mass index or blood pressure.

Keywords: BMI; chronic noncommunicable diseases; diet; lipid profile; waist-to-hip index

1. Introduction

Obesity is considered the greatest global epidemic according to the World Health Organization (1). From 1975 to 2016, obesity rates tripled worldwide, reaching 13% of the population in 2016 (2). By 2022, 43% of adults aged 18 and older were overweight, and 16% were obese (1). Currently, 3 out of 10 children and adolescents in Latin America and the Caribbean are living with excess weight. The leading causes are physical inactivity, inadequate food choices, consumption of ultra-processed foods, and sugary beverages characterized by easy access, low cost, and promotion through mass media (3). Obesity is generally associated with various comorbidities, including high blood pressure, high cholesterol, glucose intolerance, insulin resistance, fatty liver, psychological disorders, and school bullying. When a child is obese, they are more likely to maintain the same condition into adulthood. Consequently, they are at a higher risk of developing diseases such as type 2 diabetes,

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cardiovascular diseases, and certain types of cancer. Moreover, the consequences are usually more severe than those who developed obesity in adulthood (4). Within nutritional intervention strategies, efforts are made to generate incentives that promote healthy eating by introducing foods with distinctive components. Bioactive compounds and/or functional foods play a beneficial role in addressing the issue, allowing for the mitigation of comorbidities resulting from increased weight due to body fat (5, 6).

Beta-glucans are glucose polymers found naturally in various foods, particularly in mushrooms such as Ganoderma lucidum. These have significant benefits on the immune system, reducing the lipid fraction in the blood by their ability to form viscous solutions that, by prolonging gastric emptying, create a feeling of satiety(7). They inhibit the transport of triglycerides and cholesterol through the intestine and reduce concentrations of low-density lipoproteins (LDL)(8). Several studies have shown the benefits and effects they have on the glycemic index, as well as their immune system-activating properties. Currently, they are used in the food industry as soluble fibers without altering the original taste of food (9).

Blackberry (Rubus ulmifolius), a fruit native to Central America, stands out for its high content of organic and bioactive compounds; it contains mineral salts and vitamins A, B, and C. Due to its iron content, it is used to prevent anemia and cancer. Additionally, its flavonoid content (flavone, flavonols, flavanols, and anthocyanidins) prevents hypertriglyceridemia (10,11). The described foods offer significant nutritional benefits; however, their sensory acceptance is crucial for their incorporation into the diet of children and adolescents. Therefore, it is essential to associate them with familiar profiles to promote consumption (12)

According to the Codex Alimentarius (13), chocolate can be bitter, semisweet, dark, or "chocolat fondant." For such a designation, it must contain no less than 35% of total cocoa dry extract, at least 18% of which will be cocoa butter and 14% lean cocoa dry extract. It is considered the favorite food of Western children and increasingly so in other societies(14). Chocolate, a food that has been valued not only for its taste but also for its potential health benefits as a high source of polyphenols and flavanols in particular, has recently received attention for its possible role in modulating obesity due to its effect on fat and carbohydrate metabolism, providing satiety, and enhancing the activation of metabolic pathways capable of improving lipid profiles (14,15).

In other words, besides providing health benefits, chocolate could serve as a vehicle for other functional foods, given its appeal to all ages. Without local studies, this work proposes to evaluate the effect of consuming chocolate enriched with β-glucans and blackberry extract on the health of individuals of both sexes (19-25 years old). To achieve this, anthropometric and biochemical parameters were evaluated before and after the intervention.

2. Material and Methods

The study was conducted at the Pan-American Agricultural School, Zamorano. The development of the chocolate took place at the Food Innovation Plant (PIA). Microbiological analyses were performed at the Zamorano Microbiological Analysis Laboratory (LAMZ). The chocolate was donated by the company XOL CHOCOLATE in Copán, Honduras. The blackberries (Rubus ulmifolius) were purchased from the local supplier at the Zamorano horticultural plant. The β-glucans were donated by the company Progal in Colombia. The blackberries (R. ulmifolius) were received and disinfected with peracetic acid at 60 parts per million (ppm) for 15 seconds. Following this, the fruit was dehydrated in a Harvest Saber dehydrator (model HS-R-SS-1-E) at 150°C for 7 hours at wind speed number two. The fruits were placed separately to achieve a more homogeneous and faster dehydration.

The chocolate with 70% cocoa underwent a tempering process in a water bath until reaching a temperature of 49°C. Subsequently, the chocolate was cooled to 28°C, then its temperature was raised to 32°C. Half of each chocolate portion was weighed and placed in the mold. 0.3 grams of β-glucans were added, and finally 3 grams of dehydrated blackberries and the other half of the chocolate were added. They were packed in aluminum foil and stored in a refrigeration room at 4°C for nine weeks.

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A microbiological analysis was conducted to indicate safety in the food process, following the sanitary standard that establishes the microbial criteria for sanitary quality and safety in food and beverages for human consumption in Peru. For the Escherichia coli analysis, 10 grams of chocolate were weighed in a sterile bag using the Fisher Scientific Model SLF152-US balance. 90 ml of phosphate buffer at a concentration of 0.12 was added, and each sample was homogenized for 120 seconds in the Stomacher by IUL Instrument. From each of these samples (10^(-1)), 1 ml was taken with a pipette and seeded using the pour plate method. Subsequently, 15 ml of Violet Red Bile Agar with 4-methylumbelliferyl-beta-D-glucuronide (MUG) was added. Circular movements were made to the right and left to homogenize. Once the content was dry, a second, thinner layer of the same agar was applied. The plates were then incubated at 36°C for 24 hours.

To analyze fungi, 10 grams of chocolate were weighed on the Fisher Scientific Model SLF152-US balance in a sterile bag. 90 ml of phosphate buffer at a concentration of 0.12 was added, and each sample was homogenized for 120 seconds in the Stomacher by IUL Instrument. From each of these samples (10^(-1)), 1 ml was taken with a pipette and seeded using the pour plate method. Subsequently, 15 ml of Rose Bengal Agar was added. Circular movements were made to the right and left to homogenize. Finally, the plates were incubated at 25°C for three days.

The study was conducted in the Yeguare Valley, Francisco Morazán Department, Honduras; the biochemical and anthropometric analyses were performed at the Zamorano Human Nutrition Laboratory (LNHZ). This work corresponds to an observational clinical study of Phase III, case-control type, approved by the Biomedical Research Committee (CEIB No. 00003070). The sampling was non-probabilistic with acceptance desire; it consisted of male and female students aged 19-23 from Zamorano University.

The sampling was non-probabilistic with a desire to participate and signing informed consent. Participant recruitment was carried out in May 2019. The sample size was determined using equation 1.

$$n = \frac{z^2 p.q.N}{Ne^2 + Z^2 p.q} \quad [1]$$

The formula yielded a sample size of 16 individuals with 95% confidence and a 5% margin of error. Where: Z: Confidence level, p: Population proportion, q: 1-p, N: Population size, e: Margin of error, n: Sample size. Participants were assigned according to the following criteria.:

Inclusion criteria:

- Age between 18 and 25 years.
- Individuals with the ability to understand and be lucid.
- Individuals with a history of familial hypercholesterolemia.
- Body mass index between 25-29.9

Exclusion criteria:

- Individuals under 18 years old or over 25 years old.
- Individuals without a history of familial hypercholesterolemia.
- Individuals who are lucid and able to understand.
- Body mass index ≤ 24.9
- Body mass index ≥ 30

Before data collection, each participant received informed consent in which the study's objectives and procedures were declared, emphasizing the possible risks and benefits of participating. It was established that the person participated voluntarily and could withdraw from the study when appropriate. Individuals were scheduled at the Smith Falck study center at the university, where they received chocolate three times a week for nine weeks. The diet was provided in the educational dining room according to the specified distribution. The type of chocolate was randomly assigned through the "RANDOM" function in the Excel program. The population was categorized as follows:

T1: Low-fat diet and chocolate with blackberry and β-glucans.

T2: Low-fat diet and dark chocolate.

T3: Normal diet and chocolate with blackberry and β-glucans.

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T4: Normal diet and dark chocolate**.**

For each participant, anthropometric measurements of weight, height, waist circumference, and hip circumference were obtained to determine the waist-to-hip ratio. The bioelectrical impedance equipment OMRON model HBF-514C was used for this purpose; participants were asked to place their heel and instep on the electrodes in the reading position. With the data obtained, the Body Mass Index (BMI) was calculated using equation 2.

$$BMI = \frac{weight(kg)}{sice\ (m^2)} \quad [2]$$

The population's BMI values were classified into their respective categories according to the National Heart, Lung, and Blood Institute. Using a non-stretchable measuring tape SECA 201, the waist circumference was measured with the last floating rib as reference point. Additionally, the hip circumference was measured with the most prominent part at the level of the buttocks as reference point. The data was recorded in centimeters (cm), and the waist-to-hip ratio (WHR) was calculated using equation 3:

$$WHR = \frac{waist\ circumference\ (cm)}{hip\ circumference\ (cm)} \quad [3]$$

The WHR is associated with the cardiometabolic risk that a person may have; therefore, its values are divided into different categories according to the National Heart, Lung, and Blood Institute. The biochemical measurements included glucose analysis and lipid profile. Due to displacement and location of the health center, glucose was obtained capillary-wise using the portable quick test equipment Accu-Chek; for the lipid profile, the portable equipment DS Lipidocare was used. The ring finger was disinfected with 70% clinical-grade alcohol. The sample was collected with a micropipette, and 35 µL of blood was placed on the reactive strip of the device. The results were displayed after 180 seconds. The participants were classified according to the ranges stipulated by the National Institute of Diabetes and Digestive Kidney Diseases.

Table 1. Reference values for fasting plasma glucose (FPG) test.

Category	**Range (mg/dL)**
Normal	< 100
Prediabetes	100-125
Diabetes	> 126

Fuente:NIDDK (2020). National Institute of Diabetes and Digestive Kidney Diseases.

Table 2. Lipid profile parameters.

Paramater	**mg/dL**	**Category**
LDL	< 100	Optimal
	100-129	Near Optimal
	130-159	Borderline High
	160-189	High
	> 190	Very High
Cholesterol Total	< 200	Ideal
	200-239	Borderline
	> 240	High
HDL	< 40	Low
	40-60	Normal
	> 60	High
Triglycerides	< 150	Normal

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150-199	Borderline High
200-499	High
> 500	Very High

Fuente: NHLB (2011). National Heart, Lung, and Blood Institute.

A Randomized Complete Block Design (RCBD) was used, with a 2×2 factorial arrangement with repeated measures over time, analyzing the initial and final data of the four treatments. The Statistical Analysis System (SAS) program was used to evaluate changes in anthropometric, fat, and biochemical measures over time; mean separation was carried out using the Duncan test ($P < 0.05$) and LSmeans separation to analyze the interaction of factors over time. A Student's T-test analysis was used for the 24-hour count data. A 24-hour dietary recall was used as a retrospective method to evaluate food intake. Individual information on the foods consumed on the previous day at each mealtime was collected on the data collection day. This data was then entered into the "The Food Processor" SQL version 10.10 program using a format facilitating information input.

3. Results

Regarding the BMI, at the beginning of the study, all participants were classified as overweight (25–29.9). Although at the end of the study, the means of the individuals in each treatment suggest a reduction without statistical significance. According to their WHR values, men showed a very low cardiometabolic risk (0.85) to moderate risk.

Table 3. Anthropometric means at the beginning and end of the nutritional intervention.

	Anthropometric Measurements	
	Initial	**Final**
	May 2019	**Agosto 2019**
Body Mass Index (kg/m²)		
Treatment 1	27.10 ± 1.30^{Axy}	25.65 ± 1.21^{Axy}
Treatment 2	27.40 ± 1.23^{Ax}	26.87 ± 0.65^{Axy}
Treatment 3	25.80 ± 0.65^{Ay}	25.37 ± 0.79^{Ay}
Treatment 4	27.00 ± 1.36^{Axy}	26.95 ± 1.59^{Ax}
CV(%)	4.02	
Waist-to-Hip Ratio Men		
Treatment 1	0.84 ± 0.015^{Ax}	0.83 ± 0.045^{Ax}
Treatment 2	0.85 ± 0.015^{Ax}	0.80 ± 0.020^{Ax}
Treatment 3	0.83 ± 0.015^{Ax}	0.80 ± 0.005^{Ax}
Treatment 4	0.89 ± 0.03^{Ax}	0.83 ± 0.005^{Ax}
CV(%)	3.82	
Waist-to-Hip Ratio Women		
Treatment 1	0.77 ± 0.005^{Ay}	0.75 ± 0.01^{Axy}

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Treatment 2		0.89 ± 0.04^{Ax}	0.78 ± 0.02^{Bxy}
		0.82 ±	
Treatment 3	0.02^{Axy}		0.84 ± 0.01^{Ax}
		0.76 ±	
Treatment 4	0.015^{Ay}		0.80 ± 0.06^{Axy}
CV(%)		4.71	

A–B: Means followed by the same letters within a row are not statistically different (P > 0.05). x–y: Means followed by the same letters within a column are not statistically different (P > 0.05). Values are reported as mean ± SD. CV: Coefficient of variation. T1 Low-fat diet with blackberry chocolate and β-glucan. T2 Low-fat diet with dark chocolate. T3 Normal diet with blackberry chocolate and β-glucan. T4 Normal diet with dark chocolate.

For the biochemical measurements, the findings for glucose values were as follows: treatments 3 and 4 remained in the normal range (<100 mg/dL) throughout the study, while treatments 1 and 2 changed from the normal range to prediabetes (100-125 mg/dL). None of them showed a statistically significant change. Total cholesterol: all participants started the study with total cholesterol levels within the NHLBI's normal range in 2011, below 100 mg/dL, and after 9 weeks of consumption, no statistically significant changes were observed

At the beginning and after nine weeks of consumption, all treatments were within the normal range according to the parameters established by NHLBI 2011, <100 mg/dL of LDL in blood. On the other hand, the results for HDL were as follows: at the beginning of the study, participants in treatments 1 and 2 had normal HDL values (40-60 mg/dL), while treatments 3 and 4 were in the lower ranges (<40 mg/dL) according to NHLBI in 2011. At the end of the study, only participants in treatment 1 remained within their range. In contrast, treatments 2, 3, and 4 reduced their HDL levels to very low. No treatment showed statistically significant changes.

The participants in treatments 1 and 4 started the study with a high limit (150-199 mg/dL), treatment 3 in a high range (200-499 mg/dL), and treatment two at a normal level (<150 mg/dL) of blood triglycerides. All treatments ended the study with normal levels of blood triglycerides, showing significant reductions in treatments 1, 3, and 4.

Table 4. Biochemical indicators pre- and post-nutritional intervention.

	Biochemical markers	
	Initial	**Final**
Variable	**May 2019**	**Agosto 2029**
Glucose (mg/dL)		
Tratamiento 1	72.75 ± 3.49^{Ax}	100.75 ± 7.15^{Bx}
Tratamiento 2	74.75 ± 5.30^{Ax}	99.50 ± 6.72^{Bx}
Tratamiento 3	75.75 ± 6.75^{Ax}	93.50 ± 3.84^{Bx}
Tratamiento 4	70.75 ± 1.78^{Ax}	97.25 ± 12.81^{Bx}
CV(%)	8.44	
Cholesterol total (mg/dL)		
Treatment 1	159.75 ± 23.01^{Ax}	157.00 ± 26.67^{Ax}
Treatment 2	141.00 ± 11.15^{Ax}	126.00 ± 23.94^{Ax}
Treatment 3	158.00 ± 21.15^{Ax}	145.77 ± 39.33^{Ax}
Treatment 4	125.75 ± 27.07^{Ax}	153.25 ± 23.96^{Ax}
CV(%)	18.23	
Triglycerides (mg/dL)		

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Treatment 1	173.00 ± 30.90^{Axy}	138.00 ± 31.45^{Bx}
Treatment 2	145.00 ± 48.23^{Ay}	98.00 ± 15.77^{Ax}
Treatment 3	212.75 ± 78.72^{Ax}	133.75 ± 25.17^{Bx}
Treatment 4	181.00 ± 38.95^{Axy}	97.25 ± 20.10^{Bx}
CV(%)	32.42	
High-Density Lipoprotein (mg/dL)		
Treatment 1	49.50 ± 19.65^{Ax}	45.00 ± 4.63^{Ax}
Treatment 2	42.00 ± 8.45^{Ax}	36.25 ± 5.40^{Ax}
Treatment 3	37.25 ± 8.64^{Ax}	32.00 ± 5.47^{Ax}
Treatment 4	39.00 ± 7.61^{Ax}	36.50 ± 4.15^{Ax}
CV(%)	27.59	

A–B: Means followed by the same letters within a row are not statistically different (P > 0.05). x–y: Means followed by the same letters within a column are not statistically different (P > 0.05). Values are reported as mean ± SD. CV: Coefficient of variation. T1 Low-fat diet with blackberry chocolate and β-glucan. T2 Low-fat diet with dark chocolate. T3 Normal diet with blackberry chocolate and β-glucan. T4 Normal diet with dark chocolate.

For the 24-hour count, the low-fat diet after nine weeks of treatment significantly reduced the average of calories (P < 0.02), carbohydrates (P < 0.018), and sodium (P < 0.027). On the other hand, after nine weeks of consumption, the standard diet increased the amount of ingested fat (P > 0.03).

4. Discussion

After nine weeks of intervention, the study evaluated the impact of consuming dark chocolate enriched with β-glucans and blackberries (Rubus ulmifolius) on anthropometric, biochemical, and dietary intake indicators in overweight young individuals. The body mass index remained in the overweight category for all evaluated treatments. A decrease in this value was observed, although not significant, consistent with research suggesting a trend towards body weight loss with the daily consumption of 42 grams of chocolate over 12 weeks. According to a systematic review, the intake of cocoa flavanols may benefit cardiometabolic biomarkers in adults. (17, 18).

Furthermore, regarding the waist-hip ratio (WHR), it was observed that treatment 2 showed a significant reduction in women, which is relevant considering that WHR is a more sensitive predictor of cardiovascular risk than BMI. These findings suggest that controlled consumption of dark chocolate, even without functional components, positively impacts abdominal fat distribution (19).

No significant changes were observed in glucose levels, total cholesterol, or HDL between the treatments, suggesting a short study duration and a limited amount of chocolate ingested. This aligns with results reported in previous research that showed significant improvements in lipid profile only after consuming dark chocolate for extended periods and in larger quantities. Studies on consuming chocolate with cocoa extract ≥70% for ≥4 weeks showed no effects on body weight, BMI, waist circumference, triglycerides, or HDL-C. However, total cholesterol, LDL-C, and fasting blood glucose were reduced (20, 21).

Treatments 1, 3, and 4 showed a significant reduction in triglycerides. Treatment 3, which included chocolate, blackberries, and β-glucans, proved the most effective; it managed to reduce high triglyceride levels to normal values. These findings are consistent with research that mentions β-glucans can reduce lipid absorption in the intestine and improve blood lipid profile, especially concerning triglycerides and LDL cholesterol. Additionally, consuming chocolate with procyanidins has been shown to reduce plasma oxidation products and increase the blood's antioxidant capacity (17).

Beta-glucans form viscous solutions that delay gastric emptying and interfere with the contact of pancreatic enzymes with intestinal lumen substrates, reducing cholesterol concentrations in blood plasma (7). Research in animal models demonstrated that consuming 10 mg of beta-glucan daily

could normalize hypercholesterolemia values regarding triglyceride levels (18, 22). Low-carbohydrate diets impact lipid metabolism by utilizing fat reserves as an energy source, leading to overall improvements in lipid profile, notably enhancing triglyceride levels after regular consumption of dark chocolate (23, 24). Additionally, studies indicate that the combination of regular consumption of dark chocolate and a low-carbohydrate diet contributes to reducing blood triglyceride levels (25).

5. Conclusions

Chocolate was a suitable vehicle for other functional foods, such as beta-glucans. In a regular diet, chocolate with blackberries and β-glucan significantly reduced blood triglycerides. The 24-hour count of the low-fat diet showed significant differences in kcal, carbohydrates, proteins, monounsaturated fat, polyunsaturated fat, iron, and sodium. Including functional foods in an attractive product for the young population, such as chocolate, could propose a viable alternative for incorporating bioactive compounds into the diets of populations with low adherence to healthy eating habits. Despite the results obtained, it is crucial to consider the significant limitations in the study. The sample size (n = 16) restricts the generalization of the findings. Additionally, glucose measurement was performed capillary rather than plasmatic, which could compromise the accuracy of the reported values. Although the nine-week evaluation allowed for trend observation, it might have been insufficient to generate more significant changes in metabolic indicators.

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