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# Effect of calafate (Berberis microphylla) supplementation on lipid profile in rats with diet-induced obesity

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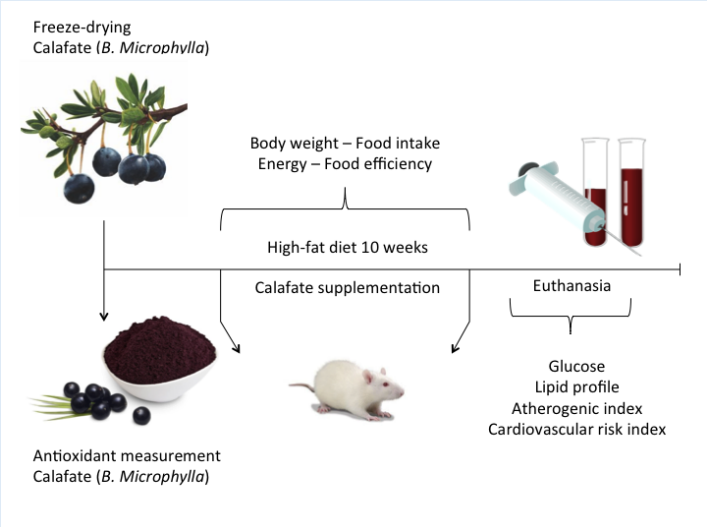
**ABSTRACT**

**Background:** Polyphenols represent a group of bioactive compounds of interest for their efficacy in the prevention and treatment of some diseases of a cardiovascular nature. Calafate (Berberis microphylla) is a native Chilean fruit, promising due to its high number of phenolic compounds, with predominance of anthocyanins delphinidin-3-hexoside and petunidin-3-hexoside.




**Methods:** Sprague Dawley rats fed a high-fat diet (HFD) were supplemented with 350 mg/kg of freeze-dried calafate for 10 weeks. Dietary variables, plasma glucose, lipid profile, as well as atherogenic and cardiovascular risk indexes were measured.

**Results:** Animals receiving calafate with their HFD diet compared to the HFD control was not associated with significant modifications in dietary variables or in total cholesterol and triglyceride concentrations. However, due

to the modest elevation of high-density lipoproteins, the Atherogenic Index and the Cardiovascular Risk Index were significantly decreased.

**Conclusion:** Based on these results, the calafate could have an antithrombotic function, favoring cardiovascular health.

**Keywords:** High-fat diet, polyphenols, calafate berry, HDL-cholesterol, Atherogenic Index.



## INTRODUCTION

Dyslipidemia is one of the main pathological events observed in obesity and is characterized by increased blood levels of total cholesterol (TC), low-density lipoproteins (c-LDL), very low-density lipoproteins (c-VLDL), triglycerides (TG) and decreased concentrations of high-density lipoproteins (c-HDL), being a key factor in the development of atherosclerosis [1] and hence cardiovascular risk (CVR) [2]. Several studies have established that hypolipidemic treatment allows c-LDL targets to be achieved in patients with concomitant pathologies such as diabetes and metabolic syndrome, but the residual CVR remains what has been attributed to low plasma concentrations of c-HDL, and the fraction of this lipoprotein may be a better marker and possible therapeutic target [3]. The dietary approach may be relevant as a complementary strategy to achieve mainly weight loss and healthy lifestyle goals, associated with favorable lipid profiles. In this context, berries, which are products of plant origin with a high content of polyphenols and bioactive compounds, are beginning to be widely studied for their protective effects against CVD [4]. The main polyphenols reported in berries are flavonoids (anthocyanins, flavonols, flavanols), tannins and phenolic acids; these chemicals are potent antioxidants with additional vascular, vasodilator activity and anti-inflammatory properties [5-6]. For this reason, the calafate (Berberis microphylla) native to Chilean Patagonia is a promising fruit due to its high number of phenolic compounds, mainly anthocyanins, comparable to those contained in other native fruits, which allows it to be considered as a therapeutic tool [7-8]. In vitro studies highlight that it could modify the inflammatory response given by the interaction between adipocytes and macrophages [9], and in human umbilical vein endothelial cells (HUVEC) calafate extract reduced intracellular reactive oxygen species (ROS) production in 51% and completely inhibited LDL oxidation and malondialdehyde (MDA) formation [10], which correlates with the high content of total polyphenols in the fruit and the concentration of anthocyanins, mainly delphinidin-3-glucoside, followed by petunidin-3-glucoside and malvidin-3-glucoside [11]. In a recent study fifty-three phenolic compounds were identified by Ultra-Liquid Chromatography with Diode Array Detector, coupled to Quadrupole-Time of Fly Mass Spectrometry (UHPLC-DAD-QTOF), where 17 of them were not previously reported in calafate [10]. The concentrations of phenolic compounds that may be influenced by the environment, which was previously described by the authors, where it was compared to the antioxidant capacity of Calafate collected at different locations in southern Chile confirming that variations in climatic conditions affect the antioxidant capacity of the fruit [12]. The aim of this study was to evaluate the effect of calafate (Berberis microphylla) supplementation on the lipid profile of rats with obesity induced by a high-fat diet.

## METHODS

***Animals:*** Male Sprague Dawley rats aged 6 – 7 weeks, obtained at the Regional Center of Advanced Studies for Life (University of Concepción, Chile) kept under standard laboratory conditions of lighting (light/dark cycle of 12 hours), temperature 25+1ºC and humidity 60%, with free access to water and food. They were housed in metal cages with an enriched environment and all manipulations were carried out between 8:00 and 12:00 hours. All efforts were made to minimize animal suffering and to reduce the number of animals used. The research was carried out under National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and was approved by the Scientific Ethics Committee of the University of La Frontera (File Nº 113_17). After a week of acclimatization, the animals were randomly divided into 3 groups (5 rats per group), equivalent to that described in previous research in the model of induced obesity, with statistically significant results [13], considering the Russell and Burch Bioethics Principles: replacement, reduction and refinement [14]. The groups were (a) Fed a high-fat diet (HFD); (b) Fed a HFD supplemented with melatonin (HFD+MEL); (c) Fed an HFD supplemented with calafate (HFD+BM).

***Diets and supplementation:*** It used a high-fat diet, with a distribution of 19% protein, 32% carbohydrates and 49% fat. Pellets produced by PragSolutions Serviços e Com. LTDA, São Paulo, Brazil (www.pragsolucoes.com.br). The major carbohydrate, protein and fat sources were starch plus sugar, casein and soya oil plus lard, respectively (Table 1). Since the beginning of the protocol in the experimental groups a daily dose of 350 mg/kg of calafate (Berberis microphylla) lyophilized, was administered through water, plus 20% of the product considering residues and/or losses, calculating the average daily consumption per animal at 80 ml/day. The evaluation of the antioxidant capacity and total polyphenol and anthocyanin content reported in a previous study by the authors (Table 2) [12]. The calafate lyophilized consumption in humans when adjusted to a rat equivalent dose based on body surface area was 56.7 mg calafate lyophilized/60 kg per person using the conversion coefficient suggested by the US FDA [15]. This dosage was equivalent to a human consumption of 3.5 g of calafate lyophilized, which might be realistic for a human. The positive control received melatonin, a drug with antioxidant properties previously described, in a single dose of 10 mg/kg/day, dissolved in drinking water [16]. Both mixtures were prepared daily to avoid oxidation of the bioactive compounds. The described interventions were carried out for 10 weeks [17].

**Table 1.** Composition of the high fat diet used in experiments.

| Composition | % Weight | Energy Density (Kcal/Kg) | % Energy |
|---|---|---|---|
| Proteins (Casein - L. Cystine) | 23.3 | 932 | 19 |
| Lipids (Soya oil - Lard) | 27.0 | 2430 | 49 |
| Carbohydrates (Starch - Sucrose) | 40.0 | 1638 | 32 |
| Fibers cel. Microcrystalline | 5.0 | 0 | 0 |
| Vitamins/Minerals | 4.5 | 0 | 0 |
| Choline bitartrate | 0.25 | 0 | 0 |
| Antioxidant-BHT | 0.001 | 0 | 0 |

Energy density: 5 kcal/g. Produced by PragSolutions Serviços e Com. LTDA, São Paulo, Brazil.

**Table 2.** Concentration of antioxidative active compounds and antioxidant capacity of calafate. (Berberis microphylla)

| Parameters | Values |
|---|---|
| Total polyphenols | 1993 + 75.7 mg gallic acid equivalents[a] |
| Total anthocyanins | 1373 + 50.2 mg cyanidin-3-glucoside |
| DPPH[b] | 8571 + 358.5 µmol Trolox |

[a] determined by Folin-Ciocalteu method

[b] 2.2-Diphenyl-1-picrylhydrazyl radical. The data is represented as mean + standard deviation. All results were done in quadruplicate (Extracted Ref.12)

***Experimentation and euthanasia:*** The body weight (g) of each animal was determined once a week at the same time, during the whole experimental protocol using EHW EC Measuretex balance with 0.5 g resolution previously calibrated. The solid food intake (g) was measured with precision balance once a day. The liquids (ml) were measured in 200 ml graduated drinkers. Both measurements represent the average value per cage weighted by the number of animals. The energy intake was quantified from the solid intake of each animal. Food efficiency ratio as body weight gain (g) and food intakes was also calculated [16]. At the end of the intervention and after an 8-hour fast, euthanasia was carried out by means of gas saturation with an Isoflurane bell, visually confirming the state of unconsciousness. The animal was placed on the dorsal ulna for exsanguination by cardiac puncture. The blood, arranged in tubes with lithium heparin, was centrifuged (3000 rpm for 15 minutes) at 4ºC, storing the plasma at -80ºC for further analysis.

***Determination of blood glucose:*** 10 µl of sample or standard and 1 ml of reagent were used (previously prepared by mixing 150 mmol/L buffer, ATP >15 mmol/L, glucose-6-phosphate dehydrogenase >10 U/ml, preservatives, pH 8.9 and 70 mmol/L buffer, hexokinase >15 U/ml, NADP >1.5 mM preservatives, pH 6.9). After agitation, the tubes were left to incubate for 15 minutes at room temperature (16-25ºC). Then, absorbance was read at 340 nm in a Snibe Biossays 240 Plus® microplate reader. To calculate the glucose concentration, the sample absorbance was compared with the glucose standard (100 mg/dL or 5.55 mmol/L) provided by the reagent supplier, according to the Biosystems® manufacturer's instructions.

***Determination of total cholesterol:*** 10 µl of sample or standard and 1 ml of reagent were used (PIPES 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase >0.2 U/ml, cholesterol oxidase >0.1 U/ml, peroxidase >0.8 U/ml, 4-AA 0.5 mmol/L, pH 7.0). After shaking, the tubes were incubated for 10 minutes at room temperature (16-25ºC). Then, absorbance was read at 500 nm in a Snibe Biossays 240 Plus® microplate reader. To calculate the cholesterol concentration, the sample absorbance was compared with the cholesterol standard (200 mg/dL or 5.18 mmol/L) provided by the reagent supplier, according to the Biosystems® manufacturer's instructions.

***Determination of HDL cholesterol:*** 0.2 ml of sample or standard and 0.5 ml of reagent (0.4 mmol/L phosphotungstate and 20 mmol/L magnesium chloride) were used. After stirring, the sample was left at room temperature for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. 50 ul of supernatant were collected and mixed with 1 ml of reagent (PIPES 70 mmol/L, cholesterol esterase >0.2 U/ml, cholesterol oxidase >0.1 U/ml, peroxidase >1 U/ml, 4-aminoantipyrine 0.5 mmol/L, sodium cholate 0.5 mmol/L, dichlorophenol-sulfonate 4 mmol/L, pH 7.0) or 50 µl of standard. After shaking, the tubes are incubated for 30 minutes at room temperature (16-25ºC), and the absorbance was read at 500 nm in a Snibe Biossays 240 Plus® microplate reader. To calculate the HDL cholesterol concentration, the sample absorbance was compared with that of the HDL cholesterol standard (52.5 mg/dL or 1.36 mmol/L) provided by the reagent supplier, according to the Biosystems® manufacturer's instructions.

***Determination of triglyceride:*** 10 µl of sample or standard and 1 ml of reagent were used (PIPES 45 mmol/L, 4-chlorophenol 6 mmol/L, magnesium chloride 5 mmol/L, lipase >100 U/ml, glycerol kinase >1.5 U/ml, glycerol-3P-oxidase >4 U/ml, peroxidase >0.8 U/ml, 4-AA 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0). After shaking, it was left to incubate for 15 minutes at room temperature (16-25ºC), and then absorbance was read at 500 nm in a Snibe Biossays 240 Plus® microplate reader. To calculate the triglyceride concentration, the sample absorbance was compared with the triglyceride standard (200 mg/dL or 2.6 mmol/L) provided by the reagent supplier, according to the Biosystems® manufacturer's instructions.

***Atherogenic index and Coronary risk index:*** The atherogenic index (non-HDL cholesterol/HDL cholesterol) and coronary risk index (cholesterol total/HDL cholesterol) was also calculated [18].

***Statistical analysis:*** The statistical analyses were performed using IBM SPSS version 21 software. The normality of the data distribution was determined through the Shapiro-Wilk test. For the analysis of body weight, feed efficiency, HDL cholesterol and triglycerides, a single factor ANOVA test was applied with the Tukey or Games-Howell post hoc adjustment, depending on the homogeneity of the variance analyzed with the Levene statistic. On the other hand, dietary intake, energy consumption, plasma glucose and atherogenic indexes were analyzed with the Kruskal-Wallis test for data without normal distribution, with pairwise comparison. The data were represented by mean + standard deviation. Statistical significance was considered with $p<0.05$.

## RESULTS AND DISCUSSION

***Body weight and dietary variables:*** Animals fed a HFD for 10 weeks put on weight and food efficiency. Comparing experimental groups revealed that animals fed HFD supplemented with calafate (HFD+BM) showed a similar progression in body weight (Figure 1A). Compared to the HFD control group, calafate feeding did not significantly influence food intake and energy consumed ($p>0.05$) (Figure 1C, D and E). Noteworthy, food efficiency ratio in animals supplemented with calafate was decreased modestly but insignificantly 15% ($p>0.05$) (Figure 1B). It should be noted that the positive control group, supplemented with melatonin, showed a significantly decreased body weight, even though the energy intake was higher than at the HFD control, as well as the food efficiency was lower ($p<0.05$). These data findings indicated that, in some way, there is increased energy expenditure in these animals.

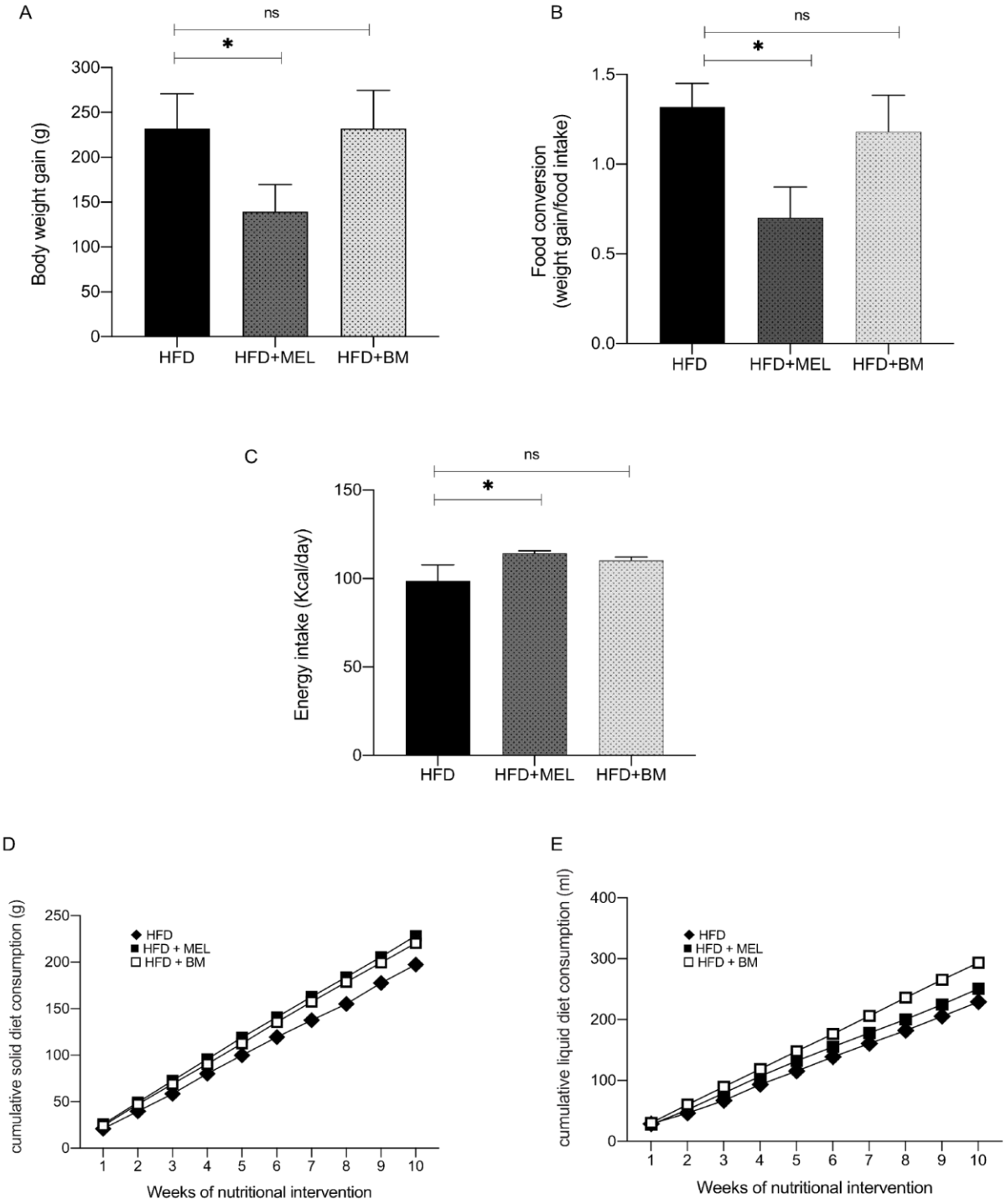



**Figure 1.** (A) Total body weight increment in grams after 10-week intervention. The graph represents the mean (SD) of the total body weight increment in experimental groups (B) Food efficiency ratio for the 10-week intervention. Calorie intake was calculated based on the energy density of the HFD. The graph represents the mean (SD) of the weekly body weight increase related to calorie intake in experimental groups. (C) Calorie intake for experimental groups during the 10-week intervention. The graph represents the mean (SD). (D) Cumulative solid intake for experimental groups during the 10-week intervention. The graph represents the mean. (E) Cumulative liquid intake for experimental groups during the 10-week intervention. The graph represents the mean. HFD: High-fat diet. MEL: melatonin. BM: Freeze-dried caulk (Berberis microphylla). n=5/group. Data are represented as mean + standard deviation. * $p < 0.05$ versus the HFD group.

***Plasma parameters, atherogenic index and coronary risk index:*** Plasma markers are presented in table 3. The results elucidated an increase in plasma total lipids, total cholesterol and a highly significant decreased HDL-cholesterol levels as compared to supplemented rats. Treatment of obese rats with calafate (HFD+BM) induced a decrease of total cholesterol (−50%), which was not significant. Triglycerides and glucose concentrations show similar levels to the control ($p>0.05$). However, the concentration of HDL cholesterol increased in groups HFD+BM and HFD+MEL, 38% and 106% respectively. The difference was significant in the animals that followed a high-fat diet supplemented with melatonin ($p<0.05$). The obesity rats exhibited a profound increase in atherogenic index (AI) and coronary risk index (CRI). The result indicated that treatment using calafate and melatonin exhibited significant decrease of AI (−81% and −93%, respectively, as compared to obesity control rats) and CRI (−62% and −72%, respectively, as compared to obesity control rats).

**Table 3.** Plasma glucose, lipid profile, atherogenic index and coronary risk index

| | HFD | HFD+MEL | HFD+BM |
|---|---|---|---|
| Glucose (mg/dl) | 217.4 + 40.9 | 202.2 + 16.6 | 227.8 + 36.4 |
| Triglyceride (mg/dl) | 106.8 + 23.8 | 106.6 + 14.2 | 111.8 + 13.9 |
| Total cholesterol (mg/dl) | 184.0 + 83.0 | 99.4 + 10.6 | 88.6 + 10.7 |
| HDL cholesterol (mg/dl) | 39.8 + 4.3 | 82.6 + 4.1** | 55.0 + 7.9 |
| Atherogenic index (#) | 3.3 + 1.8 | 0.2 + 0.1** | 0.6 + 0.1* |
| Coronary risk index (&) | 4.3 + 1.8 | 1.2 + 0.1** | 1.6 + 0.1* |

The data is represented as mean + standard deviation. n=5/group. (*) $p<0.05$; (**) $p<0.01$. #[(Total cholesterol–HDL cholesterol)/HDL cholesterol]; &[total cholesterol/HDL cholesterol]

The present study evaluated the effect of calafate (Berberis microphylla) supplementation on the lipid profile of HFD-induced obesity rats. Polyphenols represent a group of bioactive compounds present in fruits and vegetables that are of interest to the scientific community, especially due to their effectiveness in the prevention and treatment of some diseases of a cardiovascular nature such as obesity and dyslipidemia [19]. The fruit harvested for this study showed previously a higher phenol content than that reported for freeze-dried berries such as murtilla (Ugni molinae Turcz) and blueberry (Vaccinium corymbosum) [11,14]. In addition, the inhibition capacity of DPPH, expressed in Trolox µmol/100 g of dried fruit, was demonstrated, results consistent with previous research that identified several phenolic compounds for berries with similar phytochemical characteristics, such as maqui (Aristotelia chilensis), which contains antioxidants that can inhibit lipid peroxidation [20].

Polyphenols may be among the most important active plant compounds involved in beneficial effects, acting on plasma markers of cardiovascular disease. In this investigation, after a period of supplementation with calafate (Berberis microphylla), body weight did not vary significantly at the end of the experimental protocol. situation described in previous studies where final weight was not a variable that was significantly affected after consumption of a product with high antioxidant content, while other research did show a positive effect on weight loss, which can be explained by the diversity of obesity-inducing diets,

which in the case of high-fat diets can vary between 36% and 60% [21]. Specifically, calafate supplementation did not influence feeding parameters, however those animals supplemented with melatonin, as a positive control, showed lower body weight and feeding efficiency, even though their energy intake is higher and despite the lower dose used compared to previous research [22], so appetite suppression would not be the mechanism by which the lower body weight would be generated at the end of the protocol in animals supplemented with the pharmacological antioxidant. It is not known what exactly the mechanism is through which this occurs; however, there are approaches described in previous research that indicate that supplementation with melatonin in animal models generates an increase in adipose tissue, raising its thermogenic properties and thus leading to an increase in energy expenditure [23].

With regard to plasmatic markers, glucose concentration did not undergo significant variations after supplementation with calafate (Berberis microphylla). In human models the hypoglycemic effect of Berberis fruits has been observed [24], produced by a mechanism that has not yet been fully explained, although it may be associated with facilitating glucose catabolism due to the induction of the glycolysis pathway in the cell or by inhibiting the enzyme alpha-glucosidase [25]. It is known that polyphenols present in food can reach concentrations in the intestine, and can interact with the enzymes α-amylase and α-glucosidase, changing glycemic responses by inhibiting carbohydrate digestion [26], however in this research none of the antioxidants, natural or pharmacological, affected plasma glucose. With regard to lipid profile, the highest cardiovascular risk is conferred by the simultaneous presence of low HDL and total cholesterol and high triglycerides, in this context hypercholesterolemia is closely related to the pathogenesis of atherosclerosis [27]. Previous research, in an effort to achieve a marker of cardiovascular risk, has proposed AI and CRI, which have shown a correlation with lipoprotein particle size and increased LDL [28]. In this study, calafate supplementation was not associated with changes in total cholesterol and triglyceride concentrations, however, the product of elevation, although not significant, of HDL cholesterol significantly decreased AI and CRI. Previous results indicate that supplementation with antioxidants has an anti-atherogenic effect, since it significantly improves cardiovascular risk index and HDL cholesterol levels [18].

## CONCLUSION

The present study has confirmed that supplementation with melatonin can reduce cardiovascular risk, since the high atherogenic index and coronary risk index obtained by feeding the high fat diet are an indicator of high risk of developing cardiovascular disease. For calafate (Berberis microphylla) the effect is equivocal. More studies with greater numbers of animals are needed to evaluate the possible benefits of calafate at different doses.

**List of Abbreviations:** HFD: high-fat diet, TC: total cholesterol, c-LDL: low-density lipoproteins, c-VLDL: very low-density lipoproteins, TG: triglycerides, c-HDL: high-density lipoproteins, CVR: cardiovascular risk.

**Conflict of interest:** The authors have no conflicts of interest to declare

**Author´s contributions:** Carla Guzmán y Raúl Sánchez designed the study, writing- reviewing and editing. Carla Guzmán performed the experiments and analyzed the data. Raúl Sánchez supervision.

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