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FULL COMMUNICATION

Chilean Native Fruit Extracts Inhibit Inflammation Linked to the Pathogenic Interaction Between Adipocytes and Macrophages

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ABSTRACT Obesity is characterized by an increase in the infiltration of monocytes into the adipose tissue, causing an inflammatory condition associated with, for example, the development of insulin resistance. Thus, anti-inflammatory-based treatments could emerge as a novel and interesting approach. It has been reported that Chilean native fruits maqui (*Aristotelia chilensis*) and calafate (*Berberis microphylla*) present high contents of polyphenols, which are known for their antioxidant and anti-inflammatory properties. The aim of this study was to evaluate the ability of extracts of these fruits to block the pathogenic interaction between adipocytes and macrophages *in vitro* and to compare its effect with blueberry (*Vaccinium corymbosum*) extract treatment, which has been already described to possess several biomedical benefits. RAW264.7 macrophages were treated with 5 μg/mL lipopolysaccharides (LPS), with conditioned media (CM) from fully differentiated 3T3-L1 adipocytes, or in a coculture (CC) with 3T3-L1 adipocytes, in the presence or absence of 100 μM [total polyphenolic content] of each extract for 24 h. The gene expression and secretion profile of several inflammatory markers were evaluated. Nitric oxide secretion induced by LPS, CM, and CC was reduced by the presence of maqui (−12.2%, −45.6%, and −14.7%, respectively) and calafate (−27.6%, −43.9%, and −11.8%, respectively) extracts. Gene expression of inducible nitric oxide synthase and TNF-α was inhibited and of IL-10 was induced by maqui and calafate extract incubation. In conclusion, the extracts of these fruits present important inhibitory-like features over the inflammatory response of the interaction between adipocytes and macrophages, comprising a potential therapeutic tool against comorbidities associated with obesity development.

KEY WORDS: • ***adiposity*** • ***antioxidant activity*** • ***anti-inflammatory*** • ***3T3-L1 cells*** • ***RAW264.7 cells***

INTRODUCTION

OBESITY IS OFTEN ASSOCIATED with a low-grade chronic inflammation of the white adipose tissue (WAT), being a potential mechanism by which insulin resistance occurs.[1] Adipose tissue inflammation is due to macrophage infiltration, and the cross talk between these inflammatory macrophages and resident adipocytes appears as a key factor to the development of associated comorbidities.[2,3] Thus, several inflammatory products produced by this interaction, such as TNF-α, MCP-1, and nitric oxide (NO), correlate with increased body adiposity[4] and appear to participate in the induction and maintenance of the chronic inflammatory state associated with obesity.[5] Also, WAT overgrowth leads to downregulation of anti-inflammatory products, for example, adiponectin.[6] Therefore, a reduction in the inflammatory status based on anti-inflammatory agents could constitute a potential treatment to avoid adverse obesity-associated consequences, such as insulin resistance.

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In this sense, the use of native South American fruits is currently been claimed internationally,[7,8] specially driven by their important content of polyphenols. Polyphenols are metabolites with well-known positive health effects.[9] Anthocyanins, the main polyphenolic compounds, have been reported to possess antioxidant and anti-inflammatory features. It has been identified that the Chilean native fruits maqui and calafate present a high content of anthocyanins.[10] Therefore, in the present study, the ability of these fruit extracts to modulate the inflammatory response of an *in vitro* adipocyte–macrophage interaction was evaluated and further compared to the effect of blueberry (*Vaccinium corymbosum*) extracts as control. The former has been shown to possess important anti-inflammatory and antioxidant features.[11–14]

MATERIALS AND METHODS

Fruit extracts

Ripe fruits of maqui (*Aristotelia chilensis*), calafate (*Berberis microphylla*), and blueberry (*V. corymbosum*; as control) were obtained from SAAUTCHILE (Valdivia, Chile). Fruits were dried for 72 h at 40°C in a heating oven,

pulverized in a grinder, and extracted with methanol:water (1:1) for 24 h with stirring. Methanol was evaporated with a rotavapor, and the resulting aqueous extracts were maintained at −20°C until further assays were performed.

Characterization of extracts

Total polyphenolic content. Total polyphenol content (TP) was determined by the Folin-Ciocalteu colorimetric method[15] and expressed as gallic acid equivalents (GAE).

Total anthocyanins. Total anthocyanins (TA) were determined by the differential pH method.[16] Absorbance was measured at 515 and 700 nm using pH 1.0 and 4.5 buffers, respectively, with a molar extinction coefficient of 26,900. Results were expressed as mg cyanidin-3-glucoside equivalents/100 g dry weight (DW).

Antioxidant activity. The antioxidant activity (AA) was carried out by the ferric reducing/antioxidant power (FRAP) colorimetric assay[17] at 593 nm. Results were expressed as mmol Fe^{+2}/100 g DW.

Liquid chromatography coupled to mass spectrometry procedure. Samples were filtered (0.45 mm) and analyzed using a liquid chromatography coupled to mass spectrometry (LC-MS) system. The chromatographic systems LC-MS consisted of a HPLC Agilent 1100 (Agilent Technologies, Inc., Santa Clara, CA, USA) connected through a split to an Esquire 4000 ion trap LC/MS system (Bruker Daltoniks, Bremen, Germany). A C18 column (Luna 150 × 4.6 mm, 5 μm, 100 Å; Phenomenex, Inc., Torrance, CA, USA) was used; at the exit of the column, a splitter system divided the eluant in two fractions, one of them to an UV detector and the second to the mass spectrometer. A volume of 20 μL was injected. The mobile phases were water: acetonitrile:formic acid (87:3:10% v/v/v, solvent A) and water:acetonitrile:formic acid (40:50:10% v/v/v, solvent B) at a flow rate of 0.8 mL/min according to the following elution gradient: 0–15 min, 6% B; 15–30 min, 30% B; 30–35 min, 50% B; 35–41 min, 60% B; and 41–50 min, 6% B. Phenolic compounds were detected at 520 nm. The mass spectral data were acquired in a positive mode. Ionization (nebulization) was performed with nitrogen as drying gas at 55 psi, 365°C, flow rate of 10 L/min, and capillary voltage of 3000 V. The trap parameters were set in ion charge control using manufacturer default parameters. Collision-induced dissociation was performed by collisions with the helium background gas present in the trap. Fragmentation was set with Smart Frag.

Cell culture

The 3T3-L1 mouse preadipocytes and RAW264.7 mouse macrophages were obtained from the Laboratory of Cellular and Molecular Biology (INTA, University of Chile) and from the Laboratory of Biochemistry, Metabolism and Drug Resistance (ICBM, University of Chile), respectively. Both cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO_2. The 3T3-L1 cells were cultured in DMEM containing 4.5 g/L glucose and 10% calf serum. Two days after full confluence, cells were differentiated by incubation with 0.5 mM isobutylmethylxanthine, 1 mM dexamethasone, and 10 mg/mL insulin (all reagents from Sigma-Aldrich, St. Louis, MO, USA) in 4.5 g/L glucose DMEM supplemented with 10% fetal bovine serum for 2 days, and for the next 2 days with 10 mg/mL insulin in 4.5 g/L glucose DMEM supplemented with 10% fetal bovine serum. Thereafter, cells were maintained and refed every 2 or 3 days with media, without any hormones, until the cells reached a fully differentiated phenotype (14–16 days). These adipocytes were then utilized for coculture (CC) with macrophages and for conditioned media (CM) production. Regarding the preparation of CM, fully differentiated adipocytes were cultured in DMEM containing 4.5 g/L glucose and 10% fetal bovine serum for 24 h. Then, media were saved at 20°C for further assays. Macrophages were cultured in DMEM containing 4.5 g/L glucose and 10% fetal bovine serum. Cells were activated either with 5 μg/mL lipopolysaccharides (LPS; Sigma-Aldrich) or CM from adipocytes for 24 h in the presence or absence of 100 μM [TP] of each extract for 24 h. Cells and media were stored at −20°C for further determinations. Finally, a CC between adipocytes and macrophages was performed according to a previously described protocol.[18]

Secretion assays

The amount of nitrite in cell-free culture supernatants was measured using the Griess reagent according to the manufacturer's protocol (Sigma-Aldrich). IL-10 secretion to culture media was measured using the Mouse IL-10 ELISA Kit (Merck Millipore, Billerica, MA, USA). Cell viability was assessed using the LDH Cytotoxicity Assay Kit from Cayman Chemical Company (Ann Arbor, MI, USA) according to the manufacturer's indications.

Gene expression assays

Total RNA was isolated from samples using Trizol (Invitrogen, Paisley, United Kingdom), according to the supplier's protocol. Purified RNA (2 mg) was then treated with DNase (DNAfree kit; Ambion, Austin, TX, USA) and used to generate first-strand cDNA with M-MLV reverse transcriptase (Invitrogen), utilizing random hexamers (Invitrogen) and dNTP mix (Bioline, London, United Kingdom), according to the manufacturer's protocol. The resultant cDNA was amplified with specific primers for mouse inducible nitric oxide synthase (iNOS), TNF-α, IL-10, MCP-1, and ADIPOQ in a total volume of 10 μL. Real-time PCR was performed in a Stratagene Mx3000P System (Agilent Technologies) following the manufacturer's recommendations (Applied Biosystems, Foster City, CA, USA). All the expression levels of the target genes studied were normalized by the expression of cyclophilin as the selected internal control (also supplied by Applied Biosystems). Fold change between groups was calculated by the $2^{(-\Delta\Delta Ct)}$ method.

Statistical analyses

Data are expressed as mean ± SD. Differences were assessed using one-way ANOVA followed by the Tukey *post hoc* test. All statistical analyses were performed with the GraphPad Prism 6.0 statistical package (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Characterization of extracts

Table 1 shows TP, TA, and AA of the studied extracts. All three determinations presented higher levels in maqui, followed by calafate and blueberry extracts. Moreover, an LC-MS identification was performed comparing m/z signals and fragment ions of the anthocyanin pattern of all extracts (Fig. 1 and Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/jmf). Regarding maqui extracts, the main compound identified was delphinidin-3-sambubioside-5-glucoside (m/z: 759.5, peak 3, 36.5%), followed by delphinidin-3-galactoside (m/z: 465.3, peak 7, 23.8%) and delphinidin-3,5-*O*-diglucoside (m/z: 627.4, peak 4, 18.9%). Other compounds identified were glucosides of cyanidin. In the case of calafate fruit extract, the most abundant anthocyanin was delphinidin-3-glucoside (m/z: 465.3; peak 3, 36.1%), followed by petunidin and malvidin, both conjugated to 3-glucoside or 3-galactoside (m/z: 479.1, peak 7, 29.9% and m/z: 493.2, peak 10, 15.2%, respectively). Other anthocyanins detected in calafate were glycosylated derivatives of cyanidin, malvidin, peonidin, and petunidin. Finally, regarding blueberry fruit extracts (which was used as control), the major anthocyanin identified was malvidin-3-glucoside or conjugated to galactose (m/z: 493.4, peak 6, 35.4%) followed by malvidin-3-arabinoside (m/z: 463.3, peak 8, 19.9%). Glucosides of cyanidin, delphinidin, petunidin, and peonidin were also identified.

Table 1. Total Polyphenolic and Anthocyanin Content and Antioxidant Capacity of Maqui, Calafate, and Blueberry Extracts

	Maqui	*Calafate*	*Blueberry*
Total polyphenols (mg GAE/ 100 g DW)	1906.5 ± 73.2[a]	1344.2 ± 10.5[b]	1229.6 ± 20.9[b]
Total anthocyanins (mg C-3-G E/100 g DW)	72.7 ± 0.1[a]	31.5 ± 0.8[b]	20.1 ± 1.2[c]
Antioxidant activity (mmol Fe^{+2}/ 100 g DW)	38.9 ± 1.7[a]	11.7 ± 1.8[b]	5.9 ± 0.1[c]

One-way ANOVA followed by Tukey's *post hoc* tests were performed to identify statistical differences among groups.

Different letters mean statistical difference of at least $P < .05$.

GAE, gallic acid equivalents; DW, dry weight; C-3-G E, cyanidin-3-glucoside equivalents.

Maqui and calafate extracts inhibit RAW264.7 macrophage activation by LPS and 3T3-L1 CM

All extracts significantly prevented LPS-induced NO secretion by macrophages; however, the calafate extract induced the more drastic effect (−27.6%, Fig. 2A). Furthermore, neither LPS nor the extracts exerted a significant modulation of IL-10 secretion (Fig. 2B). When the effect of adipocyte CM on RAW264.7 cells was analyzed, the macrophage inflammatory activity was observed, resulting in an increase (103%) and decrease (−26.1%) of NO and IL-10 secretion, respectively (Fig. 2C, D). Regarding pretreatment, a prevention of the CM-induced NO release by maqui (−45.6%), calafate (−44%), and blueberry (−42.7%) extracts was observed, and a restoration of IL-10 secretion to control levels only by maqui extracts (−5.4% with respect to control).

To evaluate whether the extracts would modulate the gene expression of cytokines associated with certain inflammatory pathways, the relative change of *iNOS* and *TNF-α* and *IL-10* mRNA expression was determined. It was observed that LPS increased *iNOS* (106.9%) and *TNF-α* (190.4%) expression (Fig. 3A, B), indicating successful RAW264.7 macrophage activation. However, *IL-10* an anti-inflammatory cytokine was also increased with LPS treatment (325.7%) (Fig. 3C). When the effects of the extracts were assessed, a protective effect (tendency) was observed only with maqui (−32.2%) and calafate (−36.9%) extracts over *iNOS* expression (Fig. 3A). A same pattern was observed on *TNF-α* gene expression (−49.3% reduction by maqui and −32.8% by calafate extracts) (Fig. 3B). Finally, all extracts significantly prevented LPS-induced IL-10 secretion (−30.6% to −36.4%) (Fig. 3C).

On the other hand, it was observed that the CM treatment was able to significantly activate macrophages, inducing an increase (66.2%) and decrease (−63.1%) in *iNOS* and *IL-10* gene expression, respectively (Fig. 3D, F). Regarding pretreatments, it was observed that calafate extracts induced the highest inhibition (−69.7%) followed by blueberry (−68.2%) and maqui (−49.4%) extracts, when compared with CM treatment (Fig. 3D). In the case of *IL-10* gene expression, only maqui extract was able to completely revert the effect of CM treatment (to −2.2% reduction with respect to control) (Fig. 3F). No effects were observed in the *TNF-α* mRNA expression (Fig. 3E).

Maqui and calafate extracts inhibit the inflammatory response induced in a 3T3-L1 and RAW267.4 CC

To further evaluate the anti-inflammatory effects of the native fruit extracts, 3T3-L1 and RAW267.4 were cultured in direct contact with each other, aiming to obtain a more realistic model of obesity-related inflammation. In this CC, a significant increase of NO levels with respect to the control culture was observed (51.8%). Only maqui (−14.7%) and calafate (−11.8%) extracts induced a slight prevention of this inflammatory secretion profile (Fig. 4A). On the other hand, no effects were observed in *IL-10* secretion (Fig. 4B).

Finally, the expression levels of inflammatory (*MCP-1*) and anti-inflammatory (*ADIPOQ*) transcripts were assessed.

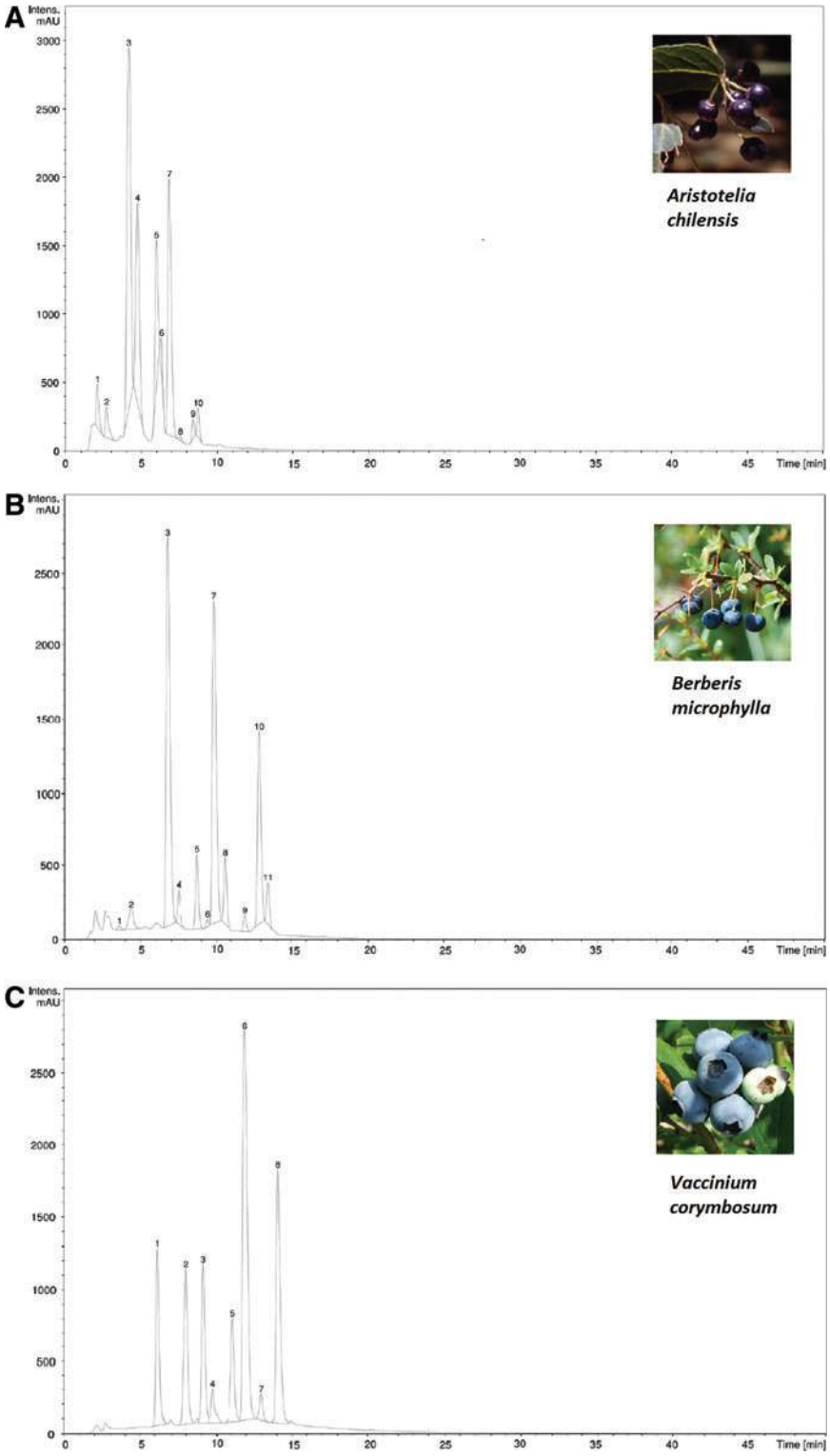

FIG. 1. HPLC-UV chromatogram detected at 520 nm of all studied extracts. In maqui extracts **(A),** peak 2: cyanidin-3,5-diglucoside; 3: delphinidin-3-sambubioside-5-glucoside; 4: delphinidin-3,5-*O*-diglucoside; 5: cyanidin-3-sambubioside-5-glucoside; 6: delphinidin-3-sambubioside; 7: delphinidin-3-galactoside or delphinidin-3-galactoside; 9: cyanidin-3-sambubioside; 10: cyanidin-3-glucoside or cyanidin-3-galactoside were identified. In calafate extracts **(B),** peak 1: cyanidin-3,5-diglucoside or cyanidin-3-soforoside; 2: petunidin-3-*O*-galactoside-5-*O*-glucoside or petunidin-3-*O*-glucoside-5-*O*-glucoside; 3: delphinidin-3- glucoside or delphinidin-3- galactoside; 5: cyanidin-3-glucoside or cyanidin-3-galactoside; 6: cyanidin-3-rutinoside; 7: petunidin-3-galactoside or petunidin-3-glucoside; 8: petunidin-3-rutinoside; 9: peonidin-3-galactoside or peonidin-3-glucoside; 10: malvidin-3-galactoside or malvidin-3-glucoside; 11: malvidin-3-rutinoside were identified. Finally, in blueberry extracts **(C),** peak 1: delphinidin-3-glucoside or delphinidin-3-galactoside; 2: delphinidin-3-arabinoside; 3: petunidin-3-galactoside or petunidin-3-glucoside; 4: cyanidin-3-arabinoside or cyanidin-3-xiloside; 5: petunidin-3-arabinoside; 6: malvidin-3-glucoside or malvidin-3-galactoside; 7: peonidin-3-arabinoside; 8: malvidin-3-arabinoside were identified. Intens., intensity; mAU, milli-absorbance units; min, minutes. Color images available online at www.liebertpub.com/jmf

Cell-to-cell interaction caused a significant increase (337%) and decrease (−87.7%) of *MCP-1* and *ADIPOQ* mRNA expression, respectively, indicating a successful establishment of the inflammatory model (Fig. 4C, D). Although extract pretreatments were not able to significantly modify both adipokine expressions, maqui (−25.1%) and calafate (−29.5%) extracts induced a slight reduction in *MCP-1* mRNA content when comparing to the CC group (Fig. 4C).

DISCUSSION

It has been recognized that increased body adiposity is habitually accompanied by an increased systemic oxidative

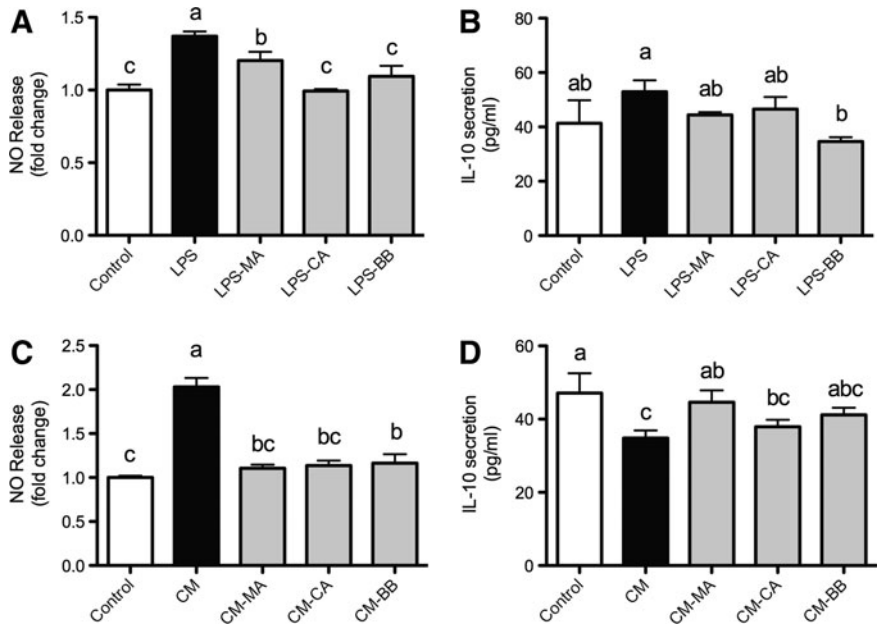

FIG. 2. Nitric oxide and IL-10 release by RAW264.7 macrophages. Nitric oxide **(A)** and IL-10 **(B)** release were detected in mouse macrophages that were pretreated for 1 h with 100 μM [total polyphenol] extracts of maqui, calafate, and blueberry, and then treated for 24 h with 5 μg/mL LPS. Likewise, both nitric oxide **(C)** and IL-10 **(D)** were detected when macrophages were treated in similar conditions, but activated with conditioned media from mature 3T3-L1 adipocytes instead of LPS. Data ($n = 3$) are expressed as mean ± SD. One-way ANOVA followed by Tukey *post hoc* test were performed to identify statistical differences among groups. NO, nitric oxide; LPS, lipopolysaccharide; CM, conditioned media; MA, maqui; CA, calafate; BB, blueberry. Different letters mean statistical difference of at least $P < .05$.

stress and a low-grade inflammation condition in the adipose tissue. In this sense, tools aiming to counteract these inflammatory processes are welcome to be investigated to set up novel protocols for prevention and/or treatment of obesity-related illnesses. The present article describes how polyphenolic-rich extracts from two Chilean native fruits inhibited inflammatory features *in vitro*.

As it has been working with crude extracts, a proper exhaustive characterization of each material used was performed. The maqui extract presented the higher antioxidant potential among all extracts analyzed. These results could be attributed to the structural features of the polyphenols and/or other compounds present in each fruit extract. Compared to previously published works, TP reported in the present study was lower than those reported in maqui (4570 mg GAE/100g DW[19]), calafate (3490 mg GAE/100 g DW[20]), and blueberry extracts (5660 mg catechin equivalents/100 g fresh weight [FW][21]). This could be explained by the use of a nonacidified hydroalcoholic extract, which probably resulted in a decrease extraction efficiency of anthocyanins

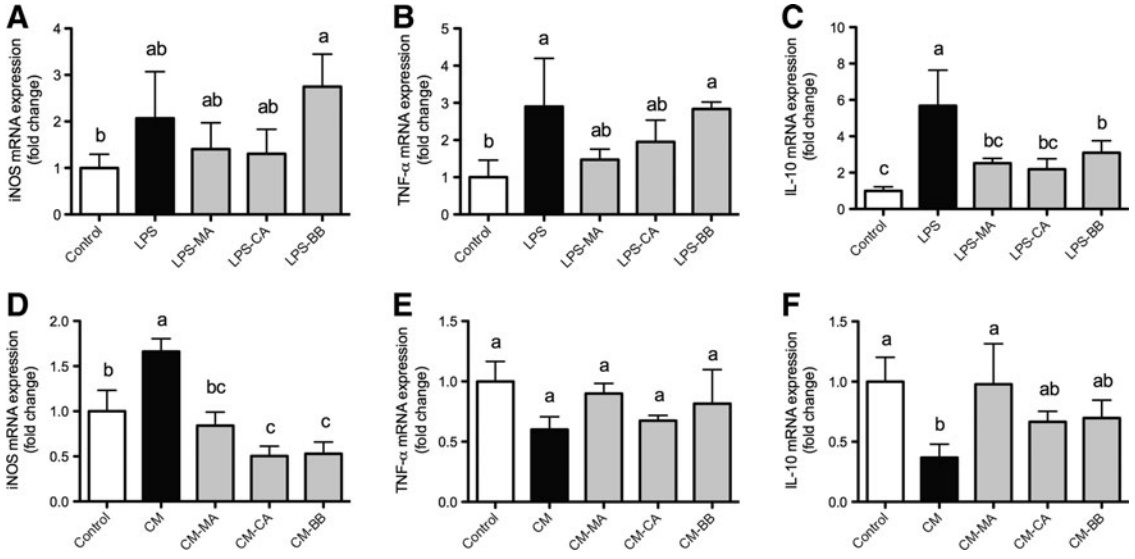

FIG. 3. Gene expression analysis in RAW264.7 macrophages. iNOS **(A)**, TNF-α **(B)**, and IL-10 **(C)** transcript contents were detected in mouse macrophages that were pretreated for 1 h with 100 μM [total polyphenol] extracts of maqui, calafate, and blueberry, and then treated for 24 h with 5 μg/mL LPS. Likewise, iNOS **(D)**, TNF-α **(E)**, and IL-10 **(F)** transcript contents were detected when macrophages were treated in similar conditions, but activated with conditioned media from mature 3T3-L1 adipocytes instead of LPS. Data ($n = 3$) are expressed as mean ± SD. One-way ANOVA followed by Tukey *post hoc* test were performed to identify statistical differences among groups. Different letters mean statistical difference of at least $P < .05$.

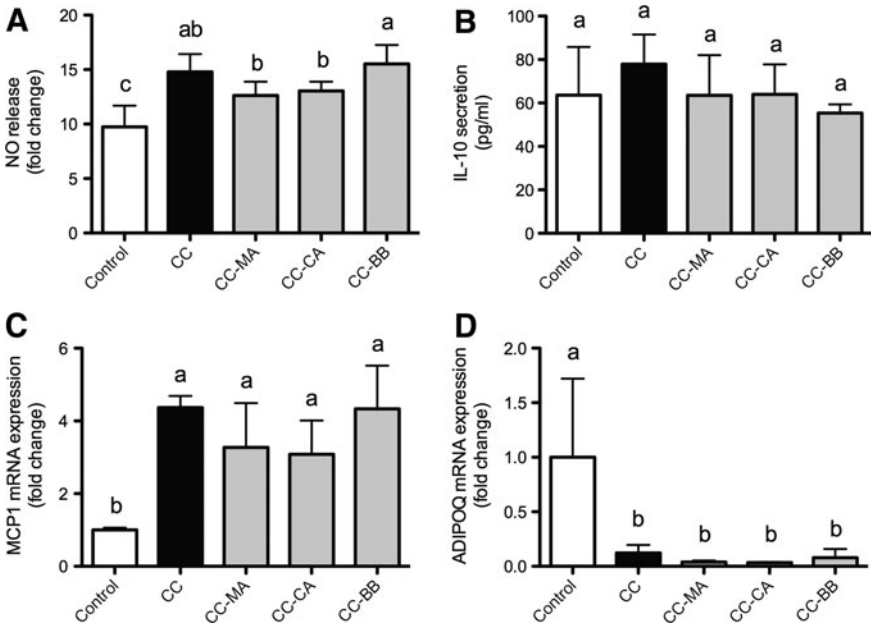

FIG. 4. Nitric oxide and IL-10 release, and gene expression analyses in coculture of 3T3-L1 adipocytes and RAW264.7 macrophages. Nitric oxide **(A)** and IL-10 **(B)** release, and MCP-1 **(C)** and ADIPOQ **(D)** transcript contents were detected in a coculture between mouse adipocytes and macrophages, in the presence of 100 μM [total polyphenol] extracts of maqui, calafate, and blueberry, after 24 h. As control, we used coculture without extracts. Data ($n = 3$) are expressed as mean ± SD. One-way ANOVA followed by Tukey *post hoc* test were performed to identify statistical differences among groups. Different letters mean statistical difference of at least $P < .05$.

and other compounds. With respect to AA and TA in methanolic extracts, in previous studies a lower AA has been reported (12.32 mmol Fe^{+2}/100 g FW), measured by the FRAP method. Although in this study a shorter extraction time was performed, that could affect the results.[22] On the other hand, a higher content of TA has been found (211.9 mg delphinidin-3-glucoside equivalents/100 g DW), although this measurement was performed by HPLC-PAD-MS.[23] Meanwhile, values of 2.53, 17.81, and 17.88 μmol/g FW of TA in blueberry, calafate, and maqui extracts have been described, respectively.[10] Regarding LC-MS compound identification, delphinidin-3-sambubioside-5-glucoside was also identified previously as a main phenolic compound in maqui.[23–25] In the case of calafate extract, the reported anthocyanin pattern agrees with the one previously found.[10,26] Finally, in blueberry extracts, the compounds observed are in accordance with those reported before,[21,27] with malvidin-3-glucoside, malvidin-3-galactoside, malvidin-3-arabinoside, and delphinidin-3-galactoside as major components. Thus, the identification of anthocyanins by LC-MS in the three extracts studied was consistent with previous reports by other authors. In maqui and calafate fruit extracts, delphinidin and petunidin (both glycosylated) predominated. On the other hand, malvidin was the major compound detected in blueberry. These differences in composition may explain, in part, the higher TP content and AA of maqui and calafate.

We found that the anthocyanin/polyphenol ratio in maqui, calafate, and blueberry extracts was 0.038, 0.023, and 0.016, respectively. Indeed, at the same concentration of TPs (100 μM) when compared with maqui extracts, calafate presented −38.5%, while blueberry presented −57.1% of TA content. These data were not related to the anti-inflammatory effect observed in the present study, suggesting that biomedical features found do not depend on concentration, but on the combination of anthocyanins. Therefore, it is necessary that future studies focus on assessing the effect of each pure compound and the synergistic interactions between them.

In the present work, we observed that LPS and CM from mature adipocytes exerted differential impact on macrophages, TNF-α and iNOS mRNA expression. One possible explication could be related to the fact that adipocytes can also secrete TNF-α,[28] and this molecule acts mediating iNOS gene expression on macrophages.[29] Therefore, CM could not cause a significant effect on TNF-α mRNA levels, but on iNOS expression.

Another controversial result observed was the differential IL-10 gene expression between LPS and CM induced in macrophages. Since IL-10 is recognized as an anti-inflammatory cytokine, a downregulation against the stimulus applied should be expected; however, the opposite outcome was observed when cells were incubated with LPS. In this sense, it has been reported that IL-10 could present a proinflammatory feature when endotoxemia is induced.[30] Furthermore, IL-10 is recognized as an anti-inflammatory cytokine secreted by the M2 macrophage subset. Thus, the upregulation observed when cells were incubated with LPS could be due to an M2b phenotype polarization. This macrophage subset is characterized with an IL-10/TNF-α secretory profile and is active with LPS and other immune complex treatments.[31,32] In this regard, the results observed in the CM induction model were of great importance, since these are closer to a real adipocyte–macrophage interaction.

Nevertheless, overall in the present article, it was reported that the pre- and co-treatment with the three fruit extracts reduced the expression/secretion of inflammatory markers on 3T3-L1 adipocytes, macrophages, and a mixed coculture. This outcome was especially evident for maqui and calafate. Thus, the fact that these former extracts present better results suggests that a combination or specific compounds could exist, ascribed only to these native fruits that are related to

higher healthy potential. Several previous reports support the present findings. Regarding the antioxidant and anti-inflammatory activities of the maqui fruit, it has been observed that this fruit extract induced a reduction in lipid peroxidation and presented important antioxidant effects on the rat heart.[33] Moreover, subfractions of maqui have been described to prevent LDL oxidation and protect against oxidative stress in human endothelial cells.[34] On the other hand, it has been described[25] that an anthocyanin-enriched fraction of maqui improves fasting glucose and glucose intolerance in diet-induced hyperglycemic obese mice, diminishes glucose production, improves insulin-stimulated glucose-6-phosphatase inhibition on hepatic cells, and increases basal and insulin-stimulated glucose uptake in myotubes. It was also observed that crude extracts of maqui inhibited the α-glucosidase and α-amylase activity, improving postprandial glucose tolerance in diabetic patients.[19] Likewise, it was described that subfractions of maqui showed anti-inflammatory effects in tetradecanoylphorbol acetate-induced formation of mouse ear edema.[35] Finally, it has been reported that maqui extracts inhibited lipid accumulation in differentiating 3T3-L1 cells, and the production of NO and prostaglandin E_2 and the expression of iNOS and cyclooxygenase-2 in RAW264.7 macrophages.[36] The present work adds novel information related to a novel obesity-related anti-inflammatory feature. Finally, regarding calafate, it has been observed that chloramphenicol-induced reactive oxygen species production was inhibited by water *Berberis* extracts in human isolated blood cells.[37] Therefore, the anti-inflammatory features observed in the present work by the calafate extract could be ascribed to its antioxidant properties.

Efforts must be made to reduce diabetes incidence worldwide. Possibly, major improvements can be accomplished counteracting the establishment of the inflammatory state in WAT. Novel strategies or specific compounds that are able to diminish this inflammation are highly valuated. In the present work, maqui and calafate extracts were able to modulate the proinflammatory state generated by the interaction between adipocytes and macrophages. These interesting outcomes must be further studied to generate new therapeutic tools against inflammation-related insulin resistance.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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