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FULL COMMUNICATION

Anthocyanins from Fermented Berry Beverages Inhibit Inflammation-Related Adiposity Response *In Vitro*

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ABSTRACT Increased adiposity has been associated with macrophage infiltration into the adipose tissue which, in turn, leads to obesity comorbidities, including type 2 diabetes. The objective of this study was to evaluate the effect of anthocyanin (ANC)-enriched fractions from blackberry–blueberry beverages on inflammation and adipogenesis in an *in vitro* model of inflammation mimicking the pathologic interaction between adipocytes and macrophages. Blend ANCs inhibited secretion of nitric oxide (17.5%), tumor necrosis factor-alpha (TNF-α) (89.4%), and phosphorylated-p65 nuclear factor kappa-B (52.1%) in lipopolysaccharide (LPS)-induced RAW264.7 macrophages after 24 h. Blends reduced intracellular fat accumulation (28.2%) when applied during 3T3-L1 adipocyte differentiation and inhibited isoproterenol-induced lipolysis (18.6%) of mature 3T3-L1 cells. In addition, blend ANCs restored adiponectin-blunted gene expression induced by the TNF-α treatment (18.2%) and reduced the glycerol release (15.9%) induced by LPS-induced macrophage-conditioned media (CM) in adipocytes. Furthermore, blends slightly restored the insulin-induced glucose uptake of adipocytes, blunted by the CM treatment. In conclusion, ANCs from blueberry and blackberry dealcoholized fermented beverages are potential inhibitors of inflammation-related adiposity response and sensitizers of insulin signaling in adipocytes.

KEY WORDS: • ***adiposity*** • ***anthocyanins*** • ***anti-inflammatory*** • ***diabetes*** • ***fermentation***

INTRODUCTION

OBESITY IS AN IMPORTANT health issue negatively affecting our society.[1] Excessive body fat accumulation initiates several associated clinical manifestations, such as type 2 diabetes (T2D) and cardiovascular disease, among others.[2]

Obesity is often accompanied by a low-grade inflammation in the adipose tissue.[3] Adipokines, cytokines, and other factors produced by this tissue during adipose hyperplasia could be responsible for the presence and prevalence of inflammation.[4] Several inflammatory proteins derived from adipose tissue, such as tumor necrosis factor-alpha (TNF-α), interleukin-6, monocyte chemoattractant protein-1 (MCP-1), and nitric oxide (NO), correlate with increased body adiposity.[5] This may lead to inflammatory macrophage infiltration into the adipose tissue. Since the cross talk between macrophages and adipocytes may negatively influence cellular insulin sensitivity,[6] this consequently aggravates the obesity state.[7] Therefore, a reduction in inflammatory status by anti-inflammatory agents could constitute a potential approach to reduce adverse obesity-associated consequences.

Dietary flavonoids have been linked to anti-inflammatory pathways,[8] and several berries or berry compounds have been investigated for their anti-inflammatory properties.[9–11] Blueberries (*Vaccinium corymbosum*) and blackberries (*Rubus spp.*) are important sources of anthocyanins (ANCs); water-soluble pigments responsible for the colors of fruits and vegetables.[12] It has been reported that the consumption of these phytochemicals exerts several positive health effects, particularly due to their high antioxidant capacity.[8,13] Research has shown that fermented juices and wines from berries inhibit inflammatory protein production or expression more than their unfermented counterparts.[14–17] The role of phenolic compounds, such as ANCs, in the interaction between the adipose and inflammatory state is not yet fully understood. Therefore, it is important to identify and evaluate specific bioactive phenolic compounds from fermented berry beverages on obesity-related inflammation.

The aim of the present study was to evaluate the effect of ANC extracts, from combinations of blueberry–blackberry dealcoholized fermented beverages, on the levels of markers of inflammation and adipogenesis in a dual cellular system, mimicking the pathogenic interaction between adipocytes and macrophages. The hypothesis was that the addition of

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antioxidant agents, such as ANCs, would lead to a reduction in the inflammatory cross talk between macrophages and adipocytes and, thus, improve insulin sensitivity as a potential approach to improve obesity-associated adverse consequences.

MATERIALS AND METHODS

Materials

Enriched ANC fractions stored from the previous work of our group were utilized for all experiments.[18] These fractions were obtained from the following blends of blackberry (Bck) and blueberry (Blue) wines: 100% Bck:0% Blue, 70% Bck:30% Blue, and 30% Bck:70% Blue. These blend ANCs were selected based on their high antioxidant and anti-inflammatory properties and chromatographic results at both 520 and 280 nm to determine the composition of both ANCs and other phenolic compounds.[18] The blends were made from wines produced from highbush blueberries (*V. corymbosum*) and blackberries (*Rubus spp.*) grown at the Dixon Springs Agricultural Center in Simpson (Illinois) and fermented with *Saccharomyces bayanus*, as previously described.[18,19] One hundred μM cyanidin-3-O-glucoside (C3G) equivalents were used for cell culture.

Murine macrophage RAW264.7, preadipocyte 3T3-L1 cell lines, and Dulbecco's modified Eagle's medium with L-glutamine (DMEM) were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Fetal bovine serum and bovine serum were from Invitrogen (Grand Island, NY, USA).

The rabbit polyclonal antibody (phosphorylated-p65 [p-p65] nuclear factor kappa-B [NFκB]) and mouse monoclonal antibody (actin) and their control lysates were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Anti-rabbit and anti-mouse IgG horseradish peroxidase-conjugated secondary antibodies were from GE Healthcare (Buckinghamshire, United Kingdom).

Cell culture

The mouse 3T3-L1 preadipocyte and RAW264.7 macrophage, (ATCC, Rockville, MD, USA) cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Experiments were conducted as follows:

I. RAW264.7 mouse macrophages were seeded in six-well plates at 70,000 cells/cm^2 density, pretreated with each blend ANC (100 μM C3G) for 1 h, and then stimulated with lipopolysachharides (LPS; 1 μg/mL) for 24 h. Cells were collected and processed immediately according to western blot sample preparation. Supernatants were collected and stored at −80°C for their use as conditioned media (CM) or until secretion analyses were performed.

II. Preadipocyte 3T3-L1 cells were cultured in six-well plates 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 μM dexamethasone, and 10 μg/mL insulin in 4.5 g/L mM glucose DMEM supplemented with 10% fetal bovine serum, using a previously described protocol.[20] To determine the impact on adipogenesis, blend ANCs were added at each media refeed until adipocytes reached 8–10 days of differentiation. Fully differentiated adipocytes were fixed and stained for triglyceride determination, as described below, or were incubated for 24 h in the presence or absence of 10 nM isoproterenol (IP) and each blend ANC for lipolysis assays. IP was used as a model of catecholamine-induced lipolysis. Cells were collected for gene expression assays, and supernatants were collected and stored at −80°C for glycerol determination.

III. Fully differentiated adipocytes were incubated for 24 h in the presence or absence of macrophage-CM (obtained as described in I) for 24 h or 4 ng/mL TNF-α (Sigma-Aldrich Company, St. Louis, MO, USA). Cells were pretreated for 1 h with each blend ANC and then incubated with TNF-α. Cells and supernatants were collected and stored at −80°C until further analysis for glycerol release, gene expression, and secretion of MCP-1 and adiponectin.

IV. Fully differentiated adipocytes were incubated for 96 h (replacing media every 24 h) with CM (obtained as described in I) for evaluating modulation of insulin sensitivity (by incubating with 100 nM insulin). Supernatants were collected and stored at −80°C for determination of glucose uptake.

The rationale for using fermented blend ANCs was that fermentation has been seen to increase the phenolic content of fruit juices and their anti-inflammatory effects.[14–17]

Cell viability assay

Cell viability was measured with the lactate dehydrogenase Cytotoxicity Assay at 24 h according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, USA).

Western blot

Western blotting was performed by loading 20 μg protein from cell lysates in 4–20% Tris–HCl gels (BioRad, Hercules, CA, USA) for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), as described previously.[18] Proteins were transferred to a PVDF Hybond-P membrane (GE Healthcare, Buckinhamshire, United Kingdom), blocked, washed, and subsequently incubated with either the p-Akt or p-p65 NFκB primary antibody (Santa Cruz Biotechnology, Inc.). Membranes were washed again and incubated with the anti-mouse (for p-Akt) or anti-rabbit (for p-p65 NFκB) IgG horseradish peroxidase-conjugated secondary antibody. The expression of proteins was visualized using a chemiluminescent reagent (GE Healthcare, Pittsburgh, PA, USA) following the manufacturer's instructions using a GL 4000 Pro Imaging system (Carestream Health, Inc., Rochester, NY, USA).

Adipogenesis assay

Adipogenesis was determined by measuring triglyceride accumulation through Oil Red O staining, according to a

modified protocol.[21] Briefly, the staining solution was prepared by dissolving Oil Red O (Sigma-Aldrich Company) in isopropanol (5 mg/mL), then filtered, and diluted 40% with distilled water. Adipocyte layers were washed with phosphate-buffered saline, fixed with 2.7% formaldehyde, washed with 60% isopropanol, allowed to dry, and then stained for 30 min with the Oil Red O solution. Wells were washed four times with distilled water before quantifying the accumulated lipid by eluting the stain from cells with 100% isopropanol and reading the absorbance at 540 nm.

Glycerol release

Glycerol production, as a measure of adipocyte lipolysis, was calculated as the increase of glycerol in the medium from adipocytes after 24 h, using a glycerol assay (RANDOX Laboratories, Crumlin, United Kingdom) according to the manufacturer's indications.

NO release measurement

The amount of nitrite in the supernatant was measured using the Griess reagent according to the manufacturer's protocol (range of standards = 0.43–65 μM nitrite) (Sigma-Aldrich Company).

Gene expression assays

All procedures were as previously described with modifications.[20] Briefly, total RNA was isolated from samples using the RNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). First-strand cDNA was then obtained using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The resultant cDNA was amplified with specific probes for mouse *MCP-1* and adiponectin (*ADIPOQ*) in a total volume of 10 μL. Real-time polymerase chain reaction (RT-PCR) was performed in an ABI PRISM 7000 HT Sequence Detection System following the manufacturer's recommendations: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. RT-PCR products were analyzed with the SDS 2.3 and RQ Manager 1.2 software (Applied Biosystems). Expression levels of target genes studied (*FAS, HSL, MCP-1, ADIPOQ*) were normalized with the expression of cyclophilin as the selected internal

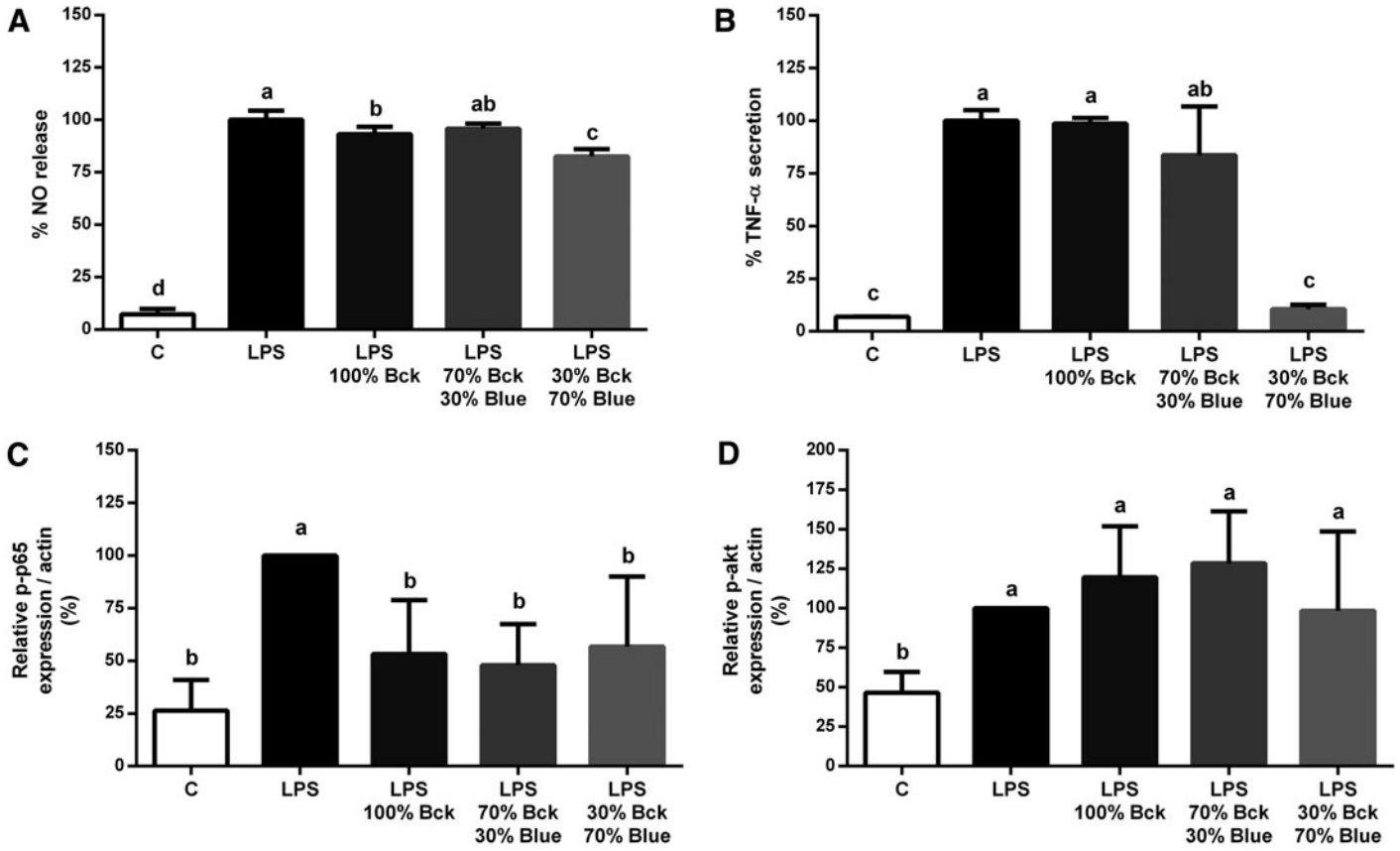

FIG. 1. LPS-stimulated macrophage response. The level of macrophage activation by LPS (1 μg/mL) treatment was determined by NO release **(A)**, TNF-α secretion **(B)**, relative p-p65 expression **(C)**, and relative p-Akt expression **(D)** after 24 h, in the presence or absence of each blend ANCs (100 μM C3G equivalents). Data ($n = 3$–6) are expressed as mean ± SD. One-way ANOVA followed by Tukey's *post hoc* test was performed to identify statistical differences. Different letters represent statistical differences of at least $P < .05$. ANC, anthocyanin; Bck, blackberry; Blue, blueberry; C3G, cyanidin-3-O-glucoside; C, Control; LPS, lipopolysachharide; NO, nitric oxide; phosphorylated-p65, p-p65; SD, standard deviation; TNF-α, tumor necrosis factor-α.

control (probe supplied by Applied Biosystems). Fold change between groups was calculated using the $2^{-\Delta\Delta Ct}$ method.

MCP-1, adiponectin, and TNF-α secretion analyses

MCP-1 secretion into culture media was measured following the manufacturer's instructions using the MCP-1 Mouse ELISA Kit (Invitrogen), adiponectin using the Adiponectin Mouse ELISA Kit (Invitrogen), and TNF-α using the TNF alpha Murine ELISA Kit (Abcam, Inc., Cambridge, MA, USA).

Glucose uptake

Glucose utilization by cells was assessed by measuring the concentration of glucose in the medium with the Autokit Glucose from Wako Chemicals (Richmond, VA, USA). After 100 nM insulin treatment for 24 h, the amount of glucose in the medium was then subtracted from the initial concentration of glucose with previously reported calculations.[22]

Statistical analyses

Results are expressed as mean ± standard deviation. All data were evaluated using one-way ANOVA, followed by the Tukey *post hoc* test. Association analyses were performed using the Pearson correlation coefficient. Significance was determined at $P < .05$. Statistical analyses were performed using the GraphPad Prism Software 6.0 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Inhibition of macrophage activation

The 100% Bck and 30% Bck:70% Blue blend ANC treatment effectively reduced LPS-induced macrophage activation as measured by NO release (Fig. 1A). Likewise, LPS-induced TNF-α release was also significantly inhibited compared to the LPS-stimulated control in the same treatment groups (Fig. 1B), with the greatest reduction by the 30% Bck:70% Blue treatment (90%). NO and TNF-α release was highly correlated ($r = 0.897$, $P < .001$). Moreover, the expression of the p-p65 subunit of NFκB was decreased due to treatments with all blend ANCs in comparison to the nontreated LPS-stimulated control (53.4% ± 25.6%, 47.9% ± 19.5%, 56.8% ± 33.2% for 100% Bck, 70% Bck:30% Blue, and 30% Bck:70% Blue, respectively vs. 100% LPS control group) (Fig. 1C). The analysis of the relative expression of p-Ser^{473}-Akt is presented in Figure 1D; no statistical

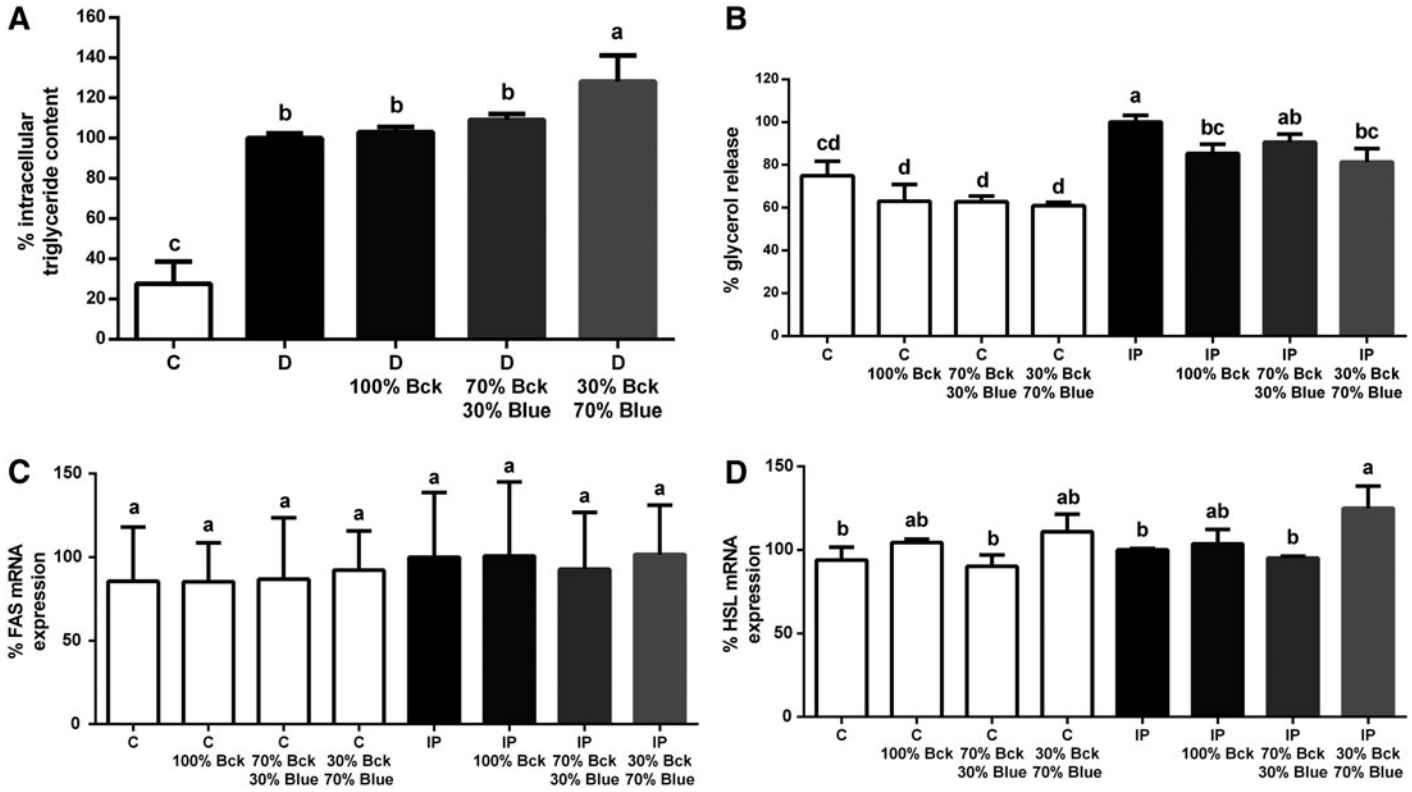

FIG. 2. Modulation of adipocyte fat accumulation. **(A)** The level of lipid accumulation was determined at the final stage of differentiation (8–10 days) of adipocytes that had previously been treated daily with each blend ANC (100 μM C3G equivalents) since day 1. Glycerol release **(B)**, FAS **(C)**, and HSL gene expression **(D)** were determined in fully differentiated adipocytes after a 24-h treatment with IP (10 nM), in the presence or absence of each blend ANC. Data ($n = 3$–6) are expressed as mean ± SD. One-way ANOVA followed by Tukey's *post hoc* test was performed to identify statistical differences. Different letters represent statistical differences of at least $P < .05$. D, differentiated; IP, isoproterenol.

differences were observed between the LPS control and blend ANC-treated groups.

Modulation of fat accumulation in adipocytes

The blend ANCs were tested for modulation of triglyceride accumulation in adipocytes during the differentiation period (Fig. 2A). Cells that were treated with 30% Bck:70% Blue blend ANCs had a higher lipid accumulation after 14 days than the differentiated control group (128.2% ± 12.9% vs. 100% ± 2.5%). Blend ANCs were then analyzed for their effect on IP-induced lipolysis of adipocytes as measured by the amount of glycerol released (Fig. 2B). As expected, IP treatment caused a higher glycerol release. The blend ANC treatment following IP treatment caused a protective effect, with significant reduction of glycerol release by 100% Bck and 30% Bck:70% Blue (85.3% ± 4.3% and 81.4% ± 6.3%, respectively vs. 100.0% ± 3.2% IP group). No significant differences were observed among groups regarding IP-induced FAS mRNA expression (Fig. 2C). In addition, treatment with the 30% Bck:70% Blue blend showed highest HSL gene expression in IP-induced adipocytes (124.9% ± 13.2% vs. 100.0% ± 0.9% IP group) (Fig. 2D).

Figure 3 shows modulation of lipolysis in adipocytes treated with TNF-α for 24 h, measured as glycerol release. A slight but not statistically significant trend toward inhibition of TNF-α-induced lipolysis was observed by treatment with 30% Bck:70% Blue blend ANCs (90.7% ± 4.5% vs. 100.0% ± 6.6% TNF-α group) (Fig. 3A). Adipocytes were also treated for 24 h with CM from macrophages that were previously treated with LPS and/or each blend ANCs. Treatment with 100% Bck and 70% Bck:30% Blue blends significantly reduced the LPS-induced glycerol release (86.1% ± 1.2% and 84.1% ± 3.7%, respectively vs. 100.0% ± 2.7% CM-LPS group) (Fig. 3B).

Inhibition of inflammatory processes involved in obesity

Figure 4 presents the effects of TNF-α and blend ANC treatments on *MCP-1* and *ADIPOQ* mRNA expression and protein secretion by adipocytes. Regarding *MCP-1* gene expression, surprisingly, the 70% Bck:30% Blue group presented a significantly higher mRNA expression ($P < .05$) compared to control (108.8% ± 5.8% vs. 100.0% ± 3.4% TNF-α group) (Fig. 4A). Both the gene expression and secretion of MCP-1 were highly correlated ($r = 0.919$; $P < .001$); however, there was no significant difference detected for MCP-1 secretion (Fig. 4B). Treatment with the 30% Bck:70% Blue blend ANCs induced a higher *ADIPOQ* gene expression than TNF-α-treated cells (118.2% ± 9.7% vs. 100.0% ± 8.3% TNF-α group) (Fig. 4C). Although an important significant correlation was observed between the gene expression and secretion of this adipokine ($r = 0.722$, $P < .001$), however, when adiponectin secretion was evaluated, no differences were observed among the TNF-α-treated groups (Fig. 4D). The same analysis was performed utilizing CM from macrophages previously treated with LPS and/or each berry blend; no differences were observed among groups (data not shown).

Effects on insulin sensitivity of adipocytes

The glucose uptake of adipocytes after a chronic treatment of macrophage-CM-LPS is presented in Figure 5. As expected, insulin (INS) induced a higher glucose uptake. LPS treatment reduced the capability of INS to induce glucose uptake in adipocytes. Whereas blend ANCs did not have an insulin-sensitizing effect ($P > .05$), from either the INS or LPS groups, however, when the independent t-test was performed among specific groups, significant differences were observed between the INS control group (100.0% ± 9.5%) versus INS-CM-LPS (56.0% ± 23.1%) ($P < .05$), versus INS-CM-LPS 70% Bck:30% Blue (67.9% ± 10.6%) ($P < .05$), and versus INS-CM-LPS 30% Bck:70% Blue (64.0% ± 7.7%) ($P < .01$).

DISCUSSION

In the current study, ANCs from dealcoholized fermented blueberry–blackberry beverage blends were applied to

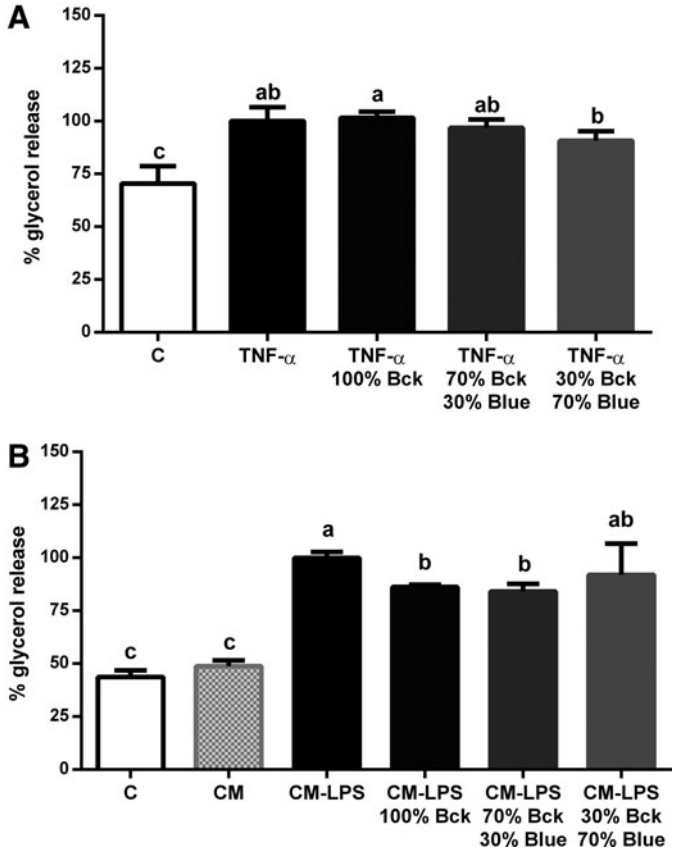

FIG. 3. Modulation of lipolysis by inflammatory inducers and fermented blend ANCs of fully differentiated adipocytes. The lipolysis modulation, measured by glycerol release, was determined after 24 h of treatment in fully differentiated adipocytes by 4 ng/mL TNF-α **(A)** and activated macrophage-conditioned media (CM-LPS) **(B)**, in the presence or absence of each blend ANC (100 μM C3G equivalents). Data ($n = 3–6$) are expressed as mean ± SD. One-way ANOVA followed by Tukey's *post hoc* test was performed to identify statistical differences. Different letters represent statistical differences of at least $P < .05$. CM, conditioned media.

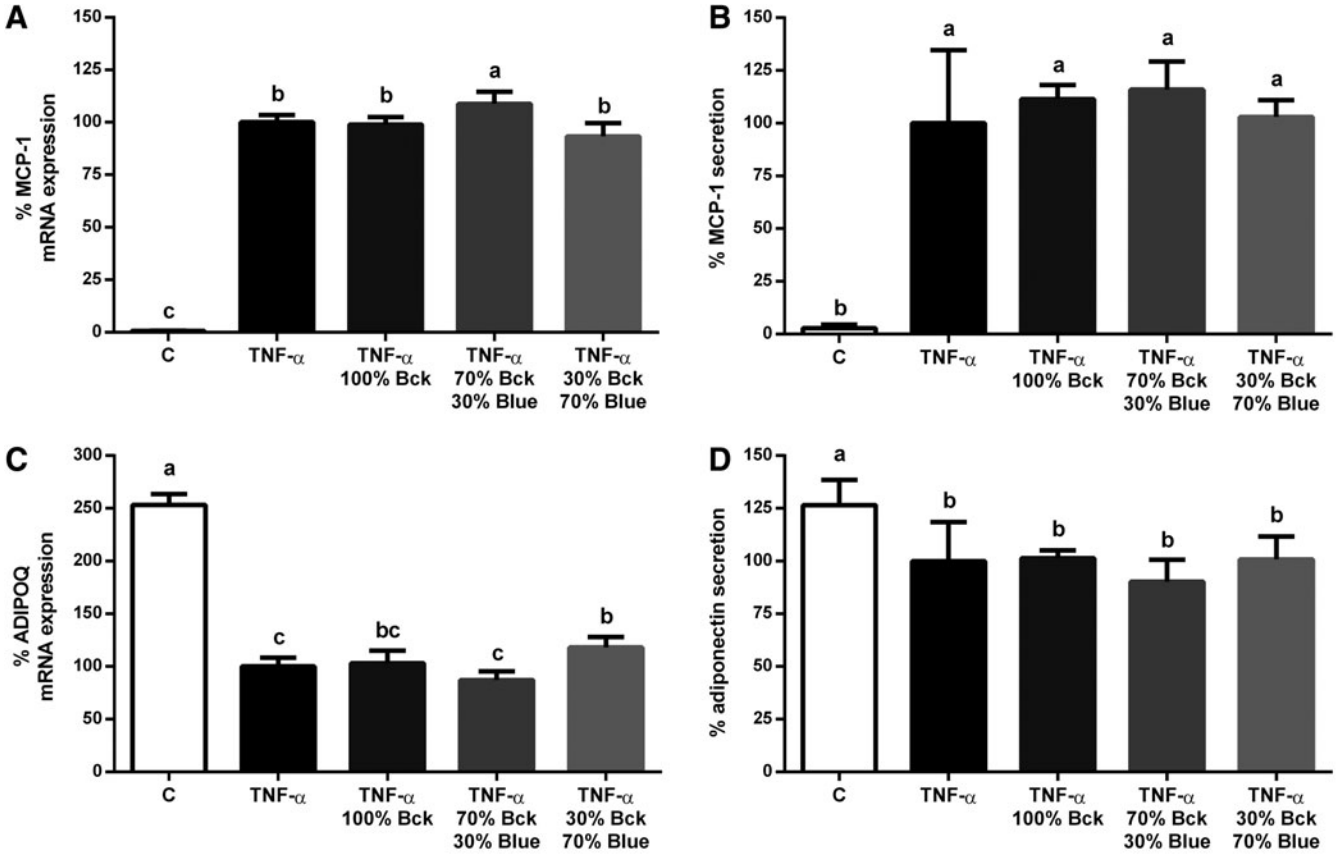

FIG. 4. Gene expression and secretion of inflammatory markers after the blend ANC treatment. mRNA expression **(A)** and secretion **(B)** of MCP-1 and mRNA expression **(C)** and secretion **(D)** of adiponectin were measured in adipocytes after 24 h of treatment with 4 ng/mL TNF-α and/or each fermented blend (100 μM C3G equivalents). Data ($n = 3$–6) are expressed as mean ± SD. One-way ANOVA followed by Tukey's *post hoc* test was performed to identify statistical differences. Different letters represent statistical differences of at least $P < .05$. MCP, monocyte chemoattractant protein-1.

macrophages and adipocytes to determine their potential to counteract the interaction between these two cells to reduce the self-perpetuating cycle of inflammation and obesity.

Our results show inhibition of NO and TNF-α secretion from macrophages following treatment with 30% Bck:70% Blue ANC, which consists of C3G as its major ANC.[18] The NO and TNF-α secretions were highly correlated, consistent with LPS activation of macrophages. The fact that the 70% Bck:30% Blue and 100% Bck treatments did not reduce TNF-α secretion may be due to their ANC composition; these blends contain mainly delphindin-3-arabinoside, with minor amounts of C3G.[18] Previous studies show that C3G is related with downregulation of proinflammatory cascades.[23] A decrease in the phosphorylation of p65 NFκB in macrophages was also observed. In this regard, polyphenols such as resveratrol have been able to reduce the phosphorylation of p65 NFκB in adipocytes.[24] Moreover, C3G has been reported to have the capability to inhibit LPS-induced expression of inflammatory mediators through decreasing I-kappa-B-alpha phosphorylation in human macrophages.[23] No differences were observed regarding the relative expression of p-Ser^{473}-Akt among LPS-treated groups, suggesting that the inflammatory mechanism of activation is

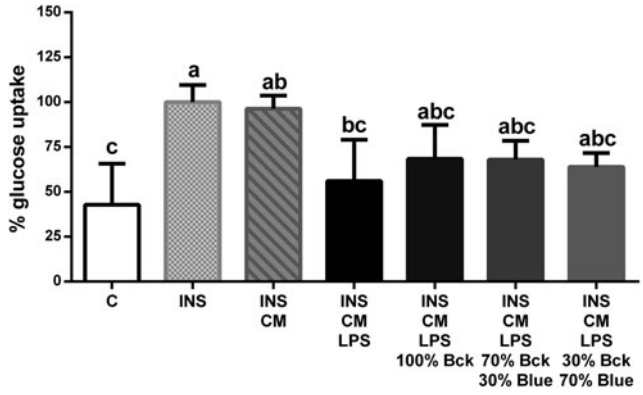

FIG. 5. Insulin sensitivity as determined by glucose uptake of adipocytes. Fully differentiated adipocytes were treated with activated macrophage-conditioned media (CM-LPS) and/or each blend ANC (100 μM C3G equivalents) for 4 days. After this period, the glucose uptake for 24 h was determined by measuring the concentration of glucose in the medium as induced by 100 nM insulin and then subtracting it from the initial concentration of glucose. Data ($n = 3$–6) are expressed as mean ± SD. One-way ANOVA followed by Tukey's *post hoc* test was performed to identify statistical differences. Different letters represent statistical differences of at least $P < .05$. INS, insulin.

most probably not driven through the PI3K pathway. These previous reports, along with the results of the present work, indicate that phenolic compounds from berry fruits have the ability to reduce the low-grade inflammatory state associated with obesity due to inhibition of the NFκB-mediated pathway in both macrophages and adipocytes.

A previous study using phenolic-enriched extracts from berries showed their ability to inhibit adipogenesis and that the highest inhibition of lipid accumulation was observed in adipocytes treated with the proanthocyanidin-enriched fraction from *Vaccinium floribundum*.[21] Another study indicated that fermented blueberry juice decreased triglyceride accumulation to levels comparable to the control; however, the nonfermented juice did not affect adipogenesis.[17] On the other hand, the higher triglyceride content induced by the 30% Bck:70% Blue ANC treatment in our study could be related to an upregulation of the peroxisome proliferator-activated receptor gamma activity, since it is a key molecule involved in adipogenesis[25] and inhibitor of NFκB signaling.[26]

In obese subjects, visceral adipose tissue lipolysis is known to be upregulated.[27] The increase in free fatty acid (FFA) levels has been directly correlated with the establishment of insulin resistance. Even though the present study was *in vitro*, some blend ANCs were able to modulate the fat accumulation in the adipocytes by exerting a protective effect on glycerol release. This fact is remarkable regarding adipose tissue inflammation; less FFA release has the potential to cause a reduction in the insulin-resistant environment. C3G has been previously related with the antilipolytic effect in 3T3-L1 cells through decreased expression of adipose triglyceride lipase.[28] Furthermore, it has been described that ANC extracts (68.3% C3G, 25.2% delphinidin-3-O-glucoside, and 6.5% petunidin-3-O-glucoside) from black soybean inhibit basal lipolysis.[29] As expected, no effects of IP treatment regarding FAS and HSL expression were observed, since both proteins are post-transcriptionally activated by catecholamines.[30] Our experiment found that only IP and 30% Bck:70% Blue ANC increased HSL expression. This apparently contradicts the obtained lipolysis results; however, this contradiction is explained by post-transcriptional modifications of HSL following stimuli. Finally, the fact that only delphinidin-enriched ANCs inhibited CM-LPS induction of glycerol release indicates prevention of lipolysis by a specific ANC in a more physiological model of obesity-related inflammation.

It has been reported that adiponectin production is reduced in subjects with visceral fat accumulation.[31] Hypoadiponectinemia induced by visceral fat accumulation has also been closely associated with T2D, lipid disorders, hypertension, and certain inflammatory diseases.[31] ANCs from dealcoholized fermented beverages did not inhibit MCP-1 induction in TNF-α-induced or LPS-induced CM adipocytes. However, TNF-α-induced *ADIPOQ* gene expression inhibition was significantly reduced by 30% Bck:70% Blue ANC treatment. This result correlated significantly with the TNF-α-induced glycerol release. To our knowledge, this is the first time that a C3G-enriched berry blend from a fermented and dealcoholized beverage has been related to the reduction of the blunted effect of adiponectin gene expression caused by an inflammatory inducer.

Our results indicate that glucose uptake was stimulated following insulin treatment. INS-CM-LPS effectively reduced glucose uptake, and thus, adipocytes were significantly insulin resistant. The fact that no significant difference was observed between INS and INS-CM-LPS 100% Bck indicates that this treatment induced some insulin-sensitizing improvement. In a previous study, it was observed that amelioration of glucose uptake in the presence of insulin by blueberry juices was highest at 6 h following LPS stimulation.[17] In this regard, ANC interventions have the potential to induce reduction of T2D incidence through modulation of insulin sensitivity and glucose utilization.[32]

In conclusion, ANC-enriched fractions from blueberry–blackberry dealcoholized fermented beverages presented a significant positive effect on an *in vitro* adipose tissue inflammatory model, in relation to several markers of adipogenesis and inflammation. Accordingly, more research is needed to unveil the promising features of these fermented beverages and their bioactive compounds *in vivo* on inflammatory-related insulin sensitivity.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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