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## Assessment of adipogenic, antioxidant, and anti-inflammatory properties of whole and whey bovine colostrum

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### ABSTRACT

Bovine colostrum (BC) has been used for nutritional purposes for animals and humans. Bovine colostrum is a complex heterogeneous product and its antimicrobial activity, antioxidant potential, and growth factors can vary depending on age and species of the cow as well as their environment. Bovine colostrum preparation in skimmed or whey fractions can also alter properties of BC. Our goal was to compare cumulative anti-inflammatory, antioxidant, and adipogenic properties of natural (whole) versus whey BC. We compared properties of whole and whey BC in 3T3-L1 preadipocytes permanently transfected with reporters responding to changes in inflammatory (*NfκB*RE/green fluorescent protein), anti-inflammatory (*Nrf2*/YFP), and adipogenic (*Fabp4*/cyan fluorescent protein) status in cells. Interleukin-6 secretion in these cells was measured by ELISA. Whole and whey BC induce IL-6 secretion from 3T3-L1 fibroblasts; however, whey preparation stimulated less IL-6 secretion. Cumulative inflammatory nuclear factor (NF)κB activation in the presence of lipopolysaccharide was reduced by both whole (−27%) and whey BC (−22%) compared with lipopolysaccharide-treated cells (100%). Treatment with whole BC was more effective in the reduction of NFκB activation compared with whey BC and occurred in a dose-dependent manner. In consonance with decreased NFκB activation, the *Nrf2* promoter activity was also reduced in response to whole (−27%) and whey (−13%) treatments compared with nontreated cells (100%). Whole and whey BC suppressed adipogenesis, measured as induction of *Fabp4*, by −27 and −13%, respectively, compared with nontreated 3T3-L1 fibro-

blasts (100%). Our results showed distinct differences in properties of whey and whole BC that could be used to attain reduced adipogenic or cumulative inflammatory responses.

**Key words:** inflammation, adipogenesis, antioxidant, lacteal secretion, cytokine

### INTRODUCTION

There is growing interest in human consumption of bovine colostrum (BC), the first mammary lacteal secretion, due to its nutritional properties as well as for pharmaceutical purposes (Ahmadi et al., 2011). Colostrum in its whole form consists of a casein fraction and a whey fraction, which has a high presence of biologically active molecules, among antibodies (Korhonen and Pihlanto, 2007). In addition to these macromolecules, a source of passive immunity for newborn ruminants, whey colostrum has enzymes with antioxidant and antimicrobial activity, minerals, hormones, and growth factors (Boudry and Thewis, 2009; Pandey et al., 2011). These and other components, including lactoferrin and vitamins A, C, and E, contribute to antioxidant activity of whole colostrum. The mechanism of action of lactoferrin, for example, includes binding of iron, which is a powerful oxidant, and oxidative stress plays a major role in inflammation-induced diseases (Yadav et al., 2016). Other biological molecules, such as lactoperoxidase, lysozyme, cytokines, and oligosaccharides, also confer antimicrobial and anti-inflammatory properties in colostrum (Yadav et al., 2016).

An et al. (2009) reported anti-inflammatory effects of BC in intestinal epithelial cells stimulated by IL-1β (An et al., 2009). The authors observed BC-mediated suppression of the nuclear factor (NF)κB transcription factor activation via different mechanisms, including inhibition of the inhibitor protein of NFκBα degradation, and blocked translocation of p65 into the nucleus. This inhibition of NFκB resulted in the suppression

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of cyclooxygenase-2 protein, as well as IL-8 and intracellular adhesion molecule-1 expression levels. This study suggests that BC may have therapeutic potential against intestinal inflammation suppressing inflammatory cytokines. On the other hand, colostrum contains inflammatory cytokines including IL-6, IL-8, IL-10, and tumor necrosis factor  $\alpha$ . The concentrations of these cytokines in colostrum can be increased in pathological settings enhancing the proinflammatory potential of colostrum (Zanardo et al., 2007). Therefore, it is critical to assess the cumulative inflammatory properties of colostrum given that age, species, and environment of the cows contribute to variation in colostrum composition. All biologically active proteins (except casein) are associated with the whey fraction and are dependent on the multiple variables described above, as well as the time of lactation and differences between primiparous and multiparous cows (Raimondo et al., 2019). Whey colostrum preparation compared with whole colostrum influences concentrations of lipophilic antioxidants, SFA, PUFA, cytokines, and antioxidant proteins. Collectively these changes in the antioxidant potential can influence anti-inflammatory properties in whey compared with whole colostrum. The effects of whole or whey formulations on inflammation and antioxidant properties have not been compared.

Nuclear factor (erythroid-derived 2)-like 2 (**NRF2**) and NF $\kappa$ B are the 2 master regulators of cellular responses to oxidative stress and inflammation, respectively (Wardyn et al., 2015). To assess the cumulative effect on inflammation in response to nutrients with a complex composition, preadipocyte fibroblast reporter cell lines containing NF $\kappa$ B response element (*Nf $\kappa$ b-RE*) have been used (Baker et al., 2011). Translocation of p65/p50 complex to the nucleus activate *Nf $\kappa$ b-RE* (Wardyn et al., 2015). In a similar fashion, activation of *Nrf2-RE* was used to assess antioxidant response of nutrients (Shen et al., 2014). The exposure to lipid-rich nutrients, such as colostrum, could have a long-term effect on metabolism via regulation of adipogenesis, development of adipose tissue, chronic inflammation related to obesity, or a combination of these (Yamashita et al., 2018). Adipogenesis is regulated by the nuclear factor peroxisome proliferator activated receptor gamma (**PPAR $\gamma$** ; Rosen and Spiegelman, 2001). The activation of PPAR $\gamma$  leads to activation of its target gene, fatty acid binding protein 4 (*Fabp4*; Tontonoz and Spiegelman, 2008). Activation of transcription factor PPAR $\gamma$  can also have anti-inflammatory effects due to its binding affinity to p65, preventing the activation of *Nf $\kappa$ b-RE* during inflammation (Chen et al., 2003). To elucidate nutrient-dependent effects, Shen et al. (2014) developed biosensor preadipocyte cell lines *Nf $\kappa$ b-RE*/GFP (**GFP** = green fluorescent protein), *Nrf2-P*/

YFP (**YFP** = yellow fluorescent protein), and *Fabp4*/CFP (**CFP** = cyan fluorescent protein) for combined screening of the interrelated anti-inflammatory, antioxidant, and anti-adipogenic pathways. We hypothesize that differences in the composition of whole and whey BC will influence their anti-inflammatory, antioxidant, and anti-adipogenic properties. Our objective was to validate a high-throughput screening approach for comparison of whole and whey BC properties.

## MATERIALS AND METHODS

### Preparation of Culture Medium and Differentiation Medium

We purchased Dulbecco's modified Eagle medium (**DMEM**) from Gibco Life Technologies (#11965, Grand Island, NY); high glucose with L-glutamine, without sodium pyruvate, penicillin/streptomycin (**P/S**) from Invitrogen (#15140-122, Karlsruhe, Germany); new born calf serum (**NBCS**) from Gibco Life Technologies (#16170-078); fetal bovine serum (**FBS**) from Gibco Life Technologies (#10437-028); bovine insulin from Sigma-Aldrich (#I-5500, St. Louis, MO); isobutylmethylxanthine (**IBMX**) from Sigma-Aldrich (#I-7018); dexamethasone from Sigma-Aldrich (#D-4902); and dimethyl sulfoxide (**DMSO**) from ISC-Bioexpress (#0231, Kaysville, UT). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (#L2762), retinoic acid from Sigma-Aldrich (#R2625), and rosiglitazone from Enzo Life Sciences (#ALX-350-125-M100, Ann Arbor, MI).

The culture medium contained 1% P/S and 10% NBCS in DMEM. Two differentiation media were used. The first is termed differentiation medium I [DMEM containing 1% P/S and 10% FBS, bovine insulin (1:1,000), IBMX diluted in DMSO (1:1,000), and dexamethasone diluted in ethanol (1:10,000)]. Differentiation medium II was prepared with the same reagents as differentiation medium I; however, medium lacked the IBMX and dexamethasone. Differentiation was induced in confluent preadipocytes by differentiation medium I (d 0). Differentiation medium II was added on d 2 and replaced every 48 h.

### Collection and Processing of BC

We compared whole and whey BC properties from 3 individual multiparous (second to fourth lactation) Jersey cows (age  $3.8 \pm 0.5$  yr). Samples were collected from the first lactation and whey colostrum extraction was performed within the first 24 h after collection. Samples in their natural form (whole colostrum) and extracted form (whey colostrum) were stored and ana-

lyzed within 1 wk (with storage at 4°C). To prepare whey colostrum, a sample of the lacteal secretion was centrifuged at  $3,600 \times g$  at 4°C for 22 min to remove the lipid fraction. Whey colostrum was obtained after incubation with rennet (Chy-Max M from Chr Hansen, Hoersholm, Denmark, material #127223, diluted as vol:vol = 1:1,000 to the sample) in a water bath for 30 min at 37°C. After centrifugation at  $16,639 \times g$  at 4° for 20 min, the supernatant (whey colostrum) was collected and stored at 4°C. The rennet control and whey colostrum contained the same concentrations of rennet (vol:vol = 1:1,000 rennet/PBS) in all cell-based experiments.

### 3T3-L1 Preadipocyte Cell Lines and Treatments

**3T3-L1 Expressing *Nfκb*-RE/GFP.** The 3T3-L1 preadipocytes were previously stably transfected with the reporter *Nfκb*-RE/GFP (Shen et al., 2014). Cells were plated into 96-well plate at 70% confluence in growth medium. After 24 h (d 0), the medium was replaced with differentiation medium I without FBS and following treatments: (1) nontreated control, (2) LPS (20 ng/mL in water), (3) 0.1% whole or whey BC, and (4) 0.5% whole or whey BC. On d 2, differentiation medium I was replaced with differentiation medium II containing the following treatments: (1) nontreated control, (2) LPS (20 ng/mL), and (3 and 4) 0.1 and 0.5% whole and whey BC with LPS (20 ng/mL). Twenty-four hours afterward, upon harvest, the differentiation medium was collected and analyzed for secreted IL-6. The GFP was measured in the living cells at 485/528 nm wavelengths for excitation and emission (**Ex/Em**). Protein content was measured in the washed and lysed cells.

**3T3-L1 Preadipocytes Expressing *Nrf2*/YFP.** The 3T3-L1 preadipocytes previously transfected with the reporter *Nrf2*/YFP (Shen et al., 2014) were used. Cells were handled as in 3.3.1. *Nrf2*/YFP cell activity was validated by comparison of cells stimulated with ethanol (vehicle, 0.1%) or retinoic acid (**RA**, 100 nM in 0.1% ethanol), a canonic regulator of this pathway. After 24 h (d 0), the medium was replaced with differentiation medium I without FBS and *Nrf2*/YFP cells were stimulated with 0.1 and 0.5% whole BC, whey BC, or remained untreated (control). On the d 2, the differentiation medium I was replaced with the differentiation medium II containing the same treatment used on d 0. Twenty-four hours afterward, the YFP in the living cells was determined at 500/530 nm Ex/Em. Protein was measured in the washed and lysed cells.

**3T3-L1 Preadipocytes Expressing *Fabp4*/CFP.** The 3T3-L1 preadipocytes previously transfected with the reporter *Fabp4*/CFP were used (Shen et al., 2014).

Cells were handled as in the previous section. *Fabp4*/CFP cell activity was validated by comparison of cells stimulated with ethanol (vehicle, 0.1%) or rosiglitazone (1 μM, **BRL**-49653), an agonist of this pathway. After 24 h (d 0), the medium was replaced with differentiation medium I without FBS containing 0.1 and 0.5% whole or whey BC, or were not treated (control). On d 2, the differentiation medium I was replaced with differentiation medium II containing the same treatments. The CFP in the living cells was determined at 426/460 nm (Ex/Em) 48 h after treatment. Protein content was measured in the washed and lysed cells.

### IL-6 and Protein Determination

Interleukin-6 in culture medium and whole and whey BC samples were measured by an ELISA kit (Invitrogen, Grand Island, NY). The protein content in RIPA cell lysates was measured using a BCA kit (Thermo Fisher Scientific, Rockford, IL).

### Statistical Analysis

Data represents measurements using whey and whole colostrum from 3 cows. Whey and whole BC from each cow is presented as an average of 8 measurements performed in independent settings. Data are shown as mean ± standard error of the mean. Group comparisons were performed using paired or unpaired Student's *t*-test, considering a probability of 5% ( $P < 0.05$ ). The IBM SPSS Statistics 23 software (IBM Corp., Armonk, NY) was used for analysis.

## RESULTS

### Whole and Whey BC Fractions Inhibit LPS-Induced NFκB Activation in Adipocytes

The 3T3-L1 fibroblasts are canonic experimental systems that allow for assessment of the effects of complex natural products on inflammation (Shen et al., 2015). Given that NFκB is a master regulator of inflammation, we used 3T3-L1 fibroblasts permanently transfected with *Nfκb* response element (*NfκbRE*)-GFP and activated by LPS (Figure 1). Activation of inflammation was measured as an increase in GFP in living cells that was normalized by protein content to account for cell proliferation. We also measured the concentrations of secreted IL-6 that serve as a specific marker of inflammation in response to BC. Both whole and whey BC markedly increased IL-6 concentration to 340 and 181%, respectively, compared with control LPS-stimulated cells (100%; Figure 1A). Both whole and whey BC exhibited dose-dependent stimulations of IL-6

secretion. This increase is consistent with the presence of IL-6 in colostrum (Figure 1A, insert; Zanardo et al., 2007) and with the known multifunctional roles of IL-6 that plays both anti- and pro-inflammatory roles in different settings (Scheller et al., 2011). However, whole BC induced higher levels of IL-6 secretion from cells than whey BC, even though whey colostrum contained higher endogenous levels of IL-6 (Figure 1A, insert). To elucidate cumulative inflammatory response, we measured the *NfκbRE* activation in the same adipocytes.

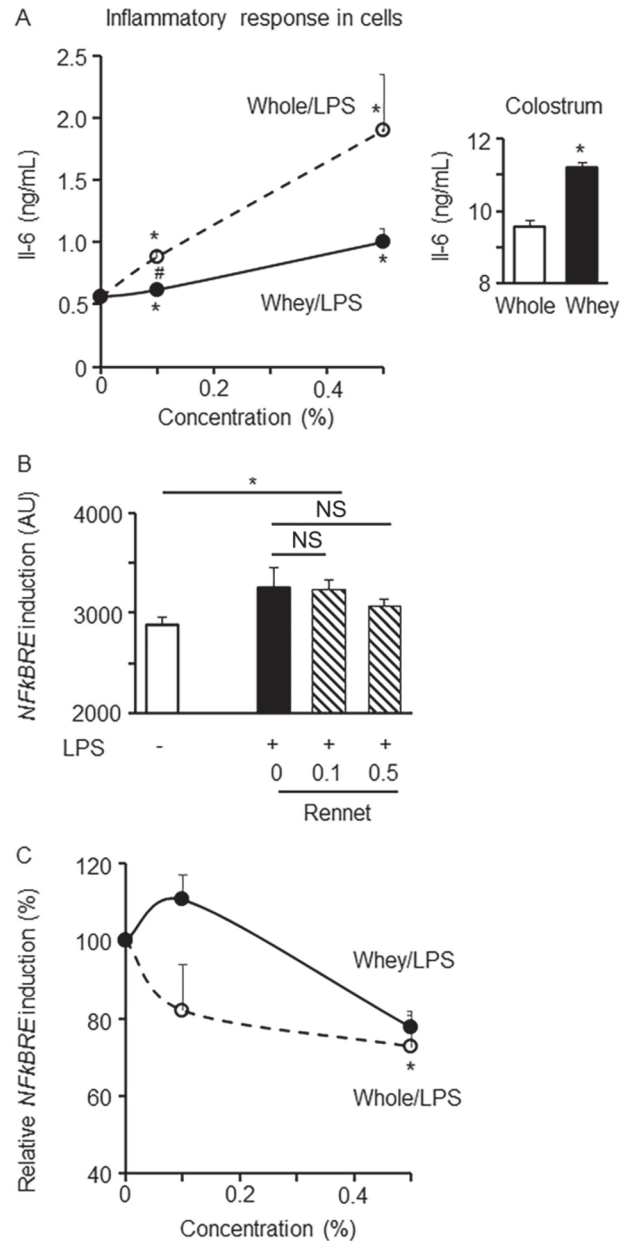
Stimulation of 3T3-L1(*NfκbRE*)-GFP fibroblasts with canonic inducer of inflammation LPS was not influenced by residual rennet from extraction (Figure 1B). Whole BC reduced LPS-dependent *NfκbRE* activation more effectively than whey BC (−27 and −22%, respectively, compared with LPS-treated cells, 100%; Figure 1C). Overall, the inhibition was more pronounced by whole BC than its whey fraction at the 0.5% concentration.

### Whole and Whey BC Alter Antioxidant Potential

Inflammatory response depends on the redox status in the cells regulated by *Nrf2* (Wardyn et al., 2015). We measured oxidative stress in 3T3-L1 adipocytes transfected with *Nfr2*-YFP reporter in response to whole and whey BC. The RA stimulation decreased *Nfr2* activation in *Nfr2*-YFP-3T3-L1 adipocytes (−10% vs. vehicle-treated control), whereas rennet moderately increased *Nfr2* activation at 0.1% concentration (110% vs. nontreated control; Figure 2A). Treatment with whole BC led to moderate dose-dependent inhibition of *Nfr2* activation to 73% compared with nonstimulated cells (100%; Figure 2B). The whey BC reduced *Nfr2* activation to a lesser extent than whole BC (−0.3 vs. −13% at 0.1% concentrations). Overall *Nfr2* status in the cells correlated with the NFκB-dependent inflammatory response in cells stimulated with whole and whey BC (Figure 2C).

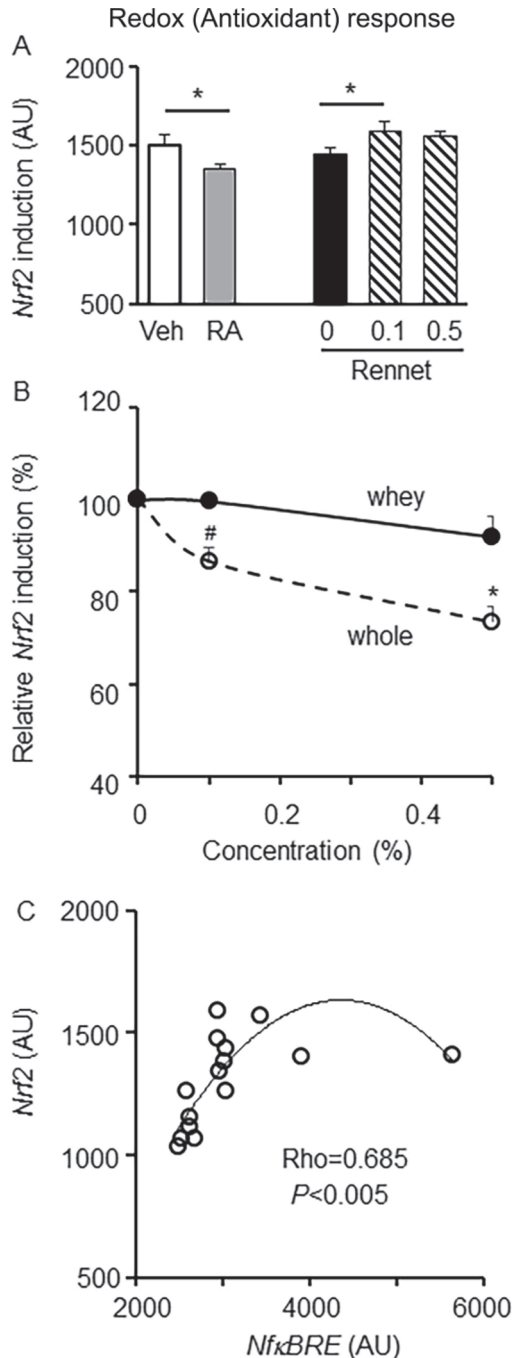
### Whole and Whey BC Reduce Adipogenic Responses 3T3-L1 Cells

Adipose fatty acid binding protein (*Fabp4*) plays a critical role in fatty acid trafficking in preadipocytes and adipocytes that leads to activation of PPAR $\gamma$ , a master regulator of adipogenesis. The 3T3-L1 preadipocytes were permanently transfected with *Fabp4*-CFP reporter to assess the adipogenic potential of these preadipocytes. The *Fabp4*-CFP preadipocytes were significantly activated by PPAR $\gamma$  ligand BRL (124 vs. 100% vehicle, Figure 3A), consistent with the low expression of *Pparg* in 3T3-L1 preadipocytes. Rennet treatment led to significant but very low activation of



**Figure 1.** Whole and whey bovine colostrum (BC) increased IL-6 secretion and decreased cumulative activation of NFκB. *NfκbRE*-green fluorescent protein (GFP) 3T3-L1 adipocytes were stimulated with LPS (20 ng/mL), rennet (0.1 or 0.5%), or indicated concentrations of whole (dashed line, open circles) and whey (solid line, solid circles) BC. Data represent measurements using whey and whole colostrum from 3 individual cows. Whey and whole BC from each cow is an average of 8 measurements performed in independent settings. Data are shown as mean  $\pm$  SEM. (A) IL-6 concentrations measured by ELISA in the culture medium collected 24 h after stimulation. \*Significance compared with LPS-stimulated control cells. #Significance between whole versus whey samples ( $P < 0.05$ ,  $t$ -test). Insert shows IL-6 concentrations in the whole and whey BC samples that were used for stimulation. (B, C) Green fluorescent protein (GFP) measured in the same living *NfκbRE*-GFP cells and normalized to protein concentrations. (B) Normalized GFP in 3T3-L1 adipocytes 24 h after stimulation with and without LPS and rennet. (C) Normalized GFP in 3T3-L1 adipocytes after LPS stimulation and treatment with whole or whey colostrum. Paired  $t$ -test.

## PROPERTIES OF WHOLE AND WHEY BOVINE COLOSTRUM



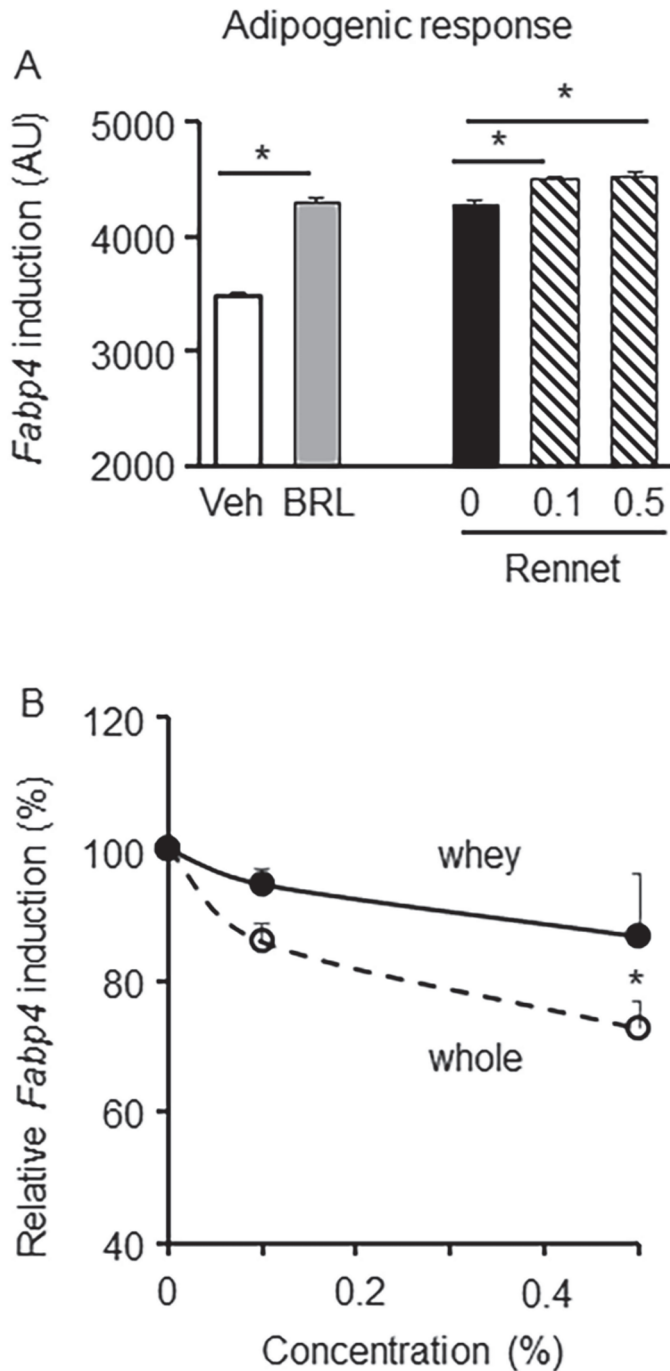
**Figure 2.** Whole and whey bovine colostrum (BC) inhibited activation of *Nrf2* promoter. Data represent measurements using whey and whole colostrum from 3 individual cows. (A) Yellow fluorescent protein (YFP) measured in living *Nrf2*-YFP3T3-L1 adipocytes 24 h after stimulation with retinoic acid (RA, 100 nM,  $n = 8$ ) and rennet (0.1 or 0.5%,  $n = 8$ ; \* represents  $P < 0.05$ ,  $t$ -test). (B) Yellow fluorescent protein with same treatment as (A) as well as indicated concentrations of whole (dashed line, open circles) and whey (solid line, solid circles) BC from 3 different cows. Data were normalized to protein concentrations and are shown as mean  $\pm$  standard error of the mean. #Significance between whole versus whey samples ( $t$ -test). (C) Spearman correlation between *Nrf2*-YFP and *NfκB*RE-GFP 3T3-L1 adipocytes with and without activation by whole and whey BC. Veh = vehicle; AU = arbitrary units.

*Fabp4* (105 and 106% activation vs. nontreated cells). Treatment with whole BC suppressed *Fabp4* activation dose-dependently, resulting in  $-27\%$  reduced activity (Figure 3B, vs. 100% in control cells). The effect of the protein-rich whey BC on *Fabp4* activation was less pronounced ( $-13\%$  compared with control). Whole BC appeared to be more effective in suppression of adipogenic responses than the whey BC fraction.

## DISCUSSION

Bovine colostrum has been extensively studied due to its large number of biologically active molecules (Boudry and Thewis, 2009; Pandey et al., 2011). Here we found that the anti-inflammatory, antioxidant, and anti-adipogenic properties are distinctly different between natural whole and whey BC fractions. Whole BC exerts stronger NFκB-dependent anti-inflammatory properties compared with whey fraction, which were related to the *Nrf2*-responsive oxidative status of these cells. However, whole BC also exhibited robust anti-adipogenic properties. Our data suggest that whole and whey BC could have specific nutritional and biomedical applications based on the differences in the regulation of inflammatory and metabolic pathways that were assessed in high-throughput reporter assays.

Antioxidative potential of BC remains a subjects of debate (Yadav et al., 2016). In humans, increased cytokine levels in colostrum were associated with neonatal jaundice (Zanardo et al., 2007). Appukutty et al. (2012), in turn, observed that supplementation with BC protects against oxidative stress induced by exercise in the skeletal muscle tissue of mice (Appukutty et al., 2012). In our study, the overall effect of BC in living cells resulted in the inhibition of *Nrf2* promoter activation. Thus, particularly whole BC, even in a small dose, could alter the redox status in cells. Several BC components could be responsible for the change in the redox status regulated by NRF2. Casein or lipid fractions (or both) of the whole BC may be involved in the regulation of antioxidant capacity. Casein is able to inhibit Fe-induced peroxidation of arachidonic acid inserted into multilamellar liposomes, indicating the antioxidant properties of milk and colostrum proteins (Cervato et al., 1999). Colostrum contains antioxidant enzymes, including catalase, glutathione peroxidase, and superoxide dismutase, as well as a high concentration (30 mg/L) of lactoperoxidase (Albera and Kankofer, 2009). Lactoperoxidase in the presence of hydrogen peroxide generates reactive species with high antimicrobial activity. Lactoferrin also provides antioxidant protection by binding free iron produced during cellular inflammation. Furthermore, colostrum's vitamins such as vitamins A, C, and E and minerals such as selenium,



**Figure 3.** Whole and whey bovine colostrum (BC) inhibited activation of *Fabp4* promoter. Data represent measurements using whey and whole colostrum from 3 individual cows. (A) Cyan fluorescent protein (CFP) measured in living *Fabp4*-CFP adipocytes 48 h after stimulation with rosiglitazone (BRL, 1  $\mu$ M) and rennet (0.1 or 0.5%), ( $n = 8$ ,  $t$ -test). (B) Cyan fluorescent protein as treated in (A) as well as with indicated concentrations of whole (dashed line, open circles) and whey (solid line, solid circles) BC from 3 different cows. Data were normalized to protein concentrations and are shown as mean  $\pm$  SEM. \* $P < 0.05$  compared with control vehicle (Veh)-stimulated cells,  $t$ -test. AU = arbitrary units.

zinc, and manganese could act as antioxidants and/or support antioxidative pathways, preventing the formation of reactive oxygen species (Ahmadi et al., 2011, Pandey et al., 2011). Glutathione in colostrum is often described as the ultimate antioxidant (Albera and Kankofer, 2009). Recent research has also focused on the assessment of overall effects of BC under oxidative stress, including neuroprotective effect of BC (Stewart, 2008; Choi et al., 2010; Janusz and Zablocka, 2010). Kim et al. (2012), in turn, reported that treatment with BC significantly decreased neuronal cell death induced by *N*-methyl-D-aspartic acid in the rodent hippocampus (Kim et al., 2012). Our study demonstrated that antioxidative effects of BC can be measured in vitro and the properties of whole BC were compared with its whey preparation. Glycomacropeptide, which is produced through cleavage of casein by rennet, is also reported to have an antioxidant function (Neelima et al., 2013). In our studies, whole colostrum had a more profound effect on *Nrf2*, indicating the higher antioxidant potential of whole compared with whey BC. Although glycomacropeptide may contribute to the effect of whey BC, the overall antioxidative effects of whey BC were moderate in our study. The dual assessment of whole and whey BC in *Nrf2* and *NfκB* reporter systems suggests that antioxidant properties of whole and whey BC were major determinants of the cellular inflammatory response. The *Nrf2* promoter activity positively correlated with *NfκB* activation in cells stimulated by whole and whey BC. Whole BC composition containing lipophilic antioxidants appeared to have marked anti-inflammatory and antioxidant potentials that exceed those seen with whey BC.

Both whey and whole BC markedly reduced *NfκBRE* activation at 0.5% concentrations compared with control nonstimulated cells, in spite of having endogenous proinflammatory cytokines, such as IL-6. These data indicate that BC has a great potential to decrease the inflammatory signaling cascade. An et al. (2009) also observed that BC protects intestinal epithelial cells from inflammation by inhibiting the NFκB pathway (An et al., 2009). The authors observed that BC inhibited IL-1β-induced IL-8, intracellular adhesion molecule-1 mRNA expression, IL-1β-induced NFκB activation, cyclooxygenase-2 protein expression, and inhibitor protein of NFκB (IκBα) degradation, besides blocking translocation of p65 into the nucleus. Based on the studies of NFκB activation in the porcine intestinal epithelial cell (IPEC-J2) stimulated by heat-inactivated bacteria *Escherichia coli* and *Salmonellae enterica*, Blais et al. (2015) proposed to use whey BC as a feed additive to prevent infections. In these studies, whey BC decreased the expression of early and late inflammatory genes

(Blais et al., 2015). In our study, both whole and whey BC could suppress NF $\kappa$ B response with similar efficacy.

Several studies showed that IL-6 is a multifunctional cytokine underlying many physiologic processes in addition to its role in inflammation (Scheller et al., 2011). Although *Nf $\kappa$ bRE* activation was suppressed, both whey and whole BC increased IL-6 secretion, suggesting that this cytokine plays a physiologic role in neonates. The secretion of IL-6 plays an important role in proliferation and repair of the intestine, where colostrum is absorbed (Kuhn et al., 2014). It is possible that the presence of IL-6 in the colostrum (Zanardo et al., 2007) and the stimulation of IL-6 secretion by BC could play a role in stimulating the development of the newborn intestinal epithelium (Garg et al., 2018). In the present work, our data suggest that measurement of *Nf $\kappa$ bRE* activation provides improved assessment of inflammation in response to complex secretory nutrients, such as BC, than specific cytokines such as IL-6.

The effects of BC on adipogenesis and the mechanisms involved in this regulation have been limited (Lyle et al., 1998; Lee and Hossner, 2002). Moreover, considering that BC is a complex heterogeneous product, it is inherently variable and can display different effects depending on environment, feed, age, and species of the cow. The BC formulations, such as natural, skimmed, or whey, can also alter adipogenic properties of BC. Enrichment of BC in proteins, fats, minerals, and vitamins, and the presence of molecules essential for promoting growth and development of the gastrointestinal tract of newborns all can influence adipogenesis (Garg et al., 2018). Growth factors, such as IGF and epidermal growth factor, are known to increase proliferation and differentiation of intestinal cells, influencing the absorption capacity of electrolytes and nutrients by newborns (Pandey et al., 2011). It is also known that IGF can stimulate a variety of cells, among them 3T3-L1 adipocytes (Boney et al., 2001). Platelet-derived growth factor is also present in high amounts in colostrum and could induce mitosis of 3T3-L1 cells (Bachmeier and Loffler, 1995). Multiple studies (Shing and Klagsbrun, 1987; Belford et al., 1997; Lee and Hossner, 2002) have found that IGF, platelet-derived growth factor, and transforming growth factor  $\beta$  have no growth-promoting activity in 3T3-L1 cells. However, Lee and Hossner observed that ultrafiltered, defatted BC stimulated adipogenesis in a manner independent of IGF and other growth factors (Lee and Hossner, 2002). The authors believe that BC contains unique growth-promoting peptides that are yet to be identified (Lyle et al., 1998). In our study, whole BC was also more effective in suppression of *Fabp4* activation, a binding protein that leads to activation of PPAR $\gamma$  and, consequently, adipogenesis.

## CONCLUSIONS

Reporter assays provide a valuable high-throughput method of assessment of colostrum differences in whole and whey preparations. These assays could have other applications, for example they could be used as a cost-effective tool to study the role of different BC components in key biological pathways to determine their beneficial or deleterious properties. Antioxidant capacity of whole and whey BC correlate with their anti-inflammatory effects in cells. Whole BC exhibits higher anti-inflammatory, anti-adipogenic, and antioxidative effects on cells compared with whey BC.

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