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Regulation of osteoblast and adipocyte differentiation from human mesenchymal stem cells by conjugated linoleic acid

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Abstract

Conjugated linoleic acid (CLA) describes a group of isomers of linoleic acid and has variable effects on bone formation and adiposity in vivo and in vitro. The variability may be due to individual effects of the predominant bioactive 9*cis*,11*trans* (9,11) and 10*trans*,12*cis* (10,12) CLA isomers. Osteoblasts and adipocytes are derived from mesenchymal stem cells (MSCs), and bone loss is accompanied by an increase in marrow adiposity. Osteoblast differentiation from MSCs requires activation of Wnt/β-catenin signaling by Wnt10b, which inhibits adipocyte differentiation by suppressing CCAAT/enhancer-binding protein (C/EBP) α. The objective of this study was to determine if 9,11 and 10,12 CLA affect osteoblast and adipocyte differentiation from MSCs and to determine whether any effects are associated with changes in Wnt10b and C/EBPα expression. Osteoblast differentiation was assessed by calcium deposition, alkaline phosphatase (ALP) activity, and the expression of Wnt10b, runx2 and osteocalcin. Adipocyte differentiation was assessed by oil red O staining and C/EBPα, PPARγ and FABP4 expression. Compared to vehicle, 9,11 CLA decreased calcium deposition (~15%), increased oil red O staining (~21-28%) and increased FABP4 (AP2) expression (~58-75%). In contrast, 10,12 CLA increased calcium deposition (~12-60%), ALP activity (~2.1-fold) and the expression of Wnt10b (~60-80%) and osteocalcin (~90%), but decreased oil red O staining (~30%) and the expression of C/EBPα (~24-38%) and PPARγ (~60%) ($P<05$). Thus, our findings demonstrate isomer-specific effects of CLA on MSC differentiation, and suggest that 10,12 CLA may be a useful therapeutic agent to promote osteoblast differentiation from MSCs.

Keywords: Conjugated linoleic acid; Mesenchymal stem cell; Osteoblast; Adipocyte; Wnt10b; C/EBPα

1. Introduction

Osteoporosis and obesity are two major public health concerns that can both be influenced by diet and often occur simultaneously within individuals [1]. Conditions that enhance bone loss and, therefore, promote osteoporosis also lead to an increase in fat accumulation within bone marrow [2]. Bone forming osteoblasts and fat forming adipocytes are both derived from mesenchymal stem cells (MSCs) [3], which are found in many tissues and are abundant in bone marrow stroma [4,5]. MSCs are able to mobilize out of the bone marrow, proliferate, differentiate, migrate and invade their targeted tissues [6] such as bone or fat. As such, factors that influence the balance between osteoblast and adipocyte differentiation within bone marrow could lead to changes in body composition.

A growing body of evidence suggests that conjugated linoleic acid (CLA) affects both bone health [7–13] and fat formation [14–19] in vivo and in vitro. CLA refers to a group of positional and geometric isomers of the essential fatty acid, linoleic acid, and is produced by the bacterial biohydrogenation of linoleic acid in the gut of ruminant animals via an enzymatic isomerase reaction [20]. CLA is found naturally in food products from these animals predominantly as the 9,11 form, whereas synthetic CLA preparations consist of a few different isomers with approximately equal amounts of 9,11 and 10,12 CLA [21]. These isomers are both biologically active and are known to have different physiological effects [22].

Several studies have examined the effects of CLA on bone formation using experimental animals, but the findings have been equivocal [8–11]. Rodent studies demonstrate that CLA supplementation increases body ash [10], has no effect on bone mineral content [23,24], increases [25] or decreases bone formation rates [11] and has variable effects

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on markers of bone formation and resorption [12]. In cultured rodent calvarial cells, CLA alters protein levels of the osteoblast-specific transcription factor, runt-related transcription factor (runx)2, and increases osteoblast differentiation [26]. Human studies demonstrate that dietary CLA is positively associated with bone mineral density (BMD) in postmenopausal women [7], while supplemental CLA has no effect on BMD in men [27,28]. In human bone cells, individual and mixed isomers of CLA variably increase alkaline phosphatase (ALP) activity [13,29], while 9,11 CLA has been shown to increase mineralized bone nodule formation [13].

CLA also appears to have isomer-specific effects on adiposity and adipocyte formation and function. Several studies have demonstrated that mixed CLA isomers reduce adiposity in vivo [30,31], reduce adipocyte formation, function and activity in vitro [15,18], and that 10,12 CLA is the anti-adipogenic isomer [32–34]. Although 10,12 CLA is known to inhibit human preadipocyte differentiation in vitro [35], these cells are committed to the adipocyte lineage and, therefore, the effects of 10,12 CLA at a non-committed stage of human adipocyte differentiation is not known. In most of the animal studies demonstrating a reduction in adiposity, feed intake was not affected by CLA treatment [31,36], suggesting that the effect of CLA on adiposity is independent of any changes in caloric intake. It has been reported that 10,12 CLA reduces adiposity by increasing adipocyte apoptosis [15,37], reducing intracellular triacylglycerol levels [15,18], increasing fatty acid oxidation [32] and inflammation [38], as well as by modulating peroxisome proliferator-activated receptor (PPAR)γ expression [39,40] and activity [39].

PPARγ is a nuclear receptor and transcription factor that is an essential regulator of lipid metabolism [41] and adipocyte differentiation [42,43]. Long-chain fatty acids, including CLA, are natural ligands for PPARγ [44], and 10,12 CLA is a known agonist that down regulates its expression in adipocytes [39,40]. The effects of 10,12 CLA on PPARγ expression may be direct or might be mediated by genes that regulate its expression. CCAAT/enhancer-binding protein (C/EBP) α is a transcription factor that stimulates and maintains PPARγ expression during adipocyte differentiation. In turn, PPARγ up-regulates C/EBPα expression to stimulate adipocyte gene expression and lipid accumulation [45]. Thus, these transcription factors work together to promote each other's expression and maintain adipogenesis. Lipid accumulation within adipocytes is promoted by the gene encoding fatty acid binding protein 4 (FABP4), which is under the transcriptional control of PPARγ and C/EBPα [46]. FABP4 (AP2) has a high affinity for a variety of fatty acids and facilitates their storage, trafficking and solubilization [47]. FABP4 has been used as a marker to follow the differentiation of adipocytes, and it is up-regulated more than 50-fold during the conversion of precursors cells to mature adipocytes [48].

PPARγ promotes adipocyte differentiation and inhibits osteoblast differentiation from MSCs by suppressing the expression [49] and activity [50] of runx2. In contrast, the osteoblast-specific transcription factor msh homeobox homolog 2 (Msx2) inhibits adipocyte differentiation by binding to C/EBPα, thereby reducing its ability to transactivate the PPARγ promoter. This reciprocal inhibitory regulation of adipogenic and osteogenic genes work together to maintain MSCs in an undifferentiated, quiescent state [51]. Commitment of MSCs to differentiate into osteoblasts or adipocytes is regulated by Wnt10b, which is expressed in bone marrow [52,53] and stimulates Wnt/β-catenin signaling [54]. Activation of Wnt signaling by Wnt10b stabilizes β-catenin and promotes MSC differentiation along the osteoblast lineage by decreasing C/EBPα and PPARγ expression [51,55].

The objective of the present study was to determine whether 9,11 or 10,12 CLA affects osteoblast or adipocyte differentiation from MSCs, and if so, to determine if these effects are associated with changes in Wnt10b and the expression of osteogenic and adipogenic transcription factors.

2. Methods and materials

2.1. Materials

Human MSCs and media were obtained from Lonza Walkersville (Walkersville, MD, USA). The 9,11 and 10,12 (>98% pure) isomers of CLA were purchased from Matreya (Pleasant Gap, PA, USA). The Bio-Rad Protein Assay reagent was purchased from Bio-Rad (Mississauga, ON, Canada). Phosphate-buffered saline (PBS) was obtained from the Central Technical Services at the University of Toronto. The Calcium Reagent Set and ALP kit were purchased from TECO Diagnostics (Anaheim, CA, USA). Oil red O and fatty acid-free bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). All other chemicals were purchased from Sigma.

2.2. Cell culture

The MSCs obtained from Lonza were derived from normal human bone marrow from a 23-year-old female, and retain their ability to differentiate into osteoblasts, adipocytes and chondrocytes. Cells were maintained in mesenchymal stem cell growth media (MSCGM). For osteogenic differentiation, MSCs were seeded at a density of 2×10^3 cells per well in 0.5 ml of MSCGM. On the following day, the medium was replaced with 0.5 ml of the osteogenic differentiation (ODM) BulletKit. For adipogenic differentiation, MSCs were seeded at a density of 4×10^4 cells per well in 0.5 ml MSCGM. On the following day, the medium was replaced with 0.5 ml of preadipocyte growth media-2 (PGM-2). For both osteogenic and adipogenic differentiation, the cells were treated four days later with varying concentrations (6.25, 12.5, 25 and 50 μM) of 9,11 or 10,12 CLA or vehicle (0.1% ethanol and 1 g/L of fatty acid-free

BSA) prepared in either the ODM BulletKit or PGM-2 BulletKit. This time period was selected to allow for the cells to stretch and increase their skeletal tension, which is required for osteoblast differentiation [56], and to allow for the cells to undergo mitotic clonal expansion, which is necessary for adipocyte differentiation [57]. The concentrations of CLA used were selected because Mosely et al. [58] demonstrated that the total serum concentration of 9,11 CLA can reach 43 μM in fasted women after consuming ^{13}C-*trans* vaccenic acid (2.5 mg/kg body weight). Others have reported that the physiological concentration of CLA in human plasma phospholipids is approximately 10 μM [59]. Thus, the concentrations selected for this study are physiologically relevant. For both osteogenic and adipogenic differentiation CLA was added to the respective serum-free BulletKit by first dissolving it in ethanol, which was then added to mesenchymal cell growth supplement (serum for osteogenic differentiation) or fetal bovine serum (adipogenic differentiation), supplemented with 1 g/L of fatty acid-free BSA. The final concentration of ethanol and BSA in each well was 0.1% and 1 g/L, respectively. The medium containing CLA or vehicle was changed twice per week.

2.3. Calcium quantification for in vitro mineralization

After 14 days of treatment, the cells grown under osteogenic conditions were washed twice with PBS and frozen in 100 μl of 0.5N HCl at −70°C. After thawing, the cells were scraped from the plates and placed on an orbital shaker overnight at 4°C. The samples were then centrifuged at 500×*g* for 2 min. The amount of calcium in the supernatant was quantified using the TECO Diagnostics colorimetric Calcium Reagent Set, according to the manufacturer's instructions. The cell pellet was resuspended in 0.1 N NaOH and 0.1% sodium dodecyl sulfate, and the protein concentration was measured at 570 nm using the Bio-Rad protein Assay reagent. The amount of calcium in the supernatant was normalized to its cellular protein concentration, calculated as units per gram of protein and expressed as percent of control.

2.4. ALP activity

After 7 days of treatment, the cells grown under osteogenic conditions were washed twice with 50 mmol/L Tris–HCl (pH 7.35). Cells were lysed in buffer containing 0.05% Triton-X-100 in 50 mmol/L Tris–HCl (pH 7.35) following one freeze-thaw cycle. ALP activity was determined in cell sonicates using a commercially available kit from TECO Diagnostics, as previously described [13]. The ALP activity of each sample was normalized to its protein concentration and expressed as percent of control.

2.5. Oil red O staining

After 7 days of treatment, the cells grown under adipogenic conditions were washed twice with PBS and fixed in 10% formalin in PBS for 10 min. The cells were then washed with 60% isopropanol and allowed to air dry. Oil red O was added to the wells at a concentration of 0.21% (w/v) for 10 min, washed four times with distilled water, washed once with 50% ethanol to remove background colorization and washed once more with distilled water. Oil red O was extracted in 100% isopropanol and absorbance was measured at 415 nm. The amount of oil red O accumulated within each well was quantified using a standard curve produced with a serial dilution of the oil red O stain in isopropanol.

2.6. RNA isolation and real-time, one-step reverse transcriptase-polymerase chain reaction

After 7 days of treatment, the cells were washed twice with PBS and lysed in 0.5 ml of nucleic acid purification solution (Applied Biosystems). Total RNA was isolated using the 6100 Nucleic Acid PrepStation (Applied Biosystems). A one-step reaction was performed in an ABI Prism 7000 Sequence Detection System (Applied Biosystems) to reverse transcribe the mRNA into cDNA, which was then amplified using the QuantiTect Multiplex reverse transcriptase-polymerase chain reaction Kit (Qiagen) and TaqMan Gene Expression Assays (Applied Biosystems). All reactions were performed in 96-well plates with a final volume of 25 μl per well. Cycling conditions were 20 min at 50°C followed by 15 min at 95°C to activate the HotStarTaq DNA Polymerase, 50 cycles of 45 s at 94°C and 45 s at 60°C. The TaqMan Gene Expression Assays used were Hs00559664_m1 (Wnt10b), Hs00231692_m1 (runx2), Hs01587813_g1 (osteocalcin), Hs00269972_s1 (C/EBPα), Hs00234592_m1 (PPARγ) and Hs00609791_m1 (FABP4). Each target gene was coamplified with VIC-labeled β-2-microglobulin (Applied Biosystems, #4326319E) as an internal control. Data were obtained as threshold cycle (C_T) values, which represent the cycle at which the first significant increase in fluorescence is detected, which corresponded to the amount of starting template in the sample. The difference in C_T values (ΔC_T) between the internal control (VIC-labeled β-2-microglobulin) and target gene of interest (FAM-labeled) was calculated to determine the relative change in C_T values between samples. The average ΔC_T of control samples was subtracted from the ΔC_T of treatment samples to derive a $\Delta\Delta C_T$ value, which represents the change in mRNA expression between treatments relative to controls. Relative mRNA levels were calculated as $2^{-\Delta\Delta C_T}$ and expressed as fold change relative to control samples that produce a $2^{-\Delta\Delta C_T}$ value of 1.

2.7. Statistical analyses

Results are expressed as mean±S.E.M. with at least three replicates in each group. Differences were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. P values <.05 were considered

significant. All data were analyzed using GraphPad Prism Software, Version 4.0.

3. Results

3.1. Osteoblast differentiation

To determine the effect of CLA on osteoblast differentiation, we first measured the amount of calcium deposited by the cells. The effect of increasing concentrations of 9,11 and 10,12 CLA on calcium deposition in MSCs is shown in Fig. 1. As shown in Fig. 1A, 12.5 and 25 μM 9,11 CLA decreased calcium deposition by 17% and 15%, respectively, compared to vehicle treated cells. In contrast, 6.25–50 μM of 10,12 CLA increased calcium deposition by 12–60% (Fig. 1B). To provide evidence that 10,12 CLA promoted extracellular calcium accumulation rather than increasing intracellular calcium levels and, therefore, osteoblast differentiation. We next determined the effect of CLA on ALP activity, which is another marker of osteoblast differentiation. The effect of increasing concentrations of 9,11 and 10,12 CLA on ALP activity in MSCs is shown in Fig. 2. The 9,11 isomer had no effect on ALP activity after 7 days of treatment (Fig. 2A). However, 50 μM 10,12 CLA significantly increased ALP activity by ~2.1-fold (Fig. 2B).

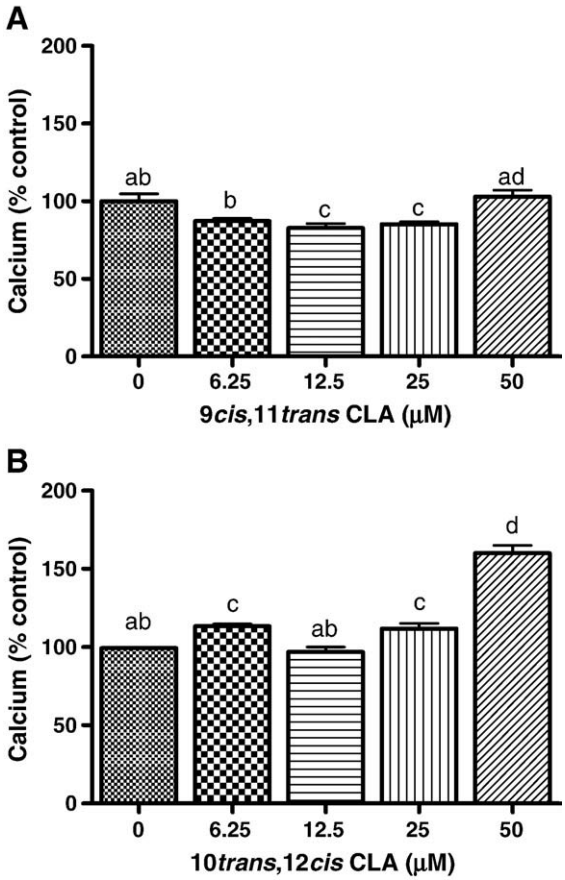

Fig. 1. Effects of individual isomers of CLA on calcium deposition in MSCs. Cells were cultured in 24-well plates and treated for 14 days in osteogenic media with either vehicle or 9,11 (A) or 10,12 (B) CLA at concentrations of 6.25, 12.5, 25 and 50 μM. Values are expressed as mean±S.E.M. The effect of concentrations of individual isomers on calcium deposition was analyzed using a 1-way ANOVA followed by Tukey's multiple comparison test. $P<.05$ was considered significant.

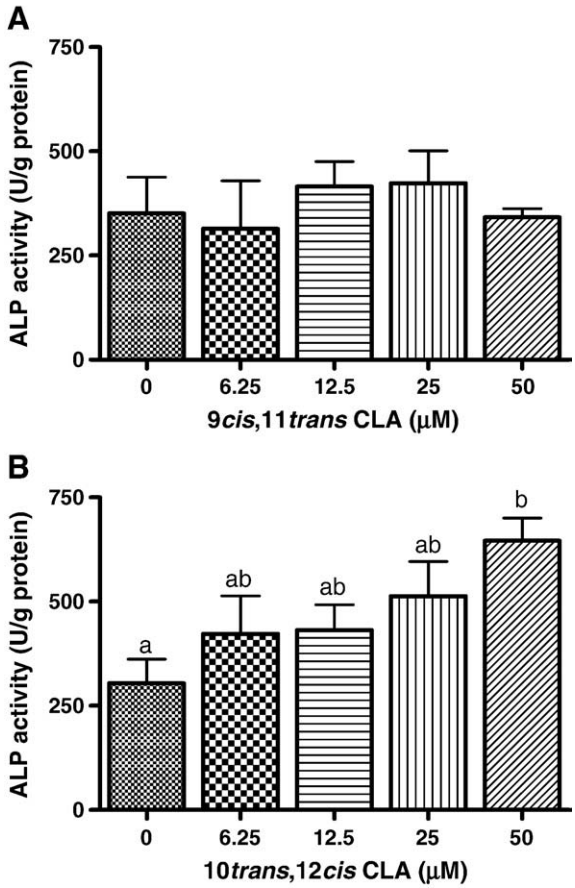

Fig. 2. Effects of individual isomers of CLA on ALP activity in MSCs. ALP activity was measured in cells treated with either vehicle or one of 9,11 (A) or 10,12 (B) CLA isomers at concentrations of 6.25, 12.5, 25 and 50 μM for 7 days in osteogenic media. Values are expressed as mean±S.E.M. The effect of concentration of individual isomers on ALP activity was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $P<.05$ was considered significant.

3.2. Oil Red O staining

To determine the effect of CLA on adipocyte differentiation, we measured the amount of oil red O taken up by the differentiated cells. The effect of increasing concentrations of 9,11 and 10,12 CLA on oil red O accumulation is shown in Fig. 3. Treatment with 9,11 CLA increased oil red O staining at 25 and 50 μM. Compared to vehicle treated cells, 25 μM 9,11 CLA increased oil red O accumulation by ~21% (or 0.58 mg/well), and 50 μM increased accumulation by ~28% (or 0.77 mg/well) (Fig. 3A). In contrast, 10,12 CLA reduced oil red O accumulation at 6.25 μM by ~31% (or 0.77 mg/well) and at 12.5 μM by ~29% (or 0.73 mg/well) (Fig. 3B).

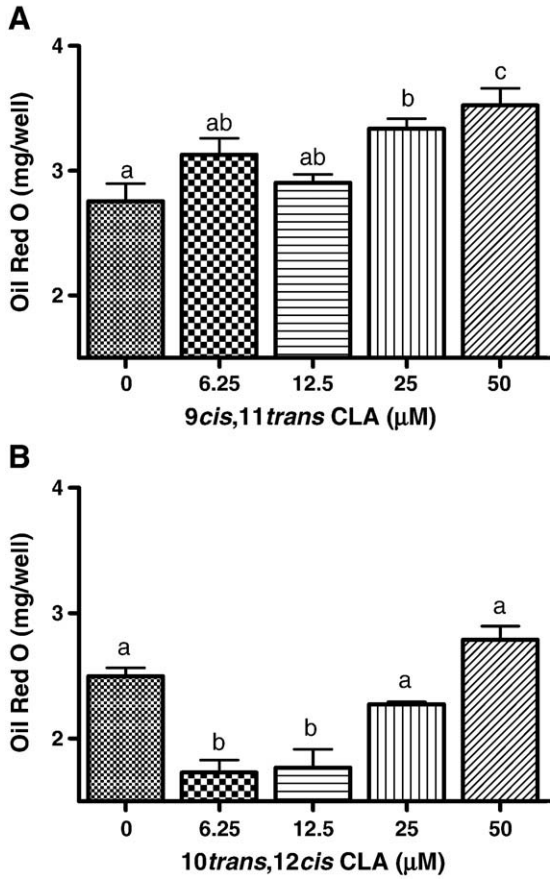

Fig. 3. Effects of individual isomers of CLA on oil red O accumulation in MSCs. Cells were treated with either vehicle or one of 9,11 (A) or 10,12 (B) CLA isomers at concentrations of 6.25, 12.5, 25 and 50 μM for 7 days in adipogenic media. After oil red O staining, the dye was extracted and absorbance at 415 nm was determined. The amount of oil red O accumulated was calculated using a standard curve of known concentrations of oil red O. Values are expressed as mean±S.E.M. The effect of concentration of individual isomers on oil red O accumulation was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $P<.05$ was considered significant.

3.3. Osteogenic gene expression

To determine whether the effects of 9,11 and 10,12 CLA on MSC differentiation into osteoblasts and adipocytes might be mediated by changes in Wnt signaling, we examined the effects of these isomers on Wnt10b expression. As shown in Fig. 4, 9,11 CLA had no effect on Wnt10b expression, whereas the 10,12 isomer increased Wnt10 expression at all concentrations tested by ~60–80%, compared to vehicle-treated cells (Fig. 4A). To provide further evidence that the effects of CLA on calcium deposition and ALP activity were due to changes in osteoblast differentiation, we investigated the effects of CLA on runx2 (Fig. 4B) and osteocalcin (Fig. 4C) gene expression. As seen in Fig. 4B, the 9,11 and 10,12 isomers of CLA had no effect on runx2 expression. Similarly, 9,11 CLA had no effect on osteocalcin gene expression as seen in Fig. 4C. In contrast, 10,12 CLA

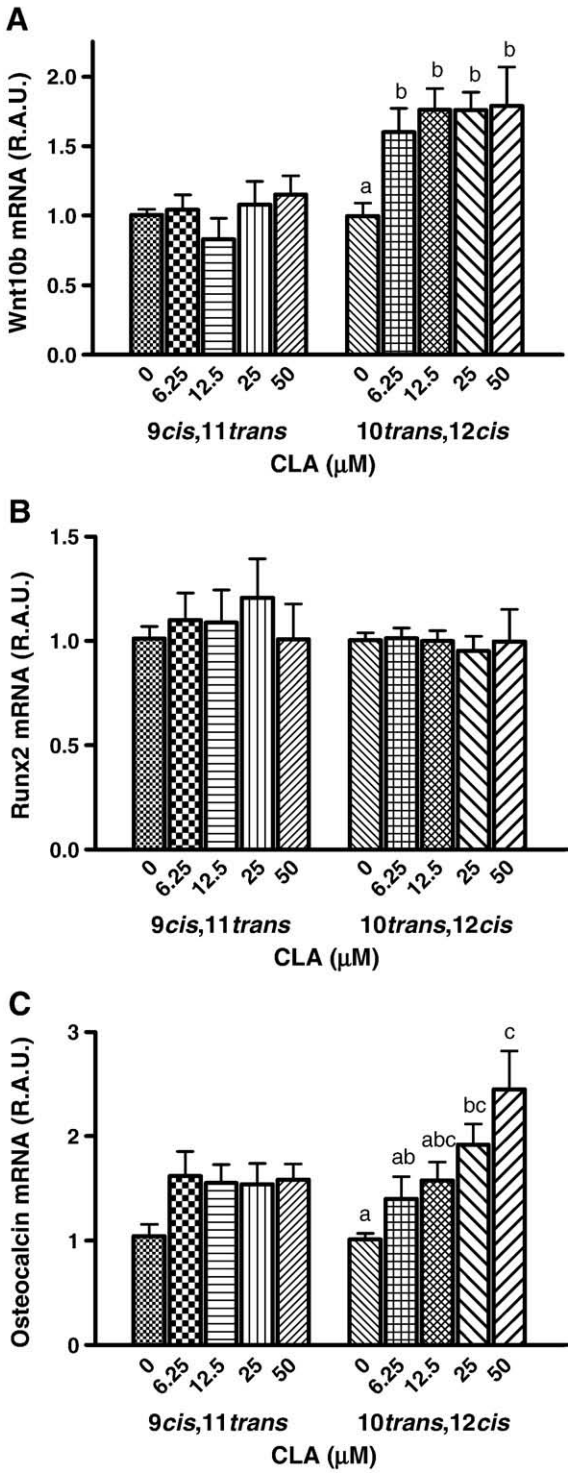

Fig. 4. Effects of individual isomers of CLA on osteogenic gene expression in MSCs treated with either vehicle or one of 9,11 or 10,12 CLA isomers at concentrations of 6.25, 12.5, 25 and 50 μM for 7 days in osteogenic media. Values are expressed as mean±S.E.M. The effect of concentration of individual isomers on Wnt10b (A), Runx2 (B) and osteocalcin (C) gene expression was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $P<.05$ were considered significant.

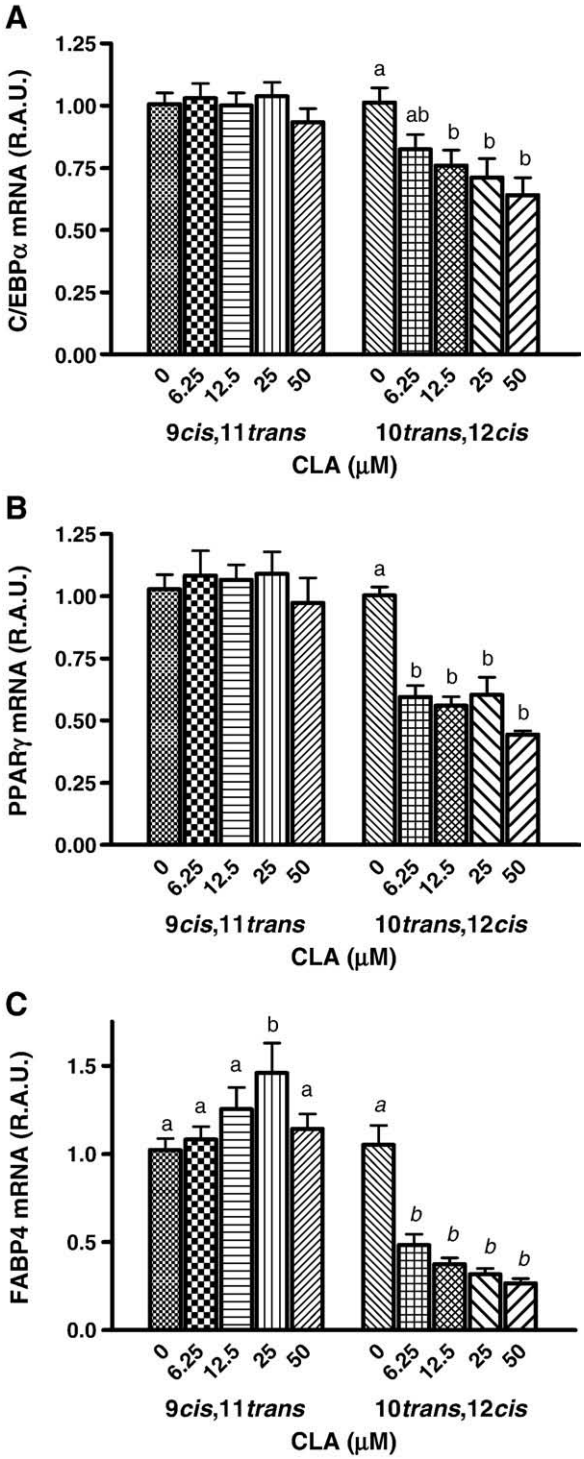

Fig. 5. Effects of individual isomers of CLA on adipogenic gene expression in MSCs treated with either vehicle or one of 9,11 or 10,12 CLA isomers at concentrations of 6.25, 12.5, 25 and 50 μM for 7 days in adipogenic media. Values are expressed as mean±S.E.M. The effect of concentration of individual isomers on C/EBPα (A), PPARγ (B) and FABP4 (C) gene expression was analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $P<05$ were considered significant.

increased osteocalcin expression by 90% at 25 μM and by 2.5-fold at 50 μM (Fig. 4C).

3.4. Adipogenic gene expression

Activation of Wnt signaling has been shown to promote osteoblast differentiation and inhibit adipocyte differentiation from MSCs by down-regulating C/EBPα expression [51]. Therefore, we determined whether the effects of 9,11 and 10,12 CLA on Wnt10b expression were accompanied by a reciprocal effect on C/EBPα expression. As seen in Fig. 5, there was no effect of 9,11 CLA on C/EBPα expression compared to vehicle treated cells (Fig. 5A). However, 1.25–50 μM 10,12 CLA significantly decreased C/EBPα expression by ~24–38% (Fig. 5A), which is consistent with the stimulatory effect of this isomer on Wnt10b expression. To test the hypothesis that CLA has isomer-specific effects on adipogenic gene expression in MSCs, we determined the effect of 9,11 and 10,12 CLA on PPARγ and FABP4 expression. The effect of increasing concentrations of 9,11 and 10,12 CLA on PPARγ expression is shown in Fig. 5. As seen in Fig. 5B, 9,11 CLA had no effect on PPARγ expression, but 10,12 CLA reduced PPARγ expression by ~60% at concentrations as low as 12.5 μM (Fig. 5B), which is consistent with the effects of this isomer on C/EBPα. The effect of increasing concentrations of 9,11 and 10,12 CLA on FABP4 expression is shown in Fig. 5C. As seen in Fig. 5C, 25 μM 9,11 CLA increased FABP4 expression by ~46%. In contrast, 10,12 CLA decreased FABP4 at all concentrations in a dose-dependent manner (~52–74%) (Fig. 5C).

4. Discussion

In the present study, we have shown that the 9,11 and 10,12 isomers of CLA have differential effects on the differentiation of MSCs into osteoblasts and adipocytes. The most abundant isomer found in food products from ruminant animals is the 9,11 isomer, which caused a dose-dependent increase in adipocyte differentiation as determined by an increase in oil red O staining and FABP4 expression. In contrast, the 10,12 isomer, which is mainly found in synthetic CLA preparations, increased osteoblast differentiation from MSCs as determined by an increase in calcium deposition, ALP activity, and the expression of Wnt10b and osteocalcin. The increase in Wnt10b expression by 10,12 CLA is consistent with the inhibitory effect we observed by this isomer on C/EBPα and PPARγ expression. Activation of Wnt signaling by Wnt10b has been shown to promote MSC differentiation along the osteoblast lineage by decreasing C/EBPα and PPARγ expression [51]. Thus, our findings suggest that 10,12 CLA promotes osteoblast differentiation from MSCs by up-regulating Wnt signaling.

The antiadipogenic properties of 10,12 CLA reported in earlier studies [32-34,60] is consistent with the findings from the present study demonstrating a down-regulation of the adipogenic transcription factor C/EBPα by this isomer. This

was accompanied by a reduction in both oil red O staining and FABP4 gene expression. We demonstrate that 10,12 CLA may reduce C/EBPα by increasing Wnt10b gene expression. Activation of the Wnt/β-catenin signaling pathway by Wnt10b has been shown to be sufficient to inhibit differentiation and stimulate apoptosis of adipocytes [54,61-63]. Thus, 10,12 CLA may increase adipocyte apoptosis, as demonstrated in previous studies [14,15,37,64], by increasing Wnt10b expression. Moreover, 10,12 CLA might further decrease adiposity by increasing fatty acid oxidation [32] and inflammation [38], by modulating PPARγ expression [33,35,39,40,64,65] and activity [39], and by reducing intracellular triacylglycerol levels [15,18,38] as suggested by its inhibitory effect on FABP4 expression. In addition to these potential mechanisms, we provide new evidence that 10,12 CLA may inhibit adipocyte differentiation and promote osteoblast differentiation from MSCs through the activation of Wnt signaling by Wnt10b.

The osteogenic effect of 10,12 CLA in MSCs is consistent with our previous findings in osteoblast-like SaOS-2 cells [13]. In both SaOS-2 cells and MSCs 10,12 CLA increased ALP activity; however, the effect of this isomer was not sufficient to induce mineralized bone nodule formation in SaOS-2 cells [13]. During osteogenic differentiation from MSCs, ALP is first detected in osteoblast progenitor cells [66], which are committed to differentiate into osteoblasts, but have yet to acquire the mature osteoblast phenotype. It is possible that 10,12 CLA promotes osteogenesis at this early stage of osteoblast differentiation and, therefore, does not affect mineralization of mature osteoblasts. Although we found no effect of either isomer on runx2 gene expression, 10,12 CLA reduced PPARγ expression. PPARγ indirectly inhibits osteoblast differentiation by suppressing runx2 gene expression and also physically inhibits osteoblast differentiation by binding to runx2 [50]. As such, a reduction in PPARγ expression by 10,12 CLA may promote osteoblast differentiation without altering runx2 expression by increasing the amount of unbound runx2, which is required for the transactivation of downstream osteogenic genes such as osterix and osteocalcin [50]. Therefore, the decrease in PPARγ expression by the 10,12 isomer in MSCs supports the hypothesis that this isomer displays osteogenic and antiadipogenic properties in these cells.

In contrast to the osteogenic effect of 10,12 CLA, 9,11 CLA did not increase osteoblast differentiation from MSCs. Under osteogenic growth conditions, 9,11 CLA had no effect on calcium deposition, ALP activity or the expression of Wnt10b, runx2 and osteocalcin. However, we have previously demonstrated that 9,11 CLA promotes the differentiation of SaOS-2 cells, as shown by a dose dependent increase in mineralized bone nodule formation and a variable increase in ALP activity after 2 weeks of treatment [13]. Similarly, in murine calvarial cells, treatment with 9,11 CLA for the same duration of time reduced runx2 gene expression in a dose-dependent manner [26]. SaOS-2 and calvarial cells are already committed along the osteoblast lineage; therefore, 9,11 CLA likely enhances the osteoblast phenotype in mature osteoblastic cells. This may explain why we observed no effect of 9,11 CLA on osteogenic differentiation from MSCs, which are not fully committed to the osteoblast lineage. It is also possible that CLA has different effects on the different cell lines because of genetic variability since the SaOS-2 cells and the MSCs are both obtained from single donors.

Although 9,11 CLA had no effect on osteoblast differentiation from MSCs, we demonstrated that this isomer promoted adipogenesis, as determined by an increase in oil red O staining and FABP4 expression. These findings are consistent with earlier reports demonstrating that 9,11 CLA increases adipocyte differentiation in vitro [67,68]. The null effect of 9,11 CLA on osteoblast differentiation from MSCs suggests that this isomer acts downstream of Wnt signaling to promote adipogenesis without affecting osteogenesis. 9,11 CLA may directly increase adiposity by increasing FABP4-mediated lipid accumulation.

Regulation of FABP4 is one mechanism by which CLA may exert its isomer-specific effects on lipid accumulation in adipocytes derived from MSCs. FABP4 is a carrier protein that transports and promotes the storage of lipids within adipocytes, and its expression can be induced by fatty acids, likely through changes in PPARγ expression or activity, both at the transcriptional and posttranscriptional level [69,70]. In accordance with its inhibitory effect on PPARγ expression, we demonstrated that 10,12 CLA down-regulates the expression of FABP4 in human MSCs, which is consistent with its effects in rodent adipocytes [39,71] and in mice [38]. The reduction in FABP4 expression by 10,12 CLA is consistent with the observed reduction in oil red O staining. Although 9,11 CLA did not alter C/EBPα or PPARγ expression, it may have increased their activity, which would explain the observed stimulatory effect of this isomer on FABP4 expression. C/EBPα expression is maintained through autoactivation [72] and stimulates and maintains PPARγ expression [45], leading to the autoactivation of PPARγ expression. Therefore, 9,11 CLA likely increased adipogenesis by promoting the activity rather than the expression of these key adipogenic genes.

The divergent effects of 9,11 and 10,12 CLA on adipocyte and osteoblast differentiation from MSCs may explain some of the variability observed in previous studies examining the effects of CLA on bone health and adiposity in vivo. Most of the rodent studies demonstrating variable effects of CLA on body ash [10,30,73], bone mineral content [19,23], bone formation rates [8,11,25] and markers of bone formation and resorption [9,12] have used commercial preparations of CLA. As such, the individual effects of 9,11 and 10,12 CLA cannot be elucidated from these studies. In one rodent study, a commercial blend of CLA containing approximately 44% 10,12 and 41% 9,11 CLA, increased bone ash and decreased fat mass [73]. These results are supported by the findings from the present study demonstrating that 10,12 CLA promotes osteogenesis. The slightly higher amount of

10,12 CLA in the diet [73] may have shifted MSC differentiation in favor of osteoblast formation. Although our results are based on bone marrow derived MSCs, the shift towards increased osteogenesis by 10,12 CLA may be significant because these cells are able to migrate from the marrow and help form or repair bone tissue [3].

To our knowledge, the present study is the first to examine the effects of 9,11 and 10,12 CLA on osteoblast and adipocyte differentiation from MSCs. We have demonstrated isomer specific effects of 9,11 and 10,12 CLA on osteoblast and adipocyte differentiation from MSCs. 10,12 CLA increases osteoblast differentiation and suppresses adipocyte differentiation, whereas 9,11 CLA increases adipocyte differentiation without affecting osteoblast differentiation. Although isolated cell cultures are useful models to test directly the effects of purified CLA on osteoblast and adipocyte differentiation, they do not account for differences in CLA metabolism or physiology. Therefore, findings from this study warrant further investigation of the individual effects of 9,11 and 10,12 CLA on MSC differentiation in vivo.

Acknowledgments

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