Effect of growth hormone on the differentiation of bovine preadipocytes into adipocytes and the role of the signal transducer and activator of transcription 5b

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ABSTRACT: We evaluated the effect of GH on the differentiation of primary bovine preadipocytes into adipocytes. Bovine preadipocytes, derived from adipose tissue explants, were induced to differentiate into adipocytes in the presence or absence of recombinant bovine GH. The differentiation status of adipocytes was assessed by Oil Red O staining and by measuring the activity of glycerol-3-phosphate dehydrogenase (G3PDH) and the rate of acetate incorporation. Fewer preadipocytes became adipocytes in the presence of GH than in the absence of GH; adipocytes formed in the presence of GH had lower G3PDH activity and lower rate of acetate incorporation than those formed without GH treatment (P < 0.05). These data suggest an inhibitory effect of GH on the differentiation of bovine preadipocytes into adipocytes. Growth hormone decreased the expression of C/EBPα and PPARγ mRNA in bovine adipocytes (P < 0.05). Because C/EBPα and PPARγ are the master regulators of adipocyte differentiation, this data suggests that GH might inhibit the differentiation of bovine preadipocytes into adipocytes by inhibiting the expression of C/EBPα and/or PPARγ. Because the signal transducer and activator of transcription 5 (STAT5) is a major component of signaling from the GH receptor, we next determined the potential role of STAT5 in GH inhibition of bovine adipocyte differentiation. Overexpression of a constitutively active form of STAT5b (STAT5bCA) in bovine preadipocytes through adenoviral transduction mimicked the effects of GH on the formation of lipid-containing adipocytes, G3PDH activity, and acetate incorporation rate. Overexpression of STAT5bCA was associated with decreased expression of C/EBPα mRNA (P < 0.05) but not that of PPARγ mRNA in bovine adipocytes. These results support a role of STAT5b in mediating GH inhibition of C/EBPα expression but not that of PPARγ expression in bovine preadipocytes. Overall, the present study suggests that GH may inhibit adipose growth in cattle in part by inhibiting adipogenesis and that GH inhibits the differentiation of bovine preadipocytes to adipocytes through STAT5b-dependent inhibition of C/EBPα expression and STAT5b-independent inhibition of PPARγ expression.

Key words: adipocyte, bovine, growth hormone, STAT5

INTRODUCTION

Growth hormone is widely known for its stimulatory effects on muscle growth and milk production (Etherton and Bauman, 1998), but it also has a po-

1This project was supported by Agriculture and Food Research Initiative competitive grant no. 2009-35205-05221 from the USDA National Institute of Food and Agriculture. Lidan Zhao was supported in part by a John Lee Pratt Fellowship in Animal Nutrition. The authors would like to thank Lee Johnson for excellent technical assistance and Smith Valley Meats (Rich Creek, VA) for tissue samples.

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Received September 3, 2013.
Accepted March 6, 2014.
Adipogenesis is the process of differentiation of preadipocytes into lipid-containing adipocytes, which is primarily controlled by the transcription factors PPARγ and C/EBPα (Rangwala and Lazar, 2000; Rosen and MacDougald, 2006). Whether GH inhibits adipose growth also through inhibition of adipogenesis is not clear. Whereas studies in primary preadipocytes indicate an inhibitory effect of GH on adipocyte differentiation (Hausman and Martin, 1989; Wabitsch and Heinzé, 1993; Wabitsch et al., 1996; Hansen et al., 1998; Gerfaut et al., 1999), those in preadipocyte cell lines show the opposite (Morikawa et al., 1982; Doglio et al., 1986; Corin et al., 1990; Clarkson et al., 1995).

The objective of this study was to further determine the effect of GH on adipogenesis using the primary bovine preadipocytes. Our results demonstrate that GH inhibits the differentiation of primary bovine preadipocytes into adipocytes. Our results also suggest that this inhibition is partially mediated by the signal transducer and activator of transcription 5b (STAT5b).

**MATERIALS AND METHODS**

**Bovine Preadipocytes Preparation and Culture**

Bovine preadipocytes were prepared as previously described (Lengi and Corl, 2010). Briefly, subcutaneous fat was collected from steers when slaughtered and transported in Dulbecco’s Modified Eagle Medium (DMEM) to the lab in less than 30 min. The tissue was minced into small pieces and then cultured in DMEM supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 1% of antibiotics–antimycotics (ABAM), and 2 mM of L-glutamine (Mediatech, Manassas, VA) at 37°C and 5% of CO₂. Medium was changed every 4 d. After 10 d, fibroblast-like cells that grew out of the explants were collected and plated onto 6-well plates or 24-well plates at a density of 2.5 × 10⁴/cm². When cells reached 100% confluence, they were induced to differentiate into adipocytes. In the first 2 d of differentiation, the cells were cultured in serum-free DMEM/F12 (1:1 vol/vol; Sigma-Aldrich, St. Louis, MO) supplemented with 10 μg/mL insulin (Sigma-Aldrich), 0.25 mM dexamethasone (Sigma-Aldrich), 0.5 mM 3-isobutyl-1-methylxantine (IBMX), and 1 μM rosiglitazone (Cayman Chemical, Ann Arbor, MI). Thereafter, cells were cultured in serum-free DMEM/F12 (1:1 vol/vol) containing 10 μg/mL insulin and 1 μM rosiglitazone. Based on Oil Red O staining (see below), bovine preadipocytes maintained in medium without adipogenic inducers (i.e., insulin, dexamethasone, IBMX, and rosiglitazone) did not form lipid-containing adipocytes. Recombinant bovine GH (National Hormone and Peptide Program, Torrance, CA) at 100 ng/mL was added to the differentiation medium from Day 1 of differentiation. The medium and GH were refreshed every other day during differentiation.

**Acetate Incorporation Assay**

Acetate incorporation assay was conducted as previously described (Lengi and Corl, 2010). In brief, the medium of differentiating adipocytes was replaced with fresh differentiation medium added with 1 μCi [1-¹⁴C]-acetate acid (ARC Inc., St. Louis, MO). The incubation was continued for another 4 h at 37°C and 5% of CO₂. The organic phase was transferred to the scintillation vials and subjected to scintillation counting. Each sample was counted in duplicate.

**Preparation of Whole Cell Lysates and Western Blot Analysis**

Cells were washed with ice-cold PBS and lysed in buffer consisting of 50 mM Tris-HCl, pH 8.0, 150 mM sodium
chloride, 1.0% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS supplemented with protease inhibitors and phosphatase inhibitors (Roche, Indianapolis, IN) and sonicated as described above. The whole cell lysates were centrifuged at 13,000 ×g for 15 min at 4°C. Protein concentrations in the supernatant were measured as described above. Equal amounts of protein samples were separated by 10% SDS-PAGE and transferred electrophoretically onto nitrocellulose membranes. Membranes were immunoblotted with anti-signal transducer and activator of transcription 5 (STAT5; 1:1,000 dilution), anti-phosphorylated STAT5 (1:1,000), or anti β-actin antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibodies were detected by 1:1,000 diluted horseradish peroxidase-coupled IgG (Santa Cruz Biotechnology). Secondary antibody was detected by incubating the membrane with enhanced chemiluminescence (ECL) substrate (Thermo Scientific, Rockford, IL). Densities of detected protein bands were quantified using ImageJ (http://imagej.nih.gov/ij/). In the western blot analysis of STAT5 phosphorylation, density of phosphorylated STAT5 protein was normalized to that of total STAT5 protein from the same sample. In the western blot analysis of overexpressed STAT5, density of total STAT5 protein was normalized to that of β-actin from the same sample.

**Total RNA Extraction and Real-Time Reverse Transcription PCR**

Total RNA from the cells was isolated using TRI reagent (MRC, Cincinnati, OH), according to the manufacturer’s instruction. The cDNA was generated using the ImProm-II Reverse Transcription System (Promega, Madison, WI), according to the manufacturer’s instruction. The PPARγ, PPARγ1, PPARγ2, and C/EBPα mRNA were quantified by real-time PCR using the Power SYBR Green PCR Fast Master Mix (Applied Biosystems, Foster City, CA). Sequences of primers are shown in Table 1. Specificity of primers was validated by gel electrophoresis and DNA sequencing, and efficiency of primers was validated by amplifying a serial dilution of cDNA. Each sample was quantified in duplicate. The PCR data were analyzed using the 2(-ΔΔCt) method (Livak and Schmittgen, 2001), using 18S rRNA as the internal control. Based on the cycle threshold (Ct) values, 18S rRNA expression was stable across the samples (P > 0.1).

**Constitutively Active STAT5b Adenovirus Generation**

The cDNA plasmid encoding constitutively active STAT5b (STAT5bCA) was kindly provided by Peter Rotwein (Oregon Health and Science University). This cDNA contained a substitution of histidine for asparagine at position 642 compared to the natural STAT5b (Woelfle et al., 2003). Adenoviruses expressing STAT5bCA were generated using the ViraPower Adenoviral Expression System (Invitrogen, Carlsbad, CA), according to the manufacturer’s instruction. Briefly, STAT5bCA cDNA was inserted into the adenoviral expression vector pAd/CMV/V5-DEST to generate pAD/CMV/STAT5bCA. This plasmid was confirmed by sequencing and transfected into 293A cells using FuGENE6 (Promega, Fitchburg, WI). Cells were harvested at the time of 70 to 80% plaque formation followed by 3 cycles of freezing (–80°C) and thawing (37°C). Crude viruses in the supernatant were collected by centrifuging at 3,000 ×g for 15 min at 4°C. The crude viruses were amplified twice in 293A cells and then purified using cesium chloride density gradient combined with ultracentrifugation. We also constructed LacZ expression adenoviruses using the construct pAd/CMV/V5-GW/LacZ from Invitrogen. The viruses were titered using the Adeno-X Rapid Titer Kit (Clontech, Mountain View, CA). The ability of the STAT5bCA adenoviruses to express constitutively active STAT5 was confirmed by an electrophoretic mobility shift assay, in which the expressed protein was able to bind to a known STAT5 binding DNA element from the bovine hepatocyte nuclear factor 3γ gene (Eleswarapu et al., 2009).

**Statistical Analyses**

Data were analyzed using General Linear Model of JMP (SAS Inst. Inc., Cary, NC). The experimental unit

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<td>Y12420.1</td>
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<td></td>
<td>Reverse: CTTCCAATGGATCTCGTTA</td>
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was the cell culture. Each experiment was repeated at least 4 times, using cells derived from different cattle. Two groups were compared by t test. Multiple groups were initially analyzed by ANOVA. When the effects of treatments were significant, treatment means were further analyzed by Tukey’s test. Differences were considered significant when P < 0.05.

RESULTS

Growth Hormone Inhibited the Differentiation of Bovine Preadipocytes into Adipocytes

To investigate the effect of GH on adipogenesis, preadipocytes derived from bovine adipose tissue explants in culture were induced to differentiate in the absence or presence of 100 ng/mL recombinant bovine GH for 6 d. At Day 0, the cells had a fibroblast-like appearance with no visible lipid droplets when observed with a microscope. At Day 6 of differentiation, the cells acquired a round shape, and lipid droplets became visible in the cytoplasm. Lipid accumulation in adipocytes was confirmed by Oil Red O staining. These changes were not observed in preadipocytes cultured extensively in growth medium without adipogenic inducers (i.e., insulin, dexamethasone, IBMX, and rosiglitazone), meaning that the preadipocytes were not “contaminated” with adipocytes and that the preadipocytes did not spontaneously differentiate into adipocytes. Addition of GH to the differentiation medium decreased the formation of adipocytes (Fig. 1A). To further determine the effect of GH on the differentiation of bovine preadipocytes into adipocytes, the activity of G3PDH was measured. This enzyme catalyzes the formation of glycerol backbone of triglyceride and is considered as a rate-limiting enzyme for triglyceride synthesis in adipocytes (Wise and Green, 1979). As can be seen in Fig. 1B, adipocytes differentiated in the presence of GH had less G3PDH activity than those in the absence of GH (P < 0.05). In ruminant adipose tissue, acetate is the primary source for de novo fatty acid synthesis (Chilliard, 1993). We measured the rate of acetate incorporation into lipids as another index of the differentiation status of adipocytes. As can be seen in Fig. 1C, the rate of acetate incorporation in adipocytes differentiated in the presence of GH was 40% less than those in the absence of GH (P < 0.05). All these data indicated that GH inhibited the differentiation of primary bovine preadipocytes into adipocytes.

Effects of GH on C/EBPα and PPARγ mRNA Expression in Bovine Adipocytes

Differentiation of preadipocytes into adipocytes is primarily driven by the transcription factors PPARγ and C/EBPα. The former is expressed as 2 splicing variants, PPARγ1 and PPARγ2, and the expression of PPARγ2 is adipose tissue specific (Rangwala and Lazar, 2000). To test the possibility that GH inhibits the differentiation of bovine preadipocytes into adipocytes by inhibiting the expression of PPARγ and/or C/EBPα, we measured the ex-
expression levels of PPARγ and C/EBPα mRNA in bovine adipocytes differentiated with or without GH. As shown in Fig. 2, adipocytes differentiated with GH expressed less total PPARγ, PPARγ1, and PPARγ2 mRNA than those differentiated without GH. We also measured the expression levels of PPARγ and C/EBPα mRNA in undifferentiated bovine preadipocytes and found that they were only 35%, and 3% of those in differentiated bovine adipocytes. These gene expression data support the possibility that GH inhibits the differentiation of bovine preadipocytes into adipocytes by inhibiting the mRNA expression of PPARγ and/or C/EBPα.

**Effect of Constitutively Active STAT5b Overexpression on Bovine Preadipocyte Differentiation**

At the cellular level, GH exerts its action by binding to the GH receptor (GHR) and subsequently activating the Janus kinase 2 (JAK2). The activated JAK2 phosphorylates several intracellular substrates including STAT5, each leading to a signaling pathway. Among the GH-activated signaling pathways, the JAK2–STAT5 pathway is considered the primary pathway that mediates GH regulation of gene transcription (Herrington et al., 2000; Piwien-Pilipuk et al., 2002). To test the possibility that GH inhibits the differentiation of bovine preadipocytes into adipocytes by the JAK2–STAT5 signaling pathway, we first determined if GH action leads to STAT5 phosphorylation in bovine preadipocytes and adipocytes. Western blot analyses showed that GH induced STAT5 phosphorylation in both undifferentiated bovine preadipocytes and differentiated bovine adipocytes (Fig. 3).

To investigate the role of STAT5 in GH inhibition of bovine preadipocyte differentiation, we determined if overexpression of STAT5bCA would mimic the inhibitory effect of GH on bovine preadipocyte differentiation. Overexpression of STAT5bCA in primary bovine preadipocytes was achieved through adenoviral transduction. Overexpression of the STAT5bCA protein was confirmed by a western blot analysis (Fig. 4A). The Oil Red O staining showed that fewer preadipocytes transduced with the STAT5bCA adenovirus became adipocytes than those transduced with the LacZ adenovirus (Fig. 4B), indicating that STAT5bCA overexpression inhibited bovine preadipocyte differentiation into adipocytes. Based on this staining, both the STAT5bCA and LacZ adenoviral transductions appeared to inhibit the differentiation compared with the no viral transduction (Fig. 4B). This inhibition was probably a side effect of adenoviral transduction.

We also determined the effects of STAT5bCA overexpression on the activity of G3PDH and the rate of acetate incorporation in differentiated adipocytes. As shown in Fig. 4C and 4D, adipocytes formed from preadipocytes transduced with the STAT5bCA adenovirus displayed less G3PDH activity and less acetate incorporation ability than those with LacZ adenovirus or untransduced cells (P < 0.05). These data supported a role of STAT5b in GH inhibition of bovine adipocyte differentiation.

**Effects of STAT5b Overexpression on PPARγ and C/EBPα Gene Expression**

To further determine the role of STAT5b in mediating the effect of GH on bovine adipocyte differentiation, we measured the expression levels of PPARγ and C/EBPα mRNA in adipocytes transduced with the STAT5bCA adenovirus, the LacZ adenovirus, or untransduced adipocytes at Day 6 of differentiation. The cells transduced with the STAT5bCA adenovirus had less C/EBPα mRNA compared with those transduced with the LacZ adenovirus or the no-adenovirus control (P < 0.05). However, these cells did not differ in the expression levels of total
Figure 4. Effect of constitutively active signal transducer and activator of transcription 5b (STAT5b; STAT5bCA) overexpression on bovine adipocyte differentiation. Bovine preadipocytes were induced to differentiate in the presence or absence (control) of LacZ adenovirus, or STAT5bCA adenovirus. Analyses of total signal transducer and activator of transcription 5 (STAT5) protein, glycerol-3-phosphate dehydrogenase (G3PDH) activity, and rate of acetate incorporation as well as Oil Red O staining were performed at Day 6 of differentiation. A) Total STAT5 protein expression detected by western blotting. Beta-actin served as a loading control. B) Oil Red O staining. C) Glycerol-3-phosphate dehydrogenase activity. D) Rate of acetate incorporation. Data are presented as mean ± SEM (n = 6 independent cell cultures). Means labeled with different letters are statistically different (P < 0.05). U = units. See online version for figure in color.
PPARγ, PPARγ1, or PPARγ2 mRNA (Fig. 5). These data indicated that STAT5b might mediate the inhibitory effect of GH on C/EBPα mRNA expression but not that on PPARγ mRNA expression in bovine adipocytes.

**DISCUSSION**

In this study, we determined the effect of GH on the differentiation of primary bovine preadipocytes into adipocytes. The hallmark of adipocyte differentiation is accumulation of lipids in the cells. In this study, we found that addition of GH to the differentiation medium of bovine preadipocytes inhibited the formation of lipid-filled adipocytes, as measured by Oil Red O staining. During the process of adipocyte differentiation, the activity of many lipogenic enzymes and hence the capacity of the cells to synthesize triglyceride increase (Wise and Green, 1979). The backbone of triglyceride comes from glucose-derived glycerol-β-phosphate, which is formed from dihydroxyacetone phosphate catalyzed by G3PDH. The activity of G3PDH increases several hundred fold from preadipocytes to adipocytes (Kuri-Harcuch et al., 1978). Therefore, G3PDH is widely considered as a marker of adipogenesis. In this study, we found that bovine adipocytes differentiated from preadipocytes in the presence of GH had less G3PDH activity than those in the absence of GH. This result again supports that GH inhibits the differentiation of bovine preadipocytes into adipocytes and suggests that GH may do so by inhibiting the G3PDH activity, a mechanism previously suggested by the study on rat preadipocytes (Wabitsch et al., 1996). Acetate is the predominant carbon source for de novo fatty acid synthesis in ruminant adipose tissue (Chilliard, 1979). The backbone of triglyceride comes from glucose-derived glycerol-β-phosphate, which is formed from dihydroxyacetone phosphate catalyzed by G3PDH. The activity of G3PDH increases several hundred fold from preadipocytes to adipocytes (Kuri-Harcuch et al., 1978). Therefore, G3PDH is widely considered as a marker of adipogenesis. In this study, we found that bovine adipocytes differentiated from preadipocytes in the presence of GH had less G3PDH activity than those in the absence of GH. This result again supports that GH inhibits the differentiation of bovine preadipocytes into adipocytes and suggests that GH may do so by inhibiting the G3PDH activity, a mechanism previously suggested by the study on rat preadipocytes (Wabitsch et al., 1996). Acetate is the predominant carbon source for de novo fatty acid synthesis in ruminant adipose tissue (Chilliard, 1993). Bovine adipocytes differentiated in the presence of GH had a lesser ability to incorporate acetate than those in the absence of GH. This provides a third line of evidence supporting an inhibitory effect of GH on the differentiation of bovine preadipocytes into adipocytes.

Differentiation of preadipocytes into adipocytes is driven primarily by the transcription factors PPARγ and C/EBPα (Rosen et al., 2000). The former is considered the master regulator of adipogenesis because PPARγ-deficient mice failed to form any forms of fat (Barak et al., 1999). Growth hormone inhibits PPARγ expression in primary rat preadipocytes (Hansen et al., 1998). Our data showed that GH inhibited PPARγ mRNA expression in primary bovine preadipocytes too. Therefore, inhibiting PPARγ gene expression may be a common mechanism by which GH inhibits the differentiation of primary preadipocytes of different species. In bovine preadipocytes, GH not only inhibited the expression of PPARγ but also that of C/EBPα, suggesting that the reduction in C/EBPα expression may also contribute to GH inhibition of differentiation of bovine preadipocytes into adipocytes. The transcription factors PPARγ and C/EBPα can stimulate each other’s expression in adipocytes (Rosen et al., 2000; Rosen and MacDougald, 2006). Thus, the GH-induced decrease in C/EBPα expression in bovine preadipocytes may be secondary to the decrease in PPARγ expression and vice versa.

The JAK2–STAT5 pathway is the primary signaling pathway that mediates GH regulation of gene transcription within a cell (Piwien-Pilipuk et al., 2002). Therefore, in this study we examined the possibility that GH inhibits the differentiation of bovine preadipocytes into adipocytes through STAT5. Our results demonstrate that the JAK2–STAT5 signaling is activated by GH in both undifferentiated and differentiated bovine preadipocytes. Overexpression of a constitutively active form of STAT5b (i.e., STAT5bCA) in bovine preadipocytes inhibited the G3PDH activity, the rate of acetate incorporation, and the number of adipocytes formed. These effects of STAT5bCA resembled those of GH, supporting a role of STAT5b in mediating GH-inhibited bovine preadipocyte differentiation. A similar role of STAT5 has been previously demonstrated in primary rat preadipocytes (Richter et al., 2003). Interestingly, in the preadipocyte cell lines 3T3-L1 and 3T3-F442A, overexpression of constitutively active STAT5 mimicked the proadipogenic effect of GH (Nanbu-Wakao et al., 2002; Floyd and Stephens, 2003; Shang and Waters, 2003; Stewart et al., 2004). Binding to its target genes, STAT5 can both activate and repress gene transcription, depending on whether it recruits a coactivator or a corepressor (Katsantoni, 2012). Perhaps it is the difference in the molecular partner recruited by STAT5 between primary preadipocytes and preadipocyte cell lines that GH has differential effects on the adipogenic differentiation of these cells.

Whereas GH inhibited the expression of both PPARγ and C/EBPα mRNA in bovine preadipocytes, overexpression of STAT5bCA only reduced that of C/EBPα mRNA. This suggests that STAT5b may mediate the inhibitory effect of GH on bovine adipocyte differentiation by inhibiting the expression of C/EBPα. The STAT5 protein binds to its target gene at the interferon γ-activated sequence (GAS) element, TTCNNNGAA, where N is A, G, C, or T (Horvath et al., 1995; Soldaini et al., 2000). The bovine C/EBPα promoter does not appear to contain a GAS element. Therefore, the inhibitory effects of STAT5bCA and GH on C/EBPα expression in bovine preadipocytes are unlikely the result of direct binding of STAT5b to the C/EBPα promoter. We speculate that GH-activated STAT5b indirectly inhibits the expression of the C/EBPα gene through another transcription factor, which directly binds to the C/EBPα promoter.

While STAT5b appears to mediate the inhibitory effect of GH on C/EBPα mRNA expression in the bovine preadipocytes, it probably does not mediate the inhibitory...
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effect of GH on PPARγ mRNA expression because PPARγ mRNA expression was not changed by STAT5bCA overexpression. The STAT5 protein has 2 isoforms, STAT5a and STAT5b, which are encoded by 2 different genes. Despite sharing more than 90% sequence identity, STAT5a and STAT5b can have different functions (Grimley et al., 1999; Herrington et al., 2000; Nakajima et al., 2001; Kornfeld et al., 2008). Therefore, it is possible that GH inhibits PPARγ gene expression in primary bovine preadipocytes through STAT5a, not STAT5b. Besides JAK2–STAT5, several additional signaling pathways are activated by GH in its target cells, including the phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and protein kinase C (PKC) signaling pathways (Herrington et al., 2000; Piwien-Pilipuk et al., 2002). It remains to be determined if GH uses any of these pathways to inhibit PPARγ mRNA expression in primary bovine preadipocytes. In the present study we induced the bovine preadipocytes to differentiate into adipocytes using a classical induction cocktail that included a high concentration of insulin, which was believed to exert its adipogenic activity through the IGF-I receptors that were expressed at high levels on preadipocytes (Smith et al., 1988; Rangwala and Lazar, 2000; Rosen and MacDougald, 2006). Long-term GH treatment is known to induce insulin resistance (Dominici et al., 2005). Therefore, we cannot rule out the possibility that GH inhibits the differentiation of bovine preadipocytes to adipocytes by antagonizing the adipogenic action of insulin.

In summary, the present study finds that GH inhibits the differentiation of primary bovine preadipocytes into adipocytes. This finding is consistent with those from studying primary rat preadipocytes (Wabitsch et al., 1996; Hansen et al., 1998), primary pig preadipocytes (Hausman and Martin, 1989; Gerfaull et al., 1999), and primary human preadipocytes (Wabitsch and Heinze, 1993) but is in stark contrast to the finding from using the 3T3-L1 and 3T3-F442A cell lines as preadipocyte models (Morikawa et al., 1982; Doglio et al., 1986; Corin et al., 1990; Clarkson et al., 1995). The reason for this discrepancy is not clear, but primary preadipocytes are conceivably more relevant than clonal preadipocyte cell lines to preadipocytes in vivo. More importantly, an inhibitory effect of GH on adipogenesis explains why GH deficiency leads to obesity or why GH administration inhibits adipose tissue growth.

LITERATURE CITED


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