Growth Hormone Can Induce Expression of Four Major Milk Protein Genes in Transfected MAC-T Cells

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ABSTRACT

Growth hormone (GH) can increase milk production in cattle, and this effect was thought to be mediated by an indirect mechanism because traditional ligand binding assays failed to detect GH binding sites in the mammary gland. However, recent findings that GH receptor (GHR) mRNA and protein are expressed in the epithelial cells of the bovine mammary gland suggest that GH may directly act on these cells to affect milk production. Therefore, the objective of this study was to determine whether GH could affect milk protein gene expression, nutrient uptake, and cell proliferation in bovine mammary epithelial cells using the bovine mammary epithelial cell-derived MAC-T cells as a model. Native MAC-T cells had low expression of GHR. Thus, we transfected them with expression plasmids for GHR and signal transducer and activator of transcription 5 (STAT5), 2 key components of GHR signaling, to maximize their GH response. Growth hormone increased the expression of αS1-casein, αS2-casein, β-casein, and α-lactalbumin mRNA 16- to 117-fold in the transfected MAC-T cells, whereas it had no effect on the expression of κ-casein, β-lactoglobulin, or insulin-like growth factor I mRNA. Cotransfection analyses showed that GH also strongly induced reporter gene expression from αS1-casein, αS2-casein, β-casein, and α-lactalbumin gene promoters. Growth hormone had no effect on the uptake of 2-deoxyglucose, an unmetabolizable glucose analog, amino acids, or oleic acid; neither did it affect cell proliferation or death. These observations together with the fact that GH receptor mRNA and protein are expressed in the epithelial cells of the bovine mammary gland raise the possibility that GH might act directly on the mammary epithelial cells in cows to stimulate transcription of major milk protein genes, as part of the mechanism by which GH stimulates milk production. Key words: growth hormone, receptor, cattle, milk production

INTRODUCTION

Growth hormone (GH), or somatotropin, is a major regulator of animal growth and metabolism (Etherton and Bauman, 1998). At the cellular level, GH exerts its action by binding to a cell membrane receptor, the GH receptor (GHR), and by subsequently activating the tyrosine kinase Janus kinase 2 (JAK2). Activated Jak2 phosphorylates signal transducer and activator of transcription 5 (STAT5) and several other intracellular substrates, leading to changes in gene expression and protein modification (Zhu et al., 2001). An example of GH-induced cellular changes is increased expression of IGF-I, which is believed to mediate many of the growth-stimulating and metabolic effects of GH (Etherton and Bauman, 1998). A widely-known action of GH in cattle is stimulation of milk production. Growth hormone increases milk yield without altering milk concentrations of fat, protein, and lactose (Bauman, 1999). This means that GH administration not only increases milk volume but also milk protein, fat and lactose syntheses in the mammary gland. The mechanism underlying these effects of GH is not clear. Earlier studies employing ligand binding assay failed to detect GH binding sites in the bovine mammary gland (Akers and Keys, 1984; Gertler et al., 1984; Keys and Djiane, 1988) and hence led many to conclude that the effects of GH administration on milk production were indirect (Bauman, 1999). It was further proposed that GH stimulate milk production by partitioning nutrients from adipose tissue and muscle, increasing blood flow to the mammary gland, increasing feed intake, and by reducing whole-body amino acid oxidation and urinary nitrogen loss (Bauman, 1999).

Recent studies using more sensitive assays clearly demonstrated that both GHR mRNA and protein are expressed in both stromal and epithelial tissue of the bovine mammary gland (Hauser et al., 1990; Jiang et al., 1999; Sinowatz et al., 2000; Plath-Gabler et al., 2001). Furthermore, GH protein expression was found to be lower in dry cows than in lactating cows (Sinowatz et al., 2000). These findings suggest that GH administration may also stimulate milk production through
direct action of GH on the mammary epithelial cells in cows. The objective of this study was to determine whether GH can directly affect milk protein gene expression, nutrient uptake, and proliferation in the mammary epithelial cells. An optimal approach to achieve this objective would be the use of mammary epithelial cells from lactating cattle. However, it is difficult to isolate and culture these cells and it is also difficult to prove the purity of them when isolated. Moreover, GH administration increases milk yield by only 10 to 15% in normal cattle (Dohoo et al., 2003). Thus, if GH changes milk protein gene expression, nutrient uptake, or cell proliferation, the changes would likely be within 10 to 15% and hence are difficult to be reliably detected by current techniques. To circumvent the first technical obstacle, we used MAC-T cells, which were originally derived from a bovine mammary epithelial cell and retain many characteristics of mammary epithelial cells (Huynh et al., 1991), as a model system for the bovine mammary epithelial cells in this study. To circumvent the second technical difficulty, we amplified GHR signaling and hence GH responses in MAC-T cells by forcing them to overexpress GHR and STAT5, 2 key components of the GHR signal transduction pathway (Zhu et al., 2001), and we measured GH responses in those cells in the absence of prior GH background. The results derived from using this model system may have implications for whether GH can directly affect milk protein gene expression, nutrient uptake, and cell proliferation in the mammary epithelia of cattle.

MATERIALS AND METHODS

Cell Culture

For routine culture, MAC-T cells were cultured in Dulbecco’s Modified Eagle’s medium supplemented with 4 mM of L-glutamine, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 10% fetal bovine serum (FBS) at 37°C under 5% CO2. All cell culture reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Cell Proliferation Assay

The MAC-T cells at ~60% confluency in 100-mm dishes were transfected with 10 μg of a bovine GHR expression plasmid (Wang and Jiang, 2005) and 10 μg of a STAT5 expression plasmid using transfection reagent FuGENE 6 (Roche Applied Science, Indianapolis, IN) as described previously (Zhou and Jiang, 2006). Twenty-four hours after the transfection, the cells (i.e., the MAC-T cells overexpressing GHR, or MAC-T-GHR cells) were split and seeded in 96-well plates at 1 × 10^4 cells/well. Four hours later, the FBS-containing medium was replaced with FBS-free medium, and the cells were cultured for another 8 h. The cells were subsequently treated with recombinant bovine GH (provided by A. F. Parlow, National Hormone and Peptide Program, Torrance, CA) at different concentrations, or PBS, or 10% FBS (positive control) for 16 h, followed by cell proliferation assay. The cell proliferation assay was performed using the nonradioactive CellTiter 96 assay kit (Promega, Madison, WI), essentially according to the manufacturer’s instructions. This cell proliferation experiment was repeated 4 times. Within each experiment, there were 4 replicates for each treatment.

2-Deoxyglucose, Amino Acids, and Oleic Acid Uptake Assays

The MAC-T cells transfected with GHR and STAT5 expression plasmids described above were seeded in 24-well plates at 3 × 10^4 cells/well. The cells were serum starved for 8 h and treated with bovine GH for 16 h before being assayed for uptake of 2-deoxyglucose, which is an unmetabolizable glucose analog; amino acids, and oleic acid, which is the most abundant long-chain fatty acid in milk (Bauman and Grinari, 2003).

For 2-deoxyglucose uptake assay, the cells were washed 3 times in Kreb-Ringer phosphate buffer (KRB) containing 128 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl2, 1.25 mM MgSO4, and 5.0 mM Na2HPO4, pH 7.4, and then incubated in KRB containing 1 μCi of 2-deoxy[3H]-glucose (PerkinElmer Life and Analytical Sciences, Waltham, MA) at 37°C for 20 min. The uptake was terminated by washing the cells 3 times with ice-cold KRB. The cells were lysed in 0.5 mL of 0.1 N NaOH for 30 min, and the lysates were neutralized by addition of 0.5 mL of 0.1 N HCl. The radioactivity and protein concentration of the cell lysates were determined by scintillation counting and using a protein assay kit (BioRad Laboratories, Hercules, CA), respectively. Noncarrier-mediated 2-deoxy[3H]glucose uptake was measured by adding excess (500 μM) glucose to the incubation. Carrier-mediated uptake of 2-deoxy[3H]glucose was obtained by subtracting the noncarrier-mediated radioactivity from the total radioactivity. The corrected 2-deoxy[3H]glucose count was divided by protein concentration of the same lysate to normalize possible difference in cell number.

For amino acids uptake assay, the cells were incubated in KRB containing 1.0 μCi of a mixture of 15[3H]-labeled amino acids, including Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Tyr, and Val (Amersham Biosciences, Piscataway, NJ) at 37°C for 20 min. The cells were then washed with cold KRB and the intracellular [3H]-amino acids were analyzed as described for 2-deoxy[3H]glucose. Noncarrier-mediated
uptake of [3H]-labeled amino acids was measured in the presence of excess (2.5 μM) unlabeled amino acids (Sigma-Aldrich).

Oleic acid uptake assay was performed as previously described (Gao and Serrero, 1999). Briefly, [3H]oleic acid (PerkinElmer) was dissolved in KRB to give a concentration of 3.2 μM (or 1 μCi/mL). Fatty acid-free BSA was added with gentle mixing to obtain a [3H]oleic acid-BSA molar ratio of 1.0. The transfected MAC-T cells were washed with room temperature KRB twice. The cells in each well were then incubated with KRB containing [3H]oleic acid-BSA mixture at room temperature for 20 min. The uptake was stopped by the addition of 1.0 mL of ice-cold KRB supplemented with 0.1% fatty acid-free BSA and 200 μM phloretin (Sigma), which inhibits transport of fatty acids across plasma membrane (Abumrad et al., 1981). The cells were then rapidly washed 3 times with ice-cold KRB and lysed as described above. Background radioactivity representing isotope trapped extracellularly and bound nonspecifically by the cells was measured by adding and immediately removing the [3H]oleic acid-BSA mixture. These uptake experiments were repeated 4 times, each time in triplicate.

**Real-time PCR and Ribonuclease Protection Assay**

Total RNA was extracted using TRI Reagent (Molecular Research Center Inc., Cincinnati, OH). The RNA integrity was confirmed by electrophoresis through formaldehyde-agarose gels. The relative abundance of αS1-casein, αS2-casein, β-casein, κ-casein, α-lactalbumin, β-LG, or GAPDH mRNA or 18S rRNA was measured by reverse transcription coupled with real-time PCR. Briefly, 2 μg of total RNA was reverse-transcribed in a 10-μL volume using TaqMan Reverse Transcribing Reagents (Applied Biosystems, Foster City, CA), for 10 min at 25°C, 30 min at 48°C, and 5 min at 95°C. Two microliters of the reverse transcription products were amplified in a total volume of 25 μL containing 12.5 μL of SyberGreen PCR Master Mix (Applied Biosystems) and 0.2 μM of gene-specific forward and reverse primers (Table 1) under 40 cycles of 95°C for 15 s and 60°C for 1 min. The real-time PCR data were analyzed using the 2−ΔΔCT method (Livak and Schmittgen, 2001), using GAPDH mRNA as internal control when the expression of GAPDH mRNA was not different across the treatments or 18S rRNA as internal control when GAPDH mRNA was different across the treatments.

Ribonuclease protection assay (RPA) was used to confirm the increased expression of GHR mRNA in the transfected MAC-T cells. This RPA was performed using the RPA II kit (Ambion, Austin, TX) essentially as previously described (Jiang et al., 2007).

**Promoter-Luciferase Reporter Gene Constructs**

A 2,013-bp bovine αS1-casein gene promoter was amplified from genomic DNA isolated from a Holstein bull by standard PCR using gene-specific primers (Table 1). The PCR product was digested with restriction enzymes Sma I and Xho I and cloned into pGL2-Basic vector (Promega) to generate plasmid bCSNAS1P/pGL2b. Similarly, a 2,195-bp bovine αS2-casein promoter and a 1,648-bp bovine β-casein promoter were cloned into pGL2-Basic vector at the Xba I and Xho I sites to generate plasmids bCSNAS2P/pGL2b and bCSNBP/pGL2b, respectively, and a 1,988-bp bovine α-LA promoter was cloned into pGL2-Basic between Kpn I and Xho I sites to generate bLALBAP/pGL2b. All new plasmids were verified by DNA sequencing, which was performed by the Virginia Bioinformatics Institute (Blacksburg, VA).

**Luciferase Reporter Assay**

The MAC-T cells were seeded in 24-well plates at a density of 3 × 104/well and cultured for 24 h. The cells in each well were then transfected with 0.5 μg of bovine milk gene promoter-luciferase plasmid, 0.5 μg of GHR expression plasmid, 0.5 μg of STAT5 expression plasmid, and 2 ng of transfection efficiency control plasmid pRL-CMV (Promega). Twenty-four hours after the transfection, the cells were serum-starved for 8 h. The cells were subsequently treated with 500 ng/mL of bovine GH or PBS for 16 h before being lysed for luciferase assay. The cell lysis and luciferase assay were done using the Dual-Luciferase Assay System (Promega), essentially following the manufacturer’s instructions. This transfection and reporter gene assay was repeated 4 times. The firefly luciferase activity expressed from the promoter construct was divided by the Renilla luciferase from pRL-CMV in the same well to normalize variation in transfection efficiency.

**Statistical Analysis**

The luciferase and mRNA expression data were analyzed using paired t-test. The cell proliferation and nutrient uptake data were analyzed using ANOVA followed by the Tukey test, which were performed using the GLM of SAS (SAS Inst. Inc., Cary, NC). All data are expressed as mean ± standard error of the mean. A difference was considered statistically significant when P < 0.05 and nonsignificant when P > 0.1.

**RESULTS**

**Establishment of GH-Responsive MAC-T Cells Through Transient Transfection**

Expression of GHR mRNA in native MAC-T cells was barely detectable by RPA compared with that in MAC-T
Table 1. Oligonucleotide primers used in this study

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1All sequences are written from 5′ to 3′. The top sequence of a pair of primers is the forward primer and the bottom sequence the reverse primer. Underlined are restriction enzyme recognition sites added for cloning.

cells transfected with GHR expression plasmid (Figure 1A). Using real-time PCR, which is more sensitive and more quantitative than RPA, GHR mRNA abundance in native MAT-C cells was estimated to be 15% of that in bovine mammary tissue, and GHR mRNA abundance in transfected MAC-T cells was approximately 600-fold of that in the mammary tissue (Figure 1B). Thus, transfection of MAC-T cells with GHR expression plasmid significantly increased the expression of GHR mRNA in these cells.

Native MAC-T cells also were not GH responsive, even when STAT5 was overexpressed in them (Zhou and Jiang, 2006). Growth hormone treatment of the MAC-T cells cotransfected with both GHR and STAT5 expression plasmids caused an 80-fold increase ($P < 0.001$) in luciferase expression from a STAT5 binding sites-containing promoter (Figure 1C). Thus, overexpression of both GHR and STAT5 significantly enhanced the GH responsiveness of MAC-T cells, and the transfected MAC-T cells provided a sensitive model for detection of direct effects of GH on the bovine mammary epithelial cells.

**GH Did Not Affect Proliferation or Death of the Transfected MAC-T Cells**

Treatment of the MAC-T cells that overexpressed GHR and STAT5 with a wide range of concentrations of bovine GH for 16 h had no effect ($P = 0.21$) on the number of viable cells (Figure 2). However, as expected, treatment of the cells with 10% FBS or 200 ng/mL of IGF-I for the same period of time increased ($P < 0.05$) the number of viable MAC-T cells (Figure 2). These results indicated that under the conditions used in this study GH has no direct effect on proliferation or death of MAC-T cells.

**GH Had No Effect on Uptake of 2-deoxyglucose, Amino Acids, or Oleic Acid in the Transfected MAC-T Cells**

Time-course experiments indicated that $^3$H-labeled 2-deoxyglucose, amino acids, or oleic acid accumulated linearly in MAC-T cells with time for at least 60 min (data not shown). Therefore, the remaining uptake assays were performed by incubating the cells with radiolabeled substrate for 20 min, a time point of the linear phase of the uptake. As shown in Figure 3A, 3B, and 3C, treatment of the transfected MAC-T cells with different concentrations of bovine GH for 16 h did not affect 2-deoxyglucose ($P = 0.56$), amino acids ($P = 0.65$), or oleic acid uptake in those cells ($P = 0.58$). These results suggest that GH probably has no direct effect on glucose, amino acid, and long-chain fatty acid uptake in MAC-T cells.

Figure 1. Establishment of growth hormone (GH)-responsive MAC-T cells by transient transfection with GHR and STAT5 expression plasmids. A) Ribonuclease protection assay (RPA) of GHR mRNA in 2 RNA samples from untransfected MAC-T cells and 6 RNA samples from transfected MAC-T cells, or MAC-T-GHR cells, each sample representing a separate transfection. GHR mRNA (indicated by an arrow) was barely detectable in untransfected MAC-T cells but became readily detectable in transfected MAC-T cells. Y RNA, yeast RNA, served as a negative control. GAPDH mRNA served as an internal control. B) Real-time PCR analysis of GHR mRNA in 2 untransfected MAC-T, 4 MAC-T-GHR, and 4 bovine mammary tissue RNA samples. In this analysis, 18S rRNA served as internal control. Note that relative GHR mRNA abundance on the y-axis is expressed on a logarithmic scale. Note that relative GHR mRNA abundance on the y-axis is expressed on a logarithmic scale. C) Demonstration of GH-responsiveness of MAC-T-GHR cells by reporter gene assays. The MAC-T-GHR cells were cotransfected with a GH-responsive promoter-luciferase plasmid and were treated with 500 ng/mL of bovine GH or phosphate buffered saline (PBS) for 16 h before luciferase assay. The GH induced an 80-fold increase in luciferase activity compared with PBS. *P < 0.001 (n = 4).

Figure 2. Effect of growth hormone (GH) on number of viable MAC-T-GHR cells. The MAC-T-GHR cells established by transient transfection were treated with bovine GH at indicated concentrations for 16 h prior to cell proliferation assay. The cell proliferation assay was done using the nonradioactive CellTiter 96 assay kit (Promega). The absorbance at 570 nm on the y-axis represents the number of viable cells. **Means (n = 4) labeled with the same letters are not different (P = 0.21). The 10% fetal bovine serum (FBS) and 200 ng/mL of IGF-I, serving as positive controls, each increased the number of viable MAC-T-GHR cells (P < 0.01).

GH Increased Expression of 4 Major Milk Protein mRNA in the Transfected MAC-T Cells

As measured by real-time PCR, GH treatment of 16 h caused a 90-fold increase in αS1-casein (P < 0.01), 117-fold increase in αS2-casein (P < 0.01), 18-fold increase in β-casein (P < 0.05), and 16-fold increase in α-lactalbumin (P < 0.05) mRNA abundance in the MAC-T cells overexpressing GHR and STAT5 (Figure 4A). The same treatment did not affect β-LG (P = 0.13) or κ-casein (P = 0.15) mRNA expression (Figure 4A). The gel images of the DNA products from 40 cycles of PCR revealed that without GH treatment, the mRNAs for the 6 milk proteins except for α-LA were barely detectable in the transfected MAC-T cells (Figure 4B) and that GH treatment clearly induced the expression of αS1-casein, αS2-casein, and β-casein mRNA and increased the expression of α-LA mRNA (Figure 4B). In the same transfected MAC-T cells, IGF-I mRNA was not detectable with or without GH treatment, suggesting that the effects of GH on milk protein gene expression in these cells were not mediated by autocrine secretion of IGF-I.

GH Stimulated Reporter Gene Expression from the Promoters of 4 Milk Protein Genes in the Transfected MAC-T Cells

A potential mechanism by which GH increased the expression of αS1-casein, αS2-casein, β-casein, and α-LA mRNA is increasing their gene transcription. To test this possibility, the effect of GH on luciferase reporter gene expression from the promoters of these milk protein genes was determined. As shown in Figure 5, GH
Figure 3. Effect of growth hormone (GH) on 2-deoxyglucose (A), amino acids (B), and oleic acid (C) uptakes in MAC-T-GHR cells. The MAC-T-GHR cells were treated with various concentrations of bovine GH for 16 h before the uptake assays. The 2-deoxyglucose, amino acids and oleic uptake assays were done by measuring the accumulation of 2-deoxy[3H]glucose, a mixture of 153H-labeled amino acids, and [3H]oleic acid, respectively, for 20 min. The accumulated radioactivity was normalized to cellular protein concentration to control for potential differences in cell number. The GH had no significant effect on 2-deoxyglucose uptake (P = 0.56; n = 4), amino acid uptake (P = 0.65; n = 4), or oleic acid uptake (P = 0.58; n = 4).

treatment of the transfected MAC-T cells caused 29-, 40-, 7-, and 3-fold increases (P < 0.001) in luciferase activity expressed from the αS1-casein, αS2-casein, β-casein, and α-LA gene promoters, respectively, further suggesting that GH can stimulate transcription of αS1-casein, αS2-casein, β-casein, and α-LA genes in MAC-T cells.

DISCUSSION

The expression of GHR mRNA and protein in bovine mammary epithelial cells suggests that GH might directly act on these cells to affect milk component biosynthesis and therefore production. There are many potential mechanisms by which GH directly acts on these cells to increase milk volume and milk protein, fat, and lactose syntheses. One is that GH increases the proliferation of these cells. Several in vivo studies have been conducted to determine whether GH administration affects mammary cell proliferation while it increases milk yield in cattle. Earlier studies indicated that GH administration did not affect mammary DNA content and hence mammary cell proliferation (Capuco et al., 1989; Binelli et al., 1995). However, a more recent
The results from these studies were conflicting. By comparing mammary arterial and venous concentrations of milk substrates, Davis et al. (1988) reported that GH injection was associated with a significant increase in mammary uptake of glucose, some increases in uptake of acetate and triglyceride, and no changes in uptake of propionate and free fatty acids in cows. However, using the same method, Miller et al. (1991) detected no significant effect of GH administration on mammary uptake of any of these nutrients in cows. One study investigated the effect of GH administration on mammary uptake of amino acids, finding that GH administration might increase mammary uptake of some amino acids, including Arg, cystathionine, Leu, and Lys (Hangan et al., 1992). In our study we found that GH had no significant effect on MAC T cell uptake of glucose; amino acids including Arg, Leu, and Lys; and oleic acid, the most abundant long-chain fatty acid in the milk (Bauman and Griinari, 2003). This result suggests that the effects of GH administration on mammary uptake of glucose, amino acids, and long chain fatty acids, if they are true, are unlikely mediated by direct action of GH on the mammary epithelial cells. A direct action of GH on mammary epithelial cell uptake of glucose is also refuted by the observation that GH had no effect on glucose uptake of the bovine mammary explants in culture (Gertler et al., 1983).

Stimulating the expression of milk protein genes in the mammary epithelial cells is obviously another potential mechanism of direct GH action that increases milk production, in particular, milk protein production. Surprisingly, the first report investigating this potential mechanism did not emerge until recently (Yang et al., 2005), where the mammary gland of GH-treated cows was shown to express 35% more β-casein mRNA than that of untreated cows. Yang et al. (2005) further concluded that this effect of GH administration resulted from a direct action of GH on the mammary gland because GH also stimulated β-casein mRNA expression in mammary explants in culture. At about the same time, Sakamoto et al. (2005) reported that GH was able to stimulate αs1-casein mRNA expression in a bovine mammary epithelial cell line named BMEC. Interestingly, neither Yang et al. (2005) nor Sakamoto et al. (2005) examined other milk protein mRNAs in their studies. In our study, we observed that GH significantly increased mRNA expression of 4 major milk protein genes, including β-casein and αs1-casein, and also markedly increased reporter gene expression from the promoters of these 4 milk protein genes, in MAC-T cells. Our observations and those of Yang et al. (2005) and Sakamoto et al. (2005) collectively suggest that GH may stimulate the expression of multiple major milk protein genes in the mammary gland by directly acting on the...
epithelial cells. That GH stimulates the expression of multiple milk protein genes seems to be a straightforward answer to why GH administration does not reduce milk protein concentrations while it increases milk volume.

Our observation that GH stimulated α-LA mRNA expression in the transfected MAC-T cells has particular implication for how GH might increase milk volume. The α-LA is not only a major protein component of milk but also a key component of lactose synthase, the enzyme that synthesizes lactose from glucose and UDP-galactose in the mammary gland (Kuhn et al., 1980). Because lactose is the major osmotic factor of milk, α-LA is believed to play a critical role in determining milk volume. This notion has indeed been supported by many studies. For example, milk from α-LA gene deletion mice was more concentrated than from wild-type mice (Stinnakre et al., 1994; Stacey et al., 1995); mammary overexpression of (Stinnakre et al., 1994; Stacey et al., 1995); mammary epithelial cells in lactating cows, thereby increasing milk yield without lowering milk protein concentrations. We have meanwhile observed that GH does not affect proliferation or death or nutrient uptake of MAC-T cells, and these observations argue against such direct actions of GH on mammary epithelial cells in cows.

REFERENCES


