Comp. Biochem. Physiol. Vol. 115B, No. 1, pp. 127–134, 1996

ISSN 0305-0491/96/$15.00
PII S0305-0491(96)00043-0

Localization and Gene Expression of Glucose Transporters in Bovine Mammary Gland

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ABSTRACT. Glucose uptake in the mammary gland is a rate-limiting step in milk synthesis. To study glucose transporters in the bovine mammary gland, the erythrocyte-type glucose transporter (GLUT1) and the insulin-responsive glucose transporter (GLUT4) proteins were assessed by Western blotting and immunohistochemical staining, using polyclonal antibodies against the C-terminal peptide of GLUT1 and GLUT4. Our results demonstrated that the bovine mammary gland expressed a relatively high level of GLUT1 protein, whereas GLUT4 protein was not detected in the mammary gland of either lactating or dry cows. The absence of GLUT4 may indicate that glucose transport is not regulated by insulin in the lactating and dry bovine mammary gland. The anti-GLUT1 antibody strongly stained the single layer of epithelial cells of mammary alveoli. The expression of GLUT1 mRNA was similar in the mammary gland of late lactation and non-lactating cows. However, a smaller molecular weight species (38 kDa) of GLUT1 protein was detected in the mammary gland of non-lactating cows where its abundance in crude membrane preparation was 80% higher than in lactating animals. There were no significant differences in GLUT1 mRNA in bovine mammary gland at 118 d and 181 d postpartum, however, GLUT1 protein expression tended to be greater at 118 d postpartum. COMP BIOCHEM PHYSIOL 115B;1:127–134, 1996.

KEY WORDS. Bovine, gene expression, lactation, localization, mammary gland, physiological states, erythrocyte-type glucose transporter, insulin-responsive glucose transporter

INTRODUCTION

Lactose constitutes about 40% of total solids in bovine milk. As lactose maintains the osmolarity of milk, the rate of lactose synthesis serves as a major control of the volume of milk produced (32). Glucose is the main precursor of lactose synthesis in the epithelial cell of the mammary gland, however, the mammary gland cannot synthesize glucose from other precursors due to the lack of glucose 6-phosphatase (37). Therefore, the mammary gland is dependent on the blood supply for its glucose needs and as a consequence, mammary glucose uptake is a rate-limiting factor for milk production (23). It has been estimated that in a lactating cow, 72 g of glucose is required to produce 1 kg of milk (23) and mammary uptake can account for as much as 60 to 85% of the total glucose entering the blood (2,11).

The epithelial cells of the mammary gland take up glucose by a passive process of facilitated diffusion (14), which is mediated by a family of structurally related glucose transporter proteins (17). To identify the relevant glucose transporter(s) in bovine mammary gland, Zhao *et al.* used five human glucose transporter cDNAs in Northern blotting analysis of bovine tissues and found that only GLUT1 mRNA was present at high levels in the mammary gland of lactating cows (39). This finding is consistent with previous studies on lactating rat mammary gland which used quantitative Western blotting and cytochalasin B-binding and revealed that GLUT1 constitutes the major glucose transporter species in the plasma membranes of rat mammary gland epithelial cells (29).

In this study we identified and localized GLUT1 isoform present in the bovine mammary gland and investigated its expression during different physiological states at the protein and mRNA levels. We also measured the GLUT4 isoform in order to investigate the possible role of insulin regulation on glucose transport in the mammary gland of the lactating cow.

MATERIALS AND METHODS

Animals and Tissues

Experiment I: Two late lactation and two nonlactating (dried-off for 3 and 10 weeks) Holstein cows, *Bos taurus*, were fed a late-lactation total mixed ration *ad libitum* for a minimum of 14 days prior to slaughter. Animals were non-

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Abbreviations–GLUT1, the erythrocyte-type glucose transporter; GLUT4, the insulin-responsive glucose transporter.

Received 7 September 1995; revised 26 January 1996; accepted 30 January 1996.

pregnant and were shipped to a slaughter house. Tissues of mammary gland, skeletal muscle (*Masseter*) and liver were collected immediately post-slaughter, frozen in liquid nitrogen, and stored at −75°C for future analysis.

Experiment II: Six nonpregnant primiparous Holstein cows were used in a randomized block design with cows allocated by date of parturition into three blocks. Within each block, two cows were started on the experiment at 55 days and 118 days after parturition, respectively. These cows were a subset of animals from a larger study (5). Cows selected for this study were the control animals that were not subjected to growth hormone or growth hormone-releasing factor treatment. Cows were fed a total mixed ration *ad libitum*. After 9 weeks, two cows within a block were slaughtered. Mammary gland tissues were obtained immediately after slaughter, frozen in liquid nitrogen, and stored at −75°C. Rat brain and leg muscle (*gastrocnemius*) were taken from three adult Sprague-Dawley rats and used as controls.

Preparation of Crude Membrane Fractions

Membrane fractions were prepared by homogenization of the tissue in 10 volumes of ice-cold 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl (pH 7.4), 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride, using a Brinkman Polytron homogenizer. Homogenates were centrifuged at 1000 **g** (3000 rpm in a Beckman J2-21 centrifuge using a JA-14 rotor) for 10 min at 4°C, and the supernatants were centrifuged at 13,000 **g** (9,500 rpm with the same rotor) for 45 min at 4°C. The cytosols were then centrifuged at 100,000 **g** (37,000 rpm with a Sorvall T647.5 rotor) for 90 min at 4°C, and the membrane pellets were resuspended in the same buffer as above. Protein concentrations were measured by the Bradford dye-binding assay (7) using the Bio-Rad Protein Assay Kit (Bio-Rad, Richmond, CA, U.S.A.) and bovine serum albumin (BSA) as a standard.

Primary Antibodies

The primary antibodies were purchased from East Acres Biologicals (Southbridge, MA, U.S.A.). The anti-GLUT1 was raised in rabbit against a synthetic peptide corresponding to the 11 amino acids in the carboxyl terminus of the rat brain glucose transporter [the same 11 amino acid peptide sequence is also present in bovine GLUT1 (6)]. The anti-GLUT4 was also raised in rabbit against a synthetic peptide specific to an 11 amino acid sequence of the carboxyl terminus of rat GLUT4. Although GLUT4 has not been cloned from bovine tissues, the same peptide sequence is conserved in the GLUT4 proteins reported for several different species (8).

Western Blotting

Membranes were resuspended in Laemmli sample buffer (26), and resolved on 10% or 12% (w/v) SDS-polyacrylamide gel using a Bio-Rad Mini-protein II Electrophoresis Cell. The proteins were electrophoretically transferred to nitrocellulose filters (BA 85, Schleicher and Schuell, Dassel, Germany). Protein markers (Rainbow markers, Amersham, Arlington Heights, IL, U.S.A.) were used as molecular mass standards and also to assess the efficiency of the transfer. The blots were blocked overnight at 4°C in TBS (20 mM Tris [pH 7.4], 137 mM NaCl) containing 5% (w/v) nonfat dried milk (Carnation Ltd., ON, Canada), and incubated for 1.5 hr at room temperature in TBS, 0.5% nonfat dry milk, containing a 1:200 dilution of either GLUT1 antiserum or GLUT4 antiserum. The filters were then washed twice at room temperature for 15 min in TBS and incubated for 1 hr at room temperature in TBS, 0.5% nonfat dried milk, with a 1:2000 dilution of goat anti-rabbit IgG conjugated with horseradish peroxidase (Calbiochem, La Jolla, CA, U.S.A.). The immune complex was detected using the Amersham ECL Western blotting system (Amersham, Arlington Heights, IL, U.S.A.) following the manufacturer's instructions. The resulting images were quantified by scanning densitometry (Imaging Densitometer GS-670, Bio-Rad).

Light Microscopy Immunolabelling

Tissues were removed from a lactating cow immediately after slaughter. Tissue samples were fixed in 4% (w/v) paraformaldehyde in PBS for 4 hr at 4°C, washed in PBS, and immersed in 0.5 M sucrose in PBS overnight. Tissue blocks were mounted on specimen holders and frozen in liquid nitrogen. Sections (10 μm) were cut and thaw-mounted on the surface of gelatin-coated slides. Immunocytochemical staining was performed according to the peroxidase-antiperoxidase procedure of Sternberger (35), with 3',3-diaminobenzidine (DAB) as cosubstrate. In brief, the endogenous peroxidase activity of samples was blocked at 22°C for 30 min in 0.25% hydrogen peroxidase in PBS. Tissue sections were then washed and incubated at 22°C for 1 hr with a 1:500 dilution (in 1% BSA in PBS) either with the anti-GLUT1 antibody or with the anti-GLUT4 antibody. After three washes with PBS, samples were incubated for 1 hr at 22°C with a 1:50 dilution (in 1% BSA in PBS) of goat anti-rabbit IgG secondary bridging antibody (Calbiochem). After several washes with PBS, samples were incubated at 22°C for 1 h with 1:200 dilution (in 1% BSA in PBS) of horseradish peroxidase-anti-peroxidase complex (Calbiochem). The sections were finally washed three times for 5 min in PBS, developed 2 to 5 min in DAB (Sigma, St Louis, MO, U.S.A.) substrate solution and rinsed in water. Some of the sections were counterstained with hematoxylin for 30 sec and all sections were mounted in Crystal/Mount (Fisher, Pittsburgh, PA, U.S.A.). Sections were visualized using a Leitz Dialux 20 (Midland, ON, Canada) and photographed with Kodak Ektachrome 400 film.

Northern Blotting

Total RNA was isolated from different tissues by a guanidinium thiocyanate-CsCl procedure (12). 30 μg of total RNA

were electrophoresed on denaturing 1% agarose/0.66 M formaldehyde gels and transferred to nylon membranes (Bio-Rad) by capillary diffusion. The integrity and relative amounts of RNA were assessed by UV-light visualization of ethidium bromide-stained RNA. Membranes were prehybridized for 2 hr at 50°C in a medium containing 60% (v/v) formamide, 1 × SSPE (0.18 M NaCl, 0.01 M sodium phosphate at pH 7.4, 1 mM EDTA), 0.5% (w/v) nonfat dried milk, 10% (w/v) dextran sulfate, 1% (w/v) sodium dodecyl sulfate, 500 μg/ml salmon testes DNA and 200 μg/ml yeast tRNA. Following prehybridization, hybridization was carried out for 16 to 18 hr at 50°C in fresh buffer containing ^{32}P-labelled antisense RNA probes (10^6 cpm/ml) generated as previously described (39). Membranes were then rinsed briefly in 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate at pH 7.0), washed for 15 min at 22°C in 2 × SSC containing 0.1% sodium dodecyl sulfate, washed at 70°C in 0.2 × SSC containing 1% sodium dodecyl sulfate, and rinsed briefly in 0.2 × SSC. Autoradiography was performed at −70°C with an intensifying screen and quantified by scanning densitometry (Imaging Densitometer GS-670, Bio-Rad).

Statistical Analysis

The significance of differences between mean values for parameters measured in Experiment II were assessed using ANOVA.

RESULTS

Characterization of the Antibodies and Distribution of GLUT1 and GLUT4

Immunoblotting analysis using 10% polyacrylamide gels showed that the anti-GLUT1 antibody and the anti-GLUT4 antibody detected a 42 kDa protein and a 43 kDa protein, respectively, in both bovine and rat tissues (Fig. 1A and Fig. 2A). These observations are in good agreement with the observed M_r of GLUT1 (9) and GLUT4 (2) and indicate that both primary antibodies used can specifically recognize GLUT1 or GLUT4 in bovine tissues. However, when 12% polyacrylamide gels were used and the gels were run for longer times, we could resolve both the 42 kDa band of GLUT1 and the 43 kDa band of GLUT4 as two separate bands: 45 kDa and 42 kDa for GLUT1, 43 kDa and 41 kDa for GLUT 4, respectively. The 42 kDa band of GLUT1 is 2.6-fold stronger than the 45 kDa band in the bovine mammary gland while both bands of GLUT4 have almost the same intensity in the bovine muscle (Fig. 1B and Fig. 2B). To date, it is not clear whether these different bands result from differential glycosylation of the proteins or other post-translational protein processing.

The anti-GLUT1 antiserum detected strong bands in rat brain and bovine mammary gland and weak bands in rat skeletal muscle with slightly different sizes (Fig. 1). The

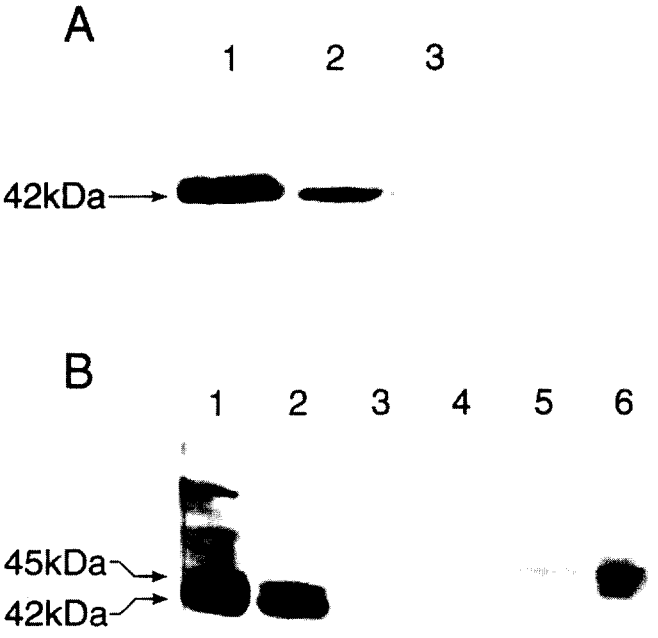

FIG. 1. Immunoblotting of rat and bovine tissues with an antibody to GLUT1. A: Membrane proteins (50 μg) of rat brain (1), 118 day lactating bovine mammary gland (2) and bovine liver (3) were resolved in 10% (w/v) SDS-polyacrylamide gel at 150 V for 1 hr. B: Membrane proteins (50 μg for bovine tissues and rat skeletal muscle and 25 μg for rat brain) of the mammary gland (2), liver (3), skeletal muscle (*Masseter*, 4), and omental fat (5) from a late lactating bovine, rat brain (1) and rat skeletal muscle (6) were resolved on 12% (w/v) SDS-polyacrylamide gel at 100 V for 2 hr.

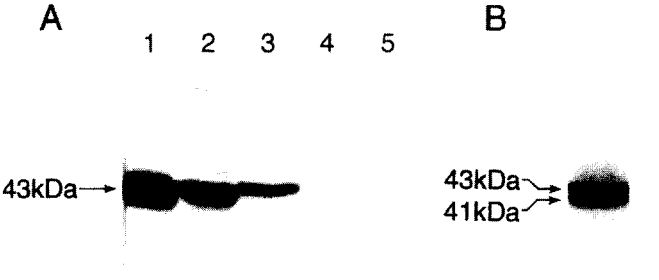

FIG. 2. Immunoblotting of rat and bovine tissues with an antibody to GLUT4. A: Membrane proteins (50 μg for bovine tissues and 25 μg for rat skeletal muscle) of the skeletal muscle (*Masseter*, 2), omental fat (3), mammary gland (4), and liver (5) from a nonlactating bovine and rat skeletal muscle (1), were resolved on 10% (w/v) SDS-polyacrylamide gel at 100 V for 2 hr. B: Membrane proteins (50 μg) of the bovine skeletal muscle (*Masseter*) were resolved on 12% (w/v) SDS-polyacrylamide gel at 150 V for 1.5 hr.

GLUT1 protein level detectable in the crude membrane of lactating bovine mammary gland was 1.5-fold less than in rat brain, unlike the situation in lactating rat mammary gland that expresses more GLUT1 than rat brain (9,10). GLUT1 protein was essentially undetectable in the liver, skeletal muscle and omental fat of lactating cow (Fig. 1). The anti-GLUT4 antiserum detected strong bands in both rat and bovine skeletal muscle and a weak band with a slightly different size in bovine omental fat (Fig. 2). The GLUT4 level in the crude membrane of bovine skeletal muscle was 1.7-fold less than in rat skeletal muscle and 1.2-fold higher than in bovine omental fat. GLUT4 protein was undetectable in the mammary gland and liver in dry animals (Fig. 2A) and lactating animals (data not shown). Although the different signal intensity could reflect different affinities of the antisera for these tissues, it is more likely to indicate the relative abundance of glucose transporters in these tissues.

Light Microscopy Immunohistochemical Localization of GLUT1 and GLUT4

Based on the high expression of GLUT1 substantiated in bovine mammary gland during lactation, the histochemical localization of GLUT1 in bovine mammary gland was studied in tissue sections using an antibody directed against the COOH-terminus of this protein. A strong anti-GLUT1 staining, and no anti-GLUT4 staining, were observed in the lactating bovine mammary gland (Fig. 3). The strong anti-GLUT1 staining was found mainly in the single layer of epithelial cells of alveoli, the functional unit of the mammary gland. Staining of GLUT1 was hardly seen in connective tissue. In fact, epithelial cells accounted for the majority of cells visualized in the mammary gland sections from the lactating bovine (Fig. 3). In histochemical controls, when the primary antibodies were omitted, there was no staining in the mammary gland. The anti-GLUT1 staining in bovine liver was only found on the cells surrounding the hepatic vein as reported previously (36) and the sinusoidal membrane of hepatocytes.

Expression of GLUT1 mRNA and Protein during Different Lactation Stages

In Experiment I, the expression of GLUT1 mRNA and protein was assessed in bovine mammary gland at different stages of lactation. To this end, total RNA and membrane fractions were isolated from lactating and nonlactating bovine mammary gland. Northern blotting analysis showed that the expression of GLUT1 mRNA was similar in the mammary gland of late lactation and nonlactating bovine (Fig. 4A). However, Western blotting analysis demonstrated that anti-GLUT1 detected a smaller molecular weight band (38 kDa) in the mammary gland of dry cows and the protein level was 80% higher than that in the lactating cow (Fig. 4B). Although differences were detected in the yield of membrane proteins per g tissue in the dry (2.6 $\pm$ 0.2 mg protein/g tissue) and lactating (3.8 $\pm$ 0.8 mg protein/g tissue) animals, the content of GLUT1 protein in the mammary gland of the dry cow, expressed as arbitrary densitometric units per g tissue, was still higher (385) than that in the lactating cow (312).

In Experiment II, the expression of GLUT1 mRNA and protein in bovine mammary gland was examined during different lactation periods. Total RNA and membrane fractions were isolated from the bovine mammary gland at 118 days and 181 days postpartum and mRNA and protein levels measured. No difference in the tissue content of GLUT1 mRNA was detected between the two groups (Fig. 5A). Although the average value of GLUT1 protein level in the 118 day postpartum mammary gland was 73% higher than that in the 181 day postpartum mammary gland, there was no statistically significant differences ($P = 0.17$) between the two groups due to the large individual difference observed within the 118 day group (Fig. 5B).

DISCUSSION

Intracellular glucose concentration may be a critical factor determining the rate of lactose synthesis and milk secretion in the mammary gland. The exposure of Golgi membrane vesicles to varying concentrations of glucose showed that apart from inhibition at high concentration, the rates of lactose synthesis follow classical Michaelis-Menten kinetics with a K_m of 1.5 mM (24), which exceeds the glucose concentration within the cell. Since a steep concentration gradient of glucose occurs across the plasma membrane, from 3.0 to 3.5 mM in plasma to 0.1 to 0.3 mM in the cell (15), the transport of glucose across the plasma membrane may be the rate-limiting step. Tracer studies have shown a linear relationship between the rate of glucose transport and milk yield in cows (23). The epithelial cells of the mammary gland take up glucose by a passive process of facilitated diffusion (14). In the present study, our Western blotting and immunostaining data showed that bovine mammary gland expresses a relatively high level of GLUT1. As only GLUT1 mRNA was detected with a high abundance in the mammary gland of lactating cows (39) and GLUT1 constitutes the major glucose transporter species in the plasma membranes of mammary gland epithelial cells of lactating rats (29), GLUT1 may also represent the major glucose transporter species in the lactating bovine mammary gland. However, considering the substantial requirement for glucose transport in the bovine mammary gland, the presence of other glucose transporters cannot be ruled out.

The expression of GLUT1 in the epithelial cells of mammary gland is consistent with the physiological behaviour of glucose metabolism in the bovine mammary gland. GLUT1 has a high affinity for glucose and its K_m has been reported to be from 2 to 20 mM (22). Bovine GLUT1 may

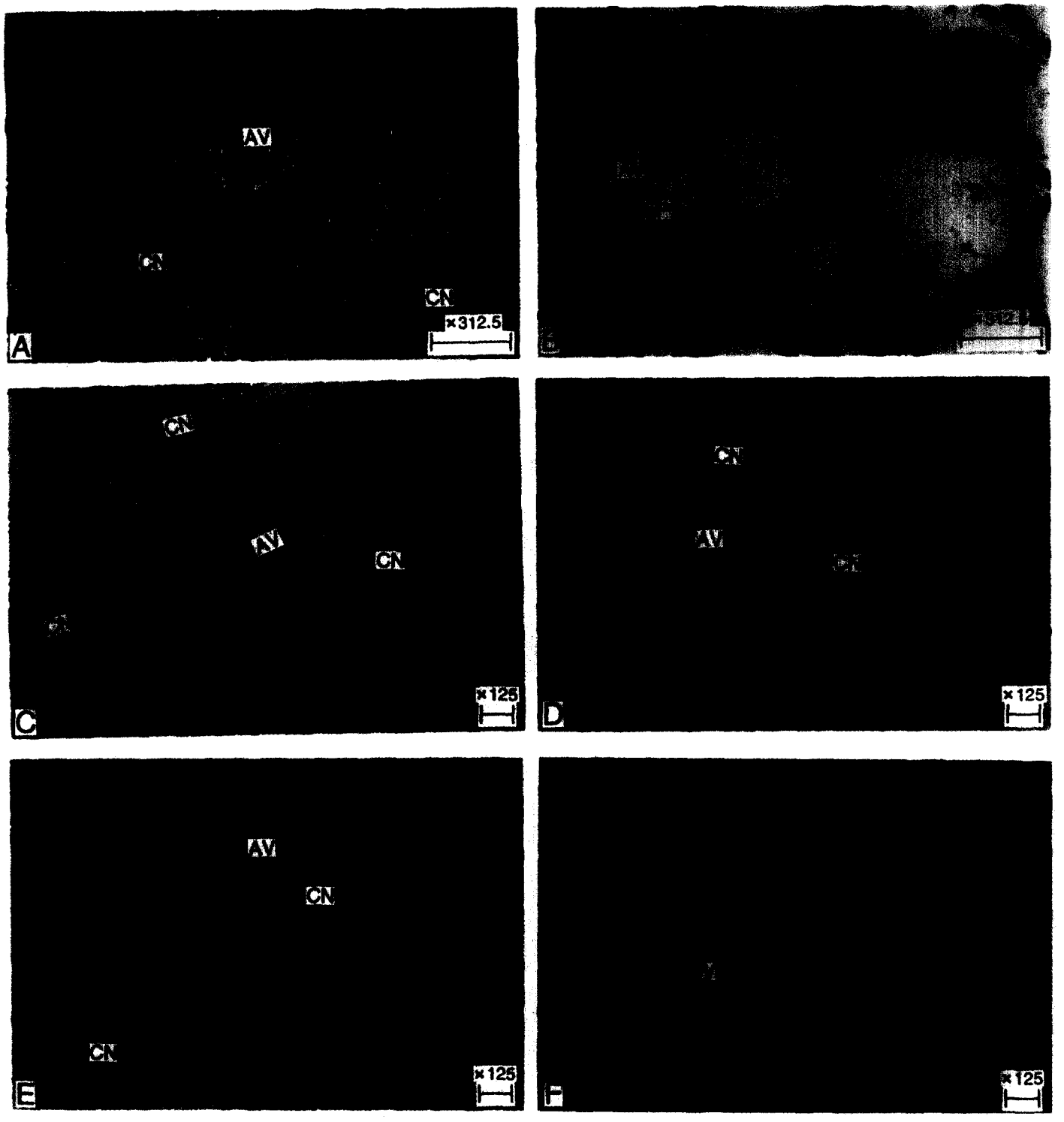

FIG. 3. Light microscopy immunohistochemical localization of GLUT1 and GLUT4 in bovine mammary gland and liver sections. Immunolocalization on cryosections from fixed bovine tissues was performed as described in the Experimental section. A–C: Photomicrographs of GLUT1-positive staining of the mammary sections from a lactating bovine. The strong anti-GLUT1 staining was found mainly in the single layer of epithelial cells (EP) of alveoli (AV). Positive staining for GLUT1 was hardly seen in connective tissue (CN). B: Section counterstained with hematoxylin. D: Photomicrograph of GLUT4-negative staining of the mammary section from the lactating bovine. E: A control section from the bovine mammary gland incubated without the first antibodies. F: A control section from the bovine liver incubated with anti-GLUT1. The anti-GLUT1 staining was only found on the cells surrounding the hepatic vein (V) and the sinusoidal membrane of hepatocytes (S: sinusoid). *Scale bars*, 48 μm.

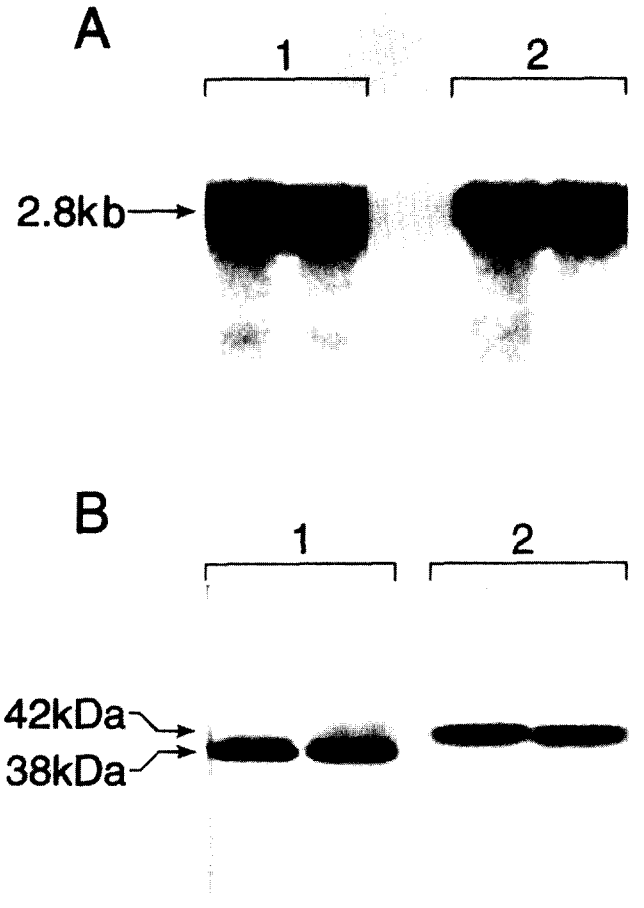

FIG. 4. Northern blotting analysis of GLUT1 mRNA (A) and Western blotting analysis of GLUT1 protein (B) in the mammary gland from two dry (1) and two late lactating (2) bovines. 50 μg crude membrane fractions were used in each lane in Western blotting analysis.

have a low K_m value for glucose and is probably nearly saturated by physiological plasma concentrations of glucose, since the physiological plasma glucose concentrations seem not to be limiting for glucose utilization in the mammary gland of the lactating cow (16). Uptake of glucose is not influenced by its arterial concentration of 2.2 to 4.7 mM (31). In accordance with this observation, Rook and Hopwood showed a direct relation between plasma glucose concentration and lactose synthesis at concentrations up to 2.2 mM (34). However, there is little further increase in lactose synthesis at higher concentrations. A value of 2.2 mM is at the lower limit of the normal range for lactating cows. Kronfeld *et al.* found that the relationship between milk production and plasma glucose concentration was linear below a plasma glucose concentration of about 3.0 mM. Raising the plasma glucose concentration above this did not increase milk production (23).

Interestingly, bovine mammary gland expresses two different species of GLUT1 protein during dry and lactation periods. This result differs from previous studies in the weaned nonlactating rat mammary gland, which failed to show smaller molecular weight species of GLUT1 (10). As our Northern blotting analysis of GLUT1 mRNA in the mammary gland of lactating cow and dry cow showed that a similar size mRNA is expressed in both mammary tissues, the difference in the apparent molecular masses of the transporter protein present in both mammary tissues probably results from posttranslational modifications. We do not know whether differences in the single *N*-linked oligosaccharide side chain could account for this observation or whether proteolysis is involved. Proteolysis occurring during the homogenization of the tissues was unlikely in the presence of the cocktail of proteinase inhibitors used. However, as we detected the transporters with an antisera specific for the COOH-terminus, any proteolytic cleavage must be restricted to the NH_2-terminus. Further studies should be carried out using the antibody specific for the NH_2-terminus and assessing the molecular mass of the transporter polypeptide itself by removing quantitatively the single *N*-linked oligosaccharide from the transporter.

The abundance of GLUT1 protein in the crude membrane preparation from the mammary gland of dry cows was surprisingly higher than in the lactating mammary gland. This result is also not consistent with the previous studies on the rat mammary gland in which there is a large reduction in the content of GLUT1 glucose transporter found after weaning (10). Explanations for our result are un-

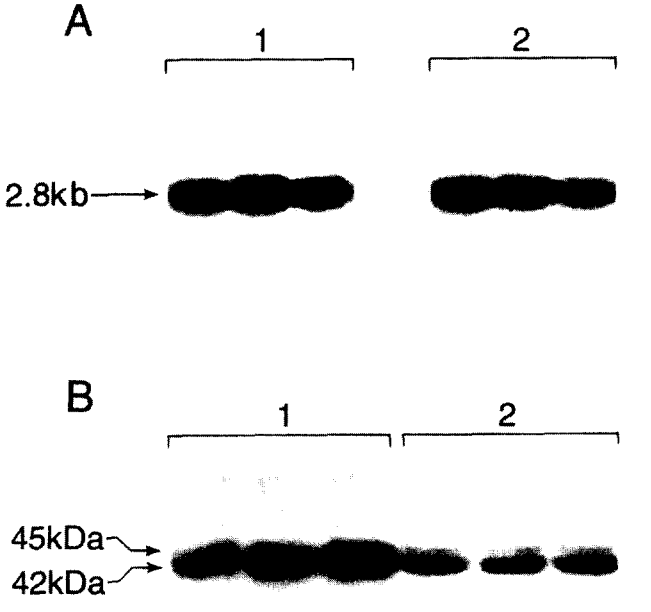

FIG. 5. Northern blotting analysis of GLUT1 mRNA (A) and Western blotting analysis of GLUT1 protein (B) in the mammary gland from three cows at 118 days of lactation (1) and three cows at 181 days of lactation (2). 50 μg crude membrane fractions were used in each lane in Western blotting analysis.

known. Linked with the observation is the question of the physiological significance of the high expression of GLUT1 protein in the dry bovine mammary gland. We speculate that our result may be due to different enrichment of plasma membrane fraction resulted from our crude membrane preparation procedures applied to lactating and nonlactating cows. Alternatively, the smaller molecular weight species of GLUT1 expressed during this period may have different kinetic properties. Since the expression of GLUT1 mRNA was similar in the mammary gland of late lactation and dry cows, any enhanced expression of GLUT1 protein in the bovine mammary gland during the dry period is due to either translational or posttranslational steps.

Generally, milk production declines from 118 days to 181 days of lactation. GLUT1 protein levels detected in the mammary gland of 118 day lactating cows was not statistically different from that observed for cows at 181 days of lactation. This lack of significance is mainly due to the large individual variation in GLUT1 protein within the 118 d group. Even so, our data show a trend of higher GLUT1 protein in earlier lactation cows. Higher glucose transporter level in the mammary gland is not necessarily a prerequisite for higher milk production. Administration of bovine growth hormone (bGH) or bGH-releasing factor (bGHRF) to lactating cows for 63 days increases milk yield, but does not change GLUT1 protein levels in the mammary gland (40). The primary action of bGH and bGHRF in increasing mammary glucose availability may result from increased blood flow rate to the mammary gland (13,28) and decreased glucose utilization in providing NADPH or α-glycerol-phosphate for triacylglycerol esterification (4).

In the present study we were unable to detect any GLUT4 in the lactating bovine mammary gland. GLUT4 is known to be the transporter isoform primarily responsible for insulin-stimulated glucose transport (21). The result indicates that glucose transport is not regulated by insulin in the mammary gland of the lactating cow. As in nonruminants, insulin is the major hormone controlling glucose utilization by extrahepatic tissues, such as skeletal muscle and adipose tissue in ruminants (38). However, in the lactating bovine mammary gland there is little evidence to suggest that insulin controls the entry of glucose into the mammary epithelial cells (19,20,25). The non-insulin-sensitive character of mammary glucose uptake may play an important role in lactation. During fasting, lactation or pregnancy, when plasma insulin concentrations are low (3,27) and less glucose is taken up by adipose tissue and muscle (30,33), a greater proportion of glucose is available for the non-insulin-responsive mammary tissues.

Surprisingly, we also did not detect any GLUT4 signals in the mammary gland of dry cows. During the dry period, involution of mammary secretory cells accelerates when milk removal is stopped, the space previously occupied by the degenerating alveoli is replaced with adipose cells. GLUT4 is a major glucose transporter isoform in adipose tissues. A possible explanation for our results is that GLUT4 content in ruminant adipose tissues is much lower than observed in nonruminant animals. It has been reported that GLUT4 protein content in cattle adipose tissue is only 14 to 22% of rat (1,18). In our Northern blotting (39) and Western blotting analysis, GLUT4 mRNA and protein are only barely detectable in omental fat using 30 μg total RNA and 50 μg crude membrane protein, respectively.

In conclusion, GLUT1 appears to be the major glucose transporter isoform in bovine mammary gland. We could not detect any GLUT4 in the mammary gland of both lactating and dry cows, which would suggest that glucose uptake is not regulated by insulin in the bovine mammary gland during dry and lactation periods.

The authors thank Dr. G. I. Bell (Howard Hughes Medical Institute, University of Chicago) for kindly providing us with the human glucose transporter cDNA clones. We also thank Dr. H. A. Tucker (Michigan State University) and the Upjohn Company for allowing us to obtain tissue samples from Holstein cattle at the Michigan State University. Thanks to the members of the Animal Reproduction Lab, Michigan State University for assistance in sample collection. The financial support of the Natural Science and Engineering Research Council of Canada and Alberta Agriculture Research Institute is gratefully acknowledged.

References

1. Abe, H.; Morimatsu, M.; Aso, H.; Shimizu, Y.; Nikami, H.; Kosaka, K.; Syuto, B.; Saito, M. Tissue distribution of major insulin-responsible glucose transporter (GLUT4) protein in cattle. Proc. Soc. Nutr. Physiol. 3:214;1994.
2. Annison, E.F.; Linzell, J.L. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their overall metabolism and to milk formation. J. Physiol. 175:372–385;1964.
3. Athanasiou, V.N.; Phillips, R.W. Effect of fasting on plasma metabolites and hormones in lactating dairy cows. Am. J. Vet. Res. 39:957–960;1968.
4. Bauman, D.E.; Peel, C.J.; Steinhour, W.D.; Reynolds, P.J.; Tyrrell, H.F.; Brown, A.C.G.; Haaland, G.L. Effect of bovine somatotropin on metabolism of lactating dairy cows: Influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J. Nutr. 118:1031–1040;1988.
5. Binelli, M.; Vanderkooi, W.K.; Chapin, L.T.; Vandehaar, M.J.; Turner, J.D.; Moseley, W.M.; Tucker, H.A. Comparison of somatotropin releasing factor and somatotropin: body growth and lactation in primiparous cows. J. Dairy Sci. 78: 2129–2139;1995.
6. Boado, R.J.; Pardridge, W.M. Molecular cloning of the bovine blood-brain barrier glucose transporter cDNA and demonstration of phylogenetic conservation of the 5′-untranslated regions. Mol. Cell. Neurosci. 1:224–232;1990.
7. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254;1976.
8. Burant, C.F.; Sivitz, W.I.; Fukumoto, H.; Kayano, T.; Nagamatsu, S.; Seino, S.; Pessin, J.E.; Bell, G.I. Mammalian glucose transporters: structure and molecular regulation. Recent Prog. Hormone Res. 47:349–388;1991.
9. Burnol, A.-F.; Leturque, A.; Loizeau, M.; Postic, C.; Girard, J. Glucose transporter expression in rat mammary gland. Biochem. J. 270:277–279;1990.

10. Camps, M.; Vilaro, S.; Testar, X.; Palacin, M.; Zorzano, A. High and polarized expression of GLUT1 glucose transporters in epithelial cells from mammary gland: acute down-regulation of GLUT1 carriers by weaning. Endocrinology 134:924–934;1994.
11. Chaiyabutr, N.; Faulkner, A.; Peaker, M. The utilization of glucose for the synthesis of milk components in the fed and starved lactating goat *in vivo*. Biochem. J. 186:301–308;1980.
12. Chirgwin, J.M.; Przybyla, A.W.; MacDonald, R.J.; Rutter, W.J. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299;1979.
13. Davis, S.R.; Collier, R.J.; McNamara, J.P.; Head, H.H.; Sussman, W. Effects of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output and mammary blood flow. J. Anim. Sci. 66:70–79;1988.
14. Delaquis, A.M.; Malo, C.; Turner, T.D. Na^+-independent glucose uptake by bovine mammary epithelial (MAC-T) cells. J. Dairy Sci. 76 (Suppl. 1), 188 (Abstr.).
15. Faulkner, A.; Chaiyabutr, N.; Peaker, M.; Carrick, D.T.; Kuhn, N.J. Metabolic significance of milk glucose. J. Dairy Res. 48:51–56;1981.
16. Forsberg, N.E.; Baldwin, R.L.; Smith, N.E. Roles of glucose and its interaction with acetate in maintenance and biosynthesis in bovine mammary tissue. J. Dairy Sci. 68:2550–2556; 1985.
17. Gould, G.W.; Holman, G.D. The glucose transporter family: structure, function and tissue-specific expression. Biochem. J. 295:329–341;1993.
18. Hocquette, J.-F.; Bornes, F.; Balage, M.; Ferre, P.; Grizard, J.; Vermorel, M. Glucose transporter (GLUT4) protein content in oxidative and glycolytic skeletal muscles from calf and goat. Biochem. J. 305:465–470;1995.
19. Hove, K. Maintenance of lactose secretion during acute insulin deficiency in lactating goats. Acta Physiol. Scand. 103: 173–179;1978.
20. Hove, K. Effects of hyperinsulinemia on lactose secretion and glucose uptake by the goat mammary gland. Acta Physiol. Scand. 104:422–430;1978.
21. James, D.E.; Strube, M.; Mueckler, M. Molecular cloning and characterization of an insulin-regulatable glucose transporter. Nature 338:83–87;1989.
22. Kahn, B.B. Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. J. Clin. Invest. 89:1367–1374;1992.
23. Kronfeld, D.S. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. J. Dairy Sci. 65:2204–2212;1982.
24. Kuhn, N.J.; Carrick, D.T.; Wilde, C.J. Lactose synthesis: the possibilities of regulation. J. Dairy Sci. 63:328–336;1980.
25. Laarveld, B.; Christensen, D.A.; Brockman, R.P. The effects of insulin on net metabolism of glucose and amino acids by the bovine mammary gland. Endocrinology (Baltimore) 108: 2217–2221;1981.
26. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685; 1970.
27. Lomax, M.A.; Baird, G.D.; Mallinson, C.B.; Symonds, H.W. Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochem. J. 180: 281–289;1979.
28. McDowell, G.H.; Gooden, J.M.; Leenanuruksa, D.; Jois, M.; English, A.W. Effects of exogenous growth hormone on milk production and nutrient uptake by muscle and mammary tissues of dairy cows in mid-lactation. Aust. J. Biol. Sci. 40:295–306;1987.
29. Madon, R.J.; Martin, S.; Davies, A.; Fawcett, H.A.C.; Flint, D.J.; Baldwin, S.A. Identification and characterization of glucose transport proteins in plasma membrane- and golgi vesicle-enriched fractions prepared from lactating rat mammary gland. Biochem. J. 272:99–105;1990.
30. Metz, S.H.M.; van den Bergh, S.G. Regulation of fat mobilization in adipose tissue of dairy cows in the period around parturition. Neth. J. Agric. Sci. 25:198–211;1977.
31. Miller, P.S.; Reis, B.L.; Calvert, C.C.; DePeters, E.J.; Baldwin, R.L. Patterns of nutrient uptake by the mammary glands of lactating cows. J. Dairy Sci. 74:3791–3799;1991.
32. Neville, M.C.; Allen, J.C.; Watters, C. The mechanisms of milk secretion. In: Neville, M.C.; Neifert, M.R. (eds). Lactation: Physiology, Nutrition, and Breast-Feeding. New York: Plenum; 1983:49–92.
33. Pethick, D.W.; Lindsay, D.B. Acetate metabolism in lactating sheep. Br. J. Nutr. 48:319–328;1982.
34. Rook, J.A.F.; Hopwood, J.B. The effects of intravenous infusions of insulin and of sodium succinate on milk secretion in the goat. J. Dairy Res. 37:193–198;1970.
35. Sternberger, L.A. Immunocytochemistry. 3rd ed. New York: John Wiley & Sons; 1986.
36. Tal, M.; Kahn, B.B.; Lodish, H.F. Expression of the low K_m GLUT1 glucose transporter is turned on in perivenous hepatocytes of insulin-deficient diabetic rat. Endocrinology 129: 1933–1941;1991.
37. Threadgold, L.C.; Kuhn, N.J. Glucose 6-phosphate hydrolysis by lactating rat mammary gland. Int. J. Biochem. 10:683–685; 1979.
38. Weekes, T.E.C. Hormonal control of glucose metabolism. In: Tsuda, T.; Sasaki, Y.; Kawashima, R. (eds). Physiology Aspects of Digestion and Metabolism in Ruminants: Proceedings of the Seventh International Symposium on Ruminant Physiology. New York: Academic Press; 1991:183–200.
39. Zhao, F.-Q.; Glimm, D.R.; Kennelly, J.J. Distribution of mammalian facilitative glucose transporter messenger RNA in bovine tissues. Int. J. Biochem. 25:1897–1903;1993.
40. Zhao, F.-Q.; Moseley, W.M.; Tucker, H.A.; Kennelly, J.J. Regulation of glucose transporter gene expression in mammary gland, muscle and fat of lactating cows by administration of bovine growth hormone and bovine growth hormone-releasing factor. J. Anim. Sci. 74:183–189;1996.