Isolation of the cDNA encoding the acid labile subunit (ALS) of the 150 kDa IGF-binding protein complex in cattle and ALS regulation during the transition from pregnancy to lactation

Jin W Kim, Robert P Rhoads, Nthabisheng Segoele, Niels B Kristensen¹, Dale E Bauman and Yves R Boisclair

Department of Animal Science, 259 Morrison Hall, Cornell University, Ithaca, New York 14853-4801, USA
¹Department of Animal Health, Welfare and Nutrition, Danish Institute of Agricultural Sciences, PO Box 50, 8830 Tjele, Denmark

Requests for offprints should be addressed to Y R Boisclair; Email: yrb1@cornell.edu

Abstract

During the transition from pregnancy to lactation, dairy cows experience a 70% reduction in plasma IGF-I. This reduction has been attributed to decreased hepatic IGF-I production. IGF-I circulates predominantly in multi-protein complexes consisting of one molecule each of IGF-I, IGF binding protein-3 and the acid labile subunit (ALS). Recent studies in the mouse have shown that absence of ALS results in accelerated turnover and severely depressed concentration of plasma IGF-I. These observations suggest that reduced plasma ALS could be a second factor contributing to the fall of plasma IGF-I in periparturient cows. This possibility has not been studied due to the lack of bovine ALS reagents. To address this, we isolated the bovine ALS cDNA and used its sequence to develop a ribonuclease protection assay (RPA) and a bovine ALS antiserum. Using the RPA, ALS mRNA abundance was approximately fivefold higher in liver than in lung, small intestine, adipose tissue, kidney and heart, but was absent in muscle and brain. The antiserum detected the highest ALS levels in plasma followed by ovarian follicular fluid, lymph and colostrum. A portion of colostrum and follicular fluid ALS appears to be synthesized locally as ALS mRNA was found in mammary epithelial cells and ovarian follicular cells. Finally, we measured plasma ALS in dairy cows during the periparturient period (days −35 and +56 relative to parturition on day 0). Plasma ALS dropped by 50% between late pregnancy and the first day of lactation and returned to prepartum levels by day +56. To determine whether this reflected a change in hepatic expression, ALS mRNA was measured in liver biopsies collected on days −35, +3 and +56. ALS mRNA expression was significantly lower on day +3 than on day −35, but recovered completely by day +56. Finally, we examined the ability of GH to increase plasma ALS abundance at selected times before and after parturition (weeks −5, −2, +1 and +5). GH increased plasma ALS at weeks −5, −2 and +5, but not at week +1. Identical effects of GH were seen when the response considered was plasma IGF-I. We conclude that the decline in plasma ALS after parturition is a consequence of hepatic GH resistance and contributes to the associated reduction of plasma IGF-I.


Introduction

During the transition from late pregnancy to early lactation, dairy cows enter a period of severe negative energy balance (Bell 1995, Boisclair et al. 2006). Under these conditions, changes in the plasma concentration of key hormones coordinate adaptations required for the preservation of metabolic homeostasis (Bell 1995, Boisclair et al. 2006). For example, plasma insulin-like growth factor-I (IGF-I) is decreased by 70% after parturition, facilitating the mobilization of amino acids from skeletal muscle in support of hepatic gluconeogenesis (Bell 1995, Boisclair et al. 2006). Based on positive correlations between plasma IGF-I concentrations and hepatic IGF-I mRNA abundance, the periparturient drop in plasma IGF-I has been attributed to decreased hepatic production (Kobayashi et al. 1999, Radcliffe et al. 2003, Kim et al. 2004).

Decreased hepatic production, however, is unlikely to be the only factor involved. This is because plasma IGF-I almost always circulates in multi-proteins, high molecular weight complexes (Baxter 1994, Boisclair et al. 2001). In adult animals, the predominant complex consists of one molecule each of IGF-I, IGF binding protein-3 or -5 (IGFBP-3 or -5), and the acid labile subunit (ALS) (Baxter 1994, Twigg & Baxter 1998, Boisclair et al. 2001). Incorporation of free IGF-I in these ternary complexes...
extends its half-life from minutes to over 12 h (Guler et al. 1989, Baxter 1994, Zapf et al. 1995). Adult animals devoid of ALS have accelerated turnover and severely depressed concentration of plasma IGF-I (Ueki et al. 2000, Yakar et al. 2002, Domene et al. 2004). ALS deficiency also results in the near disappearance of plasma IGFBP-3 whereas absence of IGFBP-3 has no effect on plasma ALS (Ueki et al. 2000, Yakar et al. 2005). These observations suggest that variation in plasma ALS could be a second factor contributing to the fall of plasma IGF-I in periparturient dairy cows.

The ALS cDNA and gene were isolated first in human, mouse and rat (Boisclair et al. 2001), and consequently most of the existing information on ALS was derived from these species. These studies have shown that ALS is synthesized primarily in liver in a growth hormone (GH)-dependent manner (Baxter 1990, Baxter & Dai 1994). The effects of GH occur via a stimulation of gene transcription, resulting in increased abundance of ALS mRNA in liver and ALS in plasma (Dai & Baxter 1994, Ooi et al. 1997, Olivecrona et al. 1999). In contrast, ALS has not been studied in dairy cattle, including during the transition from pregnancy to lactation when plasma IGF-I varies dynamically. The major factor contributing to this lack of information is the poor cross-reactivity of antibodies raised against rodent and human ALS with bovine ALS (Baxter 1990, Baxter & Dai 1994).

To address the lack of information on ALS in dairy cattle, we pursued two specific objectives. First, we cloned the bovine ALS cDNA and used this sequence to develop bovine-specific reagents. Secondly, using these reagents, we asked whether ALS is regulated during the transition from pregnancy to lactation in early lactating dairy cows as a consequence of hepatic GH resistance.

Materials and Methods

All experimental procedures involving animals were approved by the Animal Care and Use Committees of Cornell University and the Danish Animal Experiments Inspectorate.

Cloning of the bovine ALS cDNA

The full-length bovine ALS cDNA was amplified using a forward primer corresponding to nt −57 to −38 of the ovine ALS cDNA (relative to A+1TG; 5′-ATCCAGAGGCAGGAGCAGC-3′) and a poly A tail-specific reverse primer (5′-T30NNN-3′; Clontech laboratories, Palo Alto, CA, USA). PCR was performed using a high fidelity polymerase as recommended by the manufacturer (Ex Taq polymerase; Takara Bio Inc, Japan). Products were subcloned into the vector pCR 2.1-Topo (Invitrogen), sequenced and submitted to Genebank (accession number DQ444712).

Spatial ALS expression

Tissues were obtained at slaughter from two adult dairy cows that had been lactating for ~300 days (see Fig. 2 for complete listing of tissues). Ovarian follicular cells were isolated from freshly excised dominant and subdominant follicles (≥5 mm) and pooled. Mammary epithelial cells were isolated by digesting the parenchyma of a lactating dairy cow with a mixture of a collagenase, hyaluronidase and elastase (Matitashvili & Bauman 1999). Cells were subjected to gradient centrifugation to yield primarily mammary epithelial cells (>95% of isolated cells). All tissues and cells were frozen in liquid N2 immediately upon isolation.

Total RNA was extracted by the guanidium thiocyanate–phenol–chloroform method. The concentration of total RNA was determined by absorbance at 260 nm. RNA quality was verified by electrophoresis on formaldehyde agarose gel and ribosomal bands staining with Sybr Green II (Molecular Probes, Eugene, OR, USA). ALS mRNA abundance was measured by ribonuclease protection assay (RPA) with an RPA III kit (Ambion, Austin, TX, USA). The ALS probe corresponded to nt +182 to nt +599 (A+1TG) of the bovine ALS cDNA. The RPA was performed in the presence of a tenfold molar excess of a low-specific-activity 18S riboprobe generated from an 18S DNA template (Ambion). Signals were quantified by phosphorimaging and normalized to the 18S signal.

Production and validation of bovine ALS antisera

A peptide corresponding to the 28 carboxyl-terminal residues of bovine ALS (ALSc28) was conjugated to keyhole limpet hemocyanin (KLH) using sulphydryl chemistry (Bio–Synthesis Inc, Lewisville, TX, USA). Conjugated ALSc28 (100 µg) was emulsified with an equal volume of complete Freund’s adjuvant and injected subcutaneously into three New Zealand White rabbits. For secondary immunizations, emulsions were prepared with incomplete Freund’s adjuvant and injected on days 14, 42, 63, 84 and 105 (relative to primary immunization). Rabbits were bled on day 115 and serum was stored at −20 °C.

Immunoreactivity of antisera was evaluated with plasma obtained from adult animals of various species (listed in Fig. 3B). In some studies, bovine plasma was deglycosylated. Briefly, plasma (2 µl) was boiled in 0.5% SDS, 1% β-mercaptoethanol for 10 min. After adding NaHPO4 and 10% NP-40 to final concentrations of 0.05 M and 1% respectively, the samples were incubated at 37 °C in the absence or presence of 500 units of peptidase-N-glycosidase F...
After 1 h, reactions were terminated by boiling in 1× Laemmli loading buffer. Native and deglycosylated plasma were electrophoresed under reducing conditions on 10% SDS–polyacrylamide gels, and electroblotted onto nitrocellulose membranes (Protran; Schleicher & Schuell Bioscience, Keene, NH, USA). The membranes were blocked in Tris-buffered saline with Tween-20 (TBST; 0·05 M Tris, pH 7·4, 0·2 M NaCl, 0·1% Tween-20) containing 5% (w/v) non-fat dried skim milk (NFM) for 1 h at room temperature. Membranes were then incubated with bovine ALS antisera (1:4000 dilution) in blocking buffer overnight at 4°C. To establish specificity, incubation with the primary antibody was performed in the presence or absence of ALSC28. After incubation, membranes were washed in TBST and incubated with goat anti-rabbit IgG-horseradish peroxidase (HRP) (1:5000 dilution) in blocking buffer for 1 h at room temperature. Signals were detected by LumiGLO western blot chemiluminescence (KPL, Gaithersburg, MD, USA) and analyzed by densitometric scanning and NIH Image 1·63 software.

Figure 1  Nucleotide and deduced amino acid sequence of bovine ALS. Amino acid sequence is shown below the nucleotide sequence in standard one-letter abbreviation. The nucleotide and amino acid sequence are numbered on the left relative to the translation initiation codon (A+1TG). Amino acid 33 was identified by SignalP software analysis as the first amino acid residue of the mature protein and is boxed. The conserved 12 cysteine residues are indicated by bold and underlined letters. The stop codon TGA is indicated by a star.

(PNGase F; New England Biolabs, Beverly, MA, USA). After 1 h, reactions were terminated by boiling in 1× Laemmli loading buffer. Native and deglycosylated plasma were electrophoresed under reducing conditions on 10% SDS–polyacrylamide gels, and electroblotted onto nitrocellulose membranes (Protran; Schleicher & Schuell Bioscience, Keene, NH, USA). The membranes were blocked in Tris-buffered saline with Tween-20 (TBST; 0·05 M Tris, pH 7·4, 0·2 M NaCl, 0·1% Tween-20) containing 5% (w/v) non-fat dried skim milk (NFM) for 1 h at room temperature. Membranes were then incubated with bovine ALS antisera (1:4000 dilution) in blocking buffer overnight at 4 °C. To establish specificity, incubation with the primary antibody was performed in the presence or absence of ALS C28. After incubation, membranes were washed in TBST and incubated with goat anti-rabbit IgG-horseradish peroxidase (HRP) (1:5000 dilution) in blocking buffer for 1 h at room temperature. Signals were detected by LumiGLO western blot chemiluminescence (KPL, Gaithersburg, MD, USA) and analyzed by densitometric scanning and NIH Image 1·63 software.

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**Regulation of ALS during the transition from pregnancy to lactation**

Temporal profiles of plasma ALS abundance and hepatic ALS mRNA were studied in four multiparous cows in the period between 35 days prepartum and 56 days postpartum. Cows were fed a total mixed ration (TMR) according to recommended feeding standards (NRC 2001); cows were fed once daily at 1100 h and were allowed to feed ad libitum. The TMR contained 1·45 Mcal of net energy of lactation (NE\textsubscript{L}) and 125 g crude protein (CP) per kg of dry matter (DM) between days −35 and −21 (relative to parturition on day 0), 1·63 Mecal NE\textsubscript{L}/kg DM and 143 g CP/kg DM between day −21 and parturition, and 1·76 Mecal NE\textsubscript{L}/kg DM and 183 g CP/kg DM during lactation (NRC 2001). Cows were milked thrice daily at 0900, 1600 and 2300 h. Blood samples were collected between 0900 and 1000 h on days −35, −7, −1, +1, +3, +7, +10, +21 and +56 by coccygeal venipuncture. Plasma was prepared immediately and frozen at −20 °C. After blood sampling on days −35, +3 and +56, liver biopsies were obtained by a non-invasive percutaneous method (Kim et al. 2004) and snap-frozen in liquid N\textsubscript{2}.

To investigate the effect of GH on plasma ALS, a second group of 12 cows was used in the period from 5 weeks prepartum to 5 weeks postpartum. They were fed a TMR ad libitum once daily according to feeding standards (NRC 2001). The NE\textsubscript{L} and CP content of the TMR were 1·36 Mcal/kg DM and 130 g/kg DM during week −5, 1·56 Mcal/kg DM and 141 g/kg DM between week −4 and parturition, and 1·72 Mcal/kg DM and 179 g/kg DM after parturition. Cows were randomly allocated to a control or a GH group. The GH group received a daily injection of recombinant bovine GH (bovine somatotropin (bST), 45 mg i.m., lot 96J-B5128–002; Monsanto, St Louis, MO, USA) on three consecutive days during weeks −5, −2, +1 and +5. This period of GH administration produces a maximal IGF-I response that persists for over 24 h after the last injection (Cohick et al. 1989). Injections were initiated on the second day of each week. Blood samples were obtained by coccygeal venipuncture between 1000 and 1130 h, the day before the first GH injection (day 0) and the day after the last injection (day 4). Control cows were sampled on the same day of each week. Plasma was prepared immediately and frozen at −20 °C.

Abundance of plasma ALS and hepatic ALS mRNA was analyzed by western immunoblotting and RPA, respectively, as described above. Plasma IGF-I was measured by a previously described double antibody RIA (Kim et al. 2004). Bovine recombinant IGF-I was used for iodination and standard (recombinant IGF-I, lot GST–3; Monsanto). Primary antibody against IGF-I (rabbit anti-human IGF-I, lot AFP4892989) was obtained from the National Hormone and Pituitary Program (Bethesda, Maryland).

**Distribution of ALS in bovine fluids**

Catheters were surgically implanted in the thoracic duct of six dairy calves using the procedures described by Hartmann and Lascelles (1966). They were used to obtain matched jugular blood and thoracic lymph samples. Blood and follicular fluid were obtained from a second group of six dairy calves using the procedures described by Porter et al. (2000). These cows were injected with a luteolytic dose of prostaglandin F\textsubscript{2\alpha} (PGF\textsubscript{2\alpha}) between days 7 and 14 of the estrous cycle. After the onset of estrus, animals were examined by transrectal ultrasonography to identify the dominant follicle, and then ovaries were removed by colpotomy. The dominant follicle (≥9 mm) was dissected and its follicular fluid was aspirated using an 18-gauge needle. Finally, blood and colostrum were obtained from six multiparous dairy cows at day 245 of gestation (5 weeks before parturition). Plasma was prepared by heparin addition and centrifugation (3000 × g). Colostrum was centrifugated (10 000 × g) for 15 min and the aqueous phase was collected. All fluids were frozen at −20 °C until analyzed for ALS abundance by western immunoblotting (as described in previous section).

**Figure 2** Spatial ALS expression in bovine tissues. Top panel: tissues were obtained from two late lactation dairy cows. Total RNA (20 μg) was analyzed simultaneously by RPA for the abundance of ALS mRNA and 18S rRNA. Bottom panel: the ALS mRNA signal was normalized to the 18S signal and is represented as mean ± s.e. (expressed as a percentage of the liver signal).
MD, USA). The secondary antibody was a caprine anti-rabbit IgG (lot 12515; Biotech Source, Inc, Franklin, MA, USA). Inter- and intra-assay coefficients of variation averaged less than 8% and 9% respectively.

Statistical analysis

Levels of ALS in various fluids were expressed as a percentage of matched plasma levels and analyzed by a model accounting for source (lymph, follicular fluid and colostrum) as the fixed effects and animal as the random effect. Data obtained during the temporal study in transition dairy cows were analyzed by a mixed model with time as the fixed effect and animal as the random effect.

When the effect of time was significant ($P<0.05$), the variation was partitioned into linear, quadratic and cubic contrasts. For the GH study, the response was calculated for each variable as the difference between day 4 and day 0. Responses were analyzed by a model accounting for GH (control vs GH), time relative to parturition (weeks −5, −2, +1 and +5), and their interaction (GH × Time) as the fixed effects and animal as the random effect.

Results

Primary structure and spatial expression of bovine ALS

We isolated a partial 1833 bp bovine ALS cDNA containing an open reading frame of 611 amino acids (Fig. 1). The cDNA has a high degree of identity with its human (>75%) and ovine ALS counterparts (>95%). Computer analysis of the amino acid sequence predicted a signal peptide of 32 residues and a mature protein of 579 residues. The deduced mature protein has all the features previously described for ALS (Boisclair et al. 2001), including 12 cysteine residues clustered predominantly in the amino and carboxyl terminal regions, 20 leucine-rich repeats of 24 residues and 6 potential asparagine-linked glycosylation sites (NXS/T). Identities of mature bovine ALS with chicken, mouse, human, pig and sheep ALS were 60, 73, 76, 82 and 96% respectively.

We designed an RPA based on the bovine ALS cDNA and surveyed ALS expression in mature dairy cows. ALS expression was approximately fivefold higher in liver than in other tissues surveyed (Fig. 2). Expression in non-hepatic tissues was obvious in lung, small intestine, adipose tissue, kidney and heart, but nearly absent in brain and muscle.

Detection of bovine ALS in plasma and extra-vascular fluid

To raise bovine ALS antisera, 3 rabbits were immunized with a peptide corresponding to the 28 carboxyl terminal residues of ALS. For each species, amino acids differences from bovine residues are shaded. (B) Plasma (0.2 μl) from the indicated species was electrophoresed on a 10% reducing SDS–polyacrylamide gel. After electroblotting, the membranes were incubated with bovine ALS antiserum 1082, 01 or 1085. Arrows on the right correspond to the 84 kDa molecular mass marker. (C) Specificity of antiserum 1082. Bovine plasma (0.1–0.5 μl) was electrophoresed on a 10% reducing SDS–polyacrylamide gel. Immunoblotting was performed in the absence (−) or presence (+) of 10 μg/ml of the carboxyl terminal ALS<sub>C28</sub> peptide. (D) The 84 kDa protein detected by antiserum 1082 is glycosylated. Bovine plasma (2 μl) was incubated in the absence (−) or presence (+) of peptide-N-glycosidase F (PNGase F). Reactions were electrophoresed on a 10% reducing SDS–polyacrylamide gel. Immunoblotting was performed with pre-immune rabbit serum (PRE) or antiserum 1082.

![Figure 3](https://example.com/figure3.png)
90 kDa protein in plasma. All three antisera detected a protein of 84 kDa in cattle, sheep, goat and chicken plasma (Fig. 3B). Antiserum 1085 was also capable of detecting a 84 kDa signal in pig plasma, but none of these antisera detected ALS in mouse or human plasma. This cross-species reactivity profile suggests that the antisera recognized an epitope defined predominantly by the 4 amino acid residues PPSL present in bovine, sheep and chicken ALS (Fig. 3A).

Additional studies were performed to validate antiserum 1082. First, we assessed its specificity by determining whether the 84 kDa signal present in bovine plasma was eliminated when performing immunoblotting in the presence of the ALSC28 peptide (Fig. 3C). As expected for a specific signal, the 84 kDa band was completely eliminated in the presence of ALSC28. Secondly, we determined whether the 84 kDa protein is glycosylated. Bovine plasma was incubated in the presence or absence of PNGase F, followed by SDS-PAGE and immunoblotting (Fig. 3D). PNGase F incubation collapsed the immune signal to 60 kDa, the size predicted by the primary amino acid sequence of mature ALS. Overall these studies indicate that ALS is the 84 kDa protein detected in bovine plasma by antiserum 1082.

ALS abundance was compared in matched plasma and lymph samples obtained from growing cattle. As observed in humans, ALS was more abundant in plasma than lymph (Fig. 4A). ALS has also been shown to occur in other fluid compartments (Boisclair et al. 2001). To determine if this occurs in the cow, we sampled the mammary and ovarian follicular fluid compartments (Fig. 4A). Mammary secretions were obtained from dairy cows 5 weeks before parturition and again after 5 weeks of lactation. ALS was present in colostrum, but at only 15% of levels seen in matched plasma. ALS was nearly undetectable in milk (not shown). ALS was also present in fluid obtained from the dominant follicle at levels that were approximately 80% of those seen in matched plasma (Fig. 4A). These data show that ALS occurs at significant levels outside the vascular compartment.

Given the presence of ALS in colostrum and follicular fluid, we performed ALS mRNA analysis on the associated tissues. ALS mRNA was expressed in both mammary gland and ovary at levels similar to those seen in kidney (Fig. 4B). ALS mRNA was also present in isolated mammary epithelial cells and complete ovarian follicles. These data suggest that mammary and follicular cells synthesize a portion of the ALS found in associated fluid compartments.
Regulation of ALS during the periparturient period

We measured the variation in plasma IGF-I and ALS in dairy cows between day 35 prepartum and 56 postpartum. The concentration of plasma IGF-I dropped by 50% during the first week postpartum, and remained depressed until the end of the study (Fig. 5A). Plasma ALS followed an identical pattern between day 35 prepartum and day 10 postpartum (Fig. 5B). Unlike IGF-I, however, plasma ALS started to recover by day 21 postpartum and returned to prepartum levels by day 56 postpartum (Fig. 5B, quadratic \( P < 0.001 \)). To determine whether this reflected changes in hepatic expression, ALS mRNA was measured in liver biopsies collected on days -35, +3 and +56. ALS mRNA expression was significantly lower on day +3 than on day -35, but recovered completely by day +56 (Fig. 6, \( P < 0.05 \)). We conclude that hepatic ALS gene expression accounts for the variation in plasma ALS during the periparturient period.

Finally, we examined the ability of GH to increase plasma ALS abundance at selected times before (5 and 2 weeks prepartum) and after parturition (1 and 5 weeks postpartum). At each time, blood samples were collected before (day 0) and the day after a 3-day period of GH treatment (day 4). Control, untreated cows were sampled at identical times. GH significantly increased plasma ALS before parturition and 5 weeks postpartum, but this effect was abolished during the first week postpartum (Fig. 7A, GH \times Time \( P < 0.05 \)). Nearly identical effects of GH were seen when the response considered was plasma IGF-I (Fig. 7B). We conclude that plasma ALS is positively regulated by GH but that this response is abrogated during the week following parturition.

Discussion

In mature animals, the circulating IGF system consists of IGF-I and -II, IGFBP-1 to -6 and ALS (Baxter 1994, Ooi & Boisclair 1999). The effects of development, hormones and diseases on tissue expression and plasma concentrations have been studied extensively in rodents and humans (Rajaram et al. 1997, Monzavi & Cohen 2002, Le Roith 2003). Similar efforts have taken place in cattle with the notable exception of ALS (Cohick 1998, Breier 1999, Renaville et al. 2002). As a first step to address this lack of information, we measured ALS expression across tissues obtained from mature dairy cows. In agreement with surveys performed in rodents, primates and other farm animals (Boisclair et al. 2001), the highest level of ALS expression was found in liver. Non-hepatic expression has also been suggested by recent reports. For example, low levels of ALS mRNA were detected in mouse kidney (Ueki et al. 2000) by northern blot analysis, and in the rat renal cortex by in situ hybridization (Chin et al. 1994). Using an RPA, Lee et al. (2001) detected ALS expression in pig muscle, spleen, ovary and uterus. In the present study, we detected ALS mRNA in bovine heart, kidney, small intestine, adipose tissue and lung with signals ranging from 20 to 35% of that seen in liver. Excluding the liver, the highest level of expression in our study occurred in lung.
a tissue not previously known to express ALS. We also
found ALS mRNA in the mammary gland and ovary
where expression was contributed, at least in part, by
cells specific to these tissues (mammary epithelial cells
and ovarian follicular cells). In the case of the ovarian follicular
cells, we did not determine whether both theca and
granulosa cells expressed ALS, but this seems likely based
on a recent study in the pig ovary (Wandji et al. 2000).
Overall, our data indicate that the liver accounts for the
bulk of circulating ALS, as it does in other species
(Boisclair et al. 2001), but also raise the possibility that
other tissues contribute to extra-vascular ALS.

The relative abundance of ALS in plasma and other
fluid compartments has not been evaluated directly in
cattle, reflecting the lack of reactivity of antibodies raised
against human or rat ALS with their bovine counterpart
(Baxter 1990, Baxter & Dai 1994). Using the COOH-
terminal sequence of bovine ALS as an antigen we
obtained antisera detecting bovine, but not human or
mouse ALS. As previously shown in humans (Baxter
1990), we observed much higher ALS abundance in
plasma than in lymph, consistent with the inability of ALS
to cross the endothelial barrier efficiently (Binoux &
Hosenlopp 1988). We also detected ALS in colostrum
and ovarian follicular fluid. The high ALS abundance in
the latter is consistent with follicular cell synthesis, other-
wise a steep gradient would be expected between plasma
and follicular fluid.

Irrespective of its origin, a more important issue is
whether or not extra-vascular ALS is functionally import-
ant. Both IGF-I and IGF-II (IGFs) are thought to be
positive regulators of follicular growth whereas IGFBP-3
is the most abundant IGFBP in follicular fluid (Fortune
et al. 2004, Webb et al. 2004). In cattle, total concentrations
of IGF-I and IGFBP-3 do not fluctuate significantly in
fluid obtained from dominant and subordinate follicles.
Rather, IGF-I availability varies as a consequence of
changes in the abundance of IGFBP-2, -4 and -5 (Fortune
et al. 2004, Webb et al. 2004). Thus, the dominant follicle
has lower levels of IGFBP-4 and -5 and higher levels of
free IGF-I, whereas the opposite occurs in atretic follicles
(Fortune et al. 2004, Webb et al. 2004). The presence of
high levels of ALS and IGFBP-3 along with significant
levels of IGFBP-5 suggests that IGFs mostly occur as
ternary complexes in bovine follicular fluid. This has
actually been demonstrated for IGF-I in human follicular
fluid (Hughes et al. 1997). IGFs are also important
mammary growth factors (Marshman & Streuli 2002,
Akers et al. 2005), including during the period of epithelial
cell proliferation and differentiation of late pregnancy
(Brisken et al. 2002) when we detected significant levels of
ALS in colostrum. Late pregnancy is a period when
bovine colostrum contains high levels of IGF-I and
IGFBP-3 (Baumrucker & Erondo 2000), raising the
possibility of significant ternary complex formation.
Moreover, IGFBP-5 is expressed at its highest level during
lactogenesis in the bovine mammary gland (Plath-Gabler
et al. 2001). Additional studies are needed to determine
whether ternary complex formation occurs during the
different developmental stages of the bovine follicle and
mammary gland.

Periparturient dairy cows provide a unique opportunity
to examine the regulation of ALS during a physiological
condition that results in dynamic changes in plasma IGF-I.
Dairy cows experience an abrupt, 60–70% decline in
plasma IGF-I and IGFBP-3 between late pregnancy and
parturition (Kobayashi et al. 1999, Kim et al. 2004,
Boisclair et al. 2006). In agreement with others (Kobayashi
et al. 1999, Radcliff et al. 2003), we showed that the
plasma IGF-I depression is associated with reduced IGF-I

Figure 6 Hepatic ALS expression during the periparturient period. Four multiparous dairy cows were
studied during the period between day −35 and +56 (relative to parturition on day 0). Left panel: liver
total RNA (10 μg) was analyzed simultaneously for abundance of ALS mRNA and 18S RNA by RPA. Data
from two representative cows are shown. Right panel: bars represent means ± s.e. (n = 4) of the ALS mRNA
signal normalized to the 18S signal. Bars with different letters differ at P<0.05.
mRNA in liver (Kim et al. 2004). We now show that plasma ALS abundance is also decreased between pregnancy and early lactation, an effect completely accounted for by lower hepatic ALS mRNA levels. ALS has a low affinity for binary IGF:IGFBP-3 complexes and must circulate in excess over IGF-I and IGFBP-3 to efficiently recruit these proteins into long-lived ternary complexes (Holman & Baxter 1996, Boisclair et al. 2001). Therefore, reduced ALS synthesis in the days following parturition probably amplifies the effect of decreased hepatic IGF-I production on plasma IGF-I. Within 3 weeks of parturition, however, plasma ALS levels started to recover such that both hepatic ALS expression and plasma ALS had returned to pre-calving levels by day 56 of lactation. Hepatic IGF-I mRNA abundance was also completely normalized in these cows by day 56 (Kim et al. 2004), but the plasma IGF-I concentration was not. These results suggest that ALS plays little or no role in the persisting plasma IGF-I depression.

In rodents and humans, GH is the most potent positive regulator of hepatic ALS mRNA abundance and plasma ALS concentration (Baxter & Dai 1994, Dai & Baxter 1994, Olivecrona et al. 1999). The essential role of GH is illustrated by the absence of ALS and ternary complexes in the plasma of GH-deficient animals (Zapt et al. 1995). We previously showed that these effects of GH are transcriptional and mediated by STAT5 binding to a single GAS element in the proximal promoter of the mouse ALS gene (Ooi et al. 1998). This GAS element is completely conserved in the promoter of the rat, sheep and human ALS gene (Delhanty & Baxter 1997, Rhoads et al. 2000, Suwanichkul et al. 2000). A similar mechanism has recently been shown to explain the positive effects of GH on the transcription of the rat IGF-I gene (Woelfle et al. 2003, Chia et al. 2006). In the present study, GH treatment increased levels of both ALS and IGF-I during pregnancy and week 5 of lactation, but was ineffective 1 week after parturition. In this context, we recently demonstrated that the liver-specific GH receptor transcript and hepatic GH receptor abundance were decreased immediately after parturition but returned to normal within 3 weeks of parturition (Kim et al. 2004). Therefore, loss of hepatic GH action probably accounts for decreased production of both ALS and IGF-I in the immediate postparturient period.

Dairy cattle offer other opportunities to study the regulation of ALS, including other conditions where plasma IGF-I increases (euglycemic–hyperinsulinemic clamp) or decreases rapidly (50–80% decline during undernutrition) (McGuire et al. 1995a, 1995b). Cattle are also recognized as a valuable model for studying mammary and ovarian follicular development (Fortune et al. 2004, Akers et al. 2005) and have easily accessible fluid compartments associated with these tissues. The reagents we have developed will facilitate studies on the role of ALS and ternary complexes in plasma and other fluid compartments of cattle.

Figure 7. Effect of GH on plasma ALS during the periparturient period. Twelve multiparous cows were studied in the period between –5 and +5 weeks (relative to parturition on day 0). Cows were randomly allocated to remain untreated (control, n=6) or to receive three consecutive daily injections of recombinant GH (45 mg/injection, n=6) during each of weeks –5, –2, +1 and +5. During these weeks, blood samples were obtained immediately before (day 0) and the day following the 3-day period of GH injection (day 4). Blood samples were obtained at similar times from the control group. (A) Top panel: plasma ALS was measured for each sample by western immunoblotting. Data from two representative cows are shown. Bottom panel: the response in plasma ALS (Δ plasma ALS) was calculated as the plasma abundance difference between day 4 and day 0. Bars represent means ± S.E. (n=6) of the response in plasma ALS. (B) Plasma IGF-I was measured by RIA. The response in plasma IGF-I (Δ plasma IGF-I) was calculated as the plasma concentration difference between day 4 and day 0. Bars represent means ± S.E. (n=6) of the plasma IGF-I response.
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