

# Regulation of the acid-labile subunit of the 150-kDa IGF-binding protein complex and its role in the circulating IGF system<sup>1</sup>

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**ABSTRACT:** In postnatal animals, most of the IGF-I and IGF-II circulates in ternary complexes of 150 kDa composed of one molecule each of IGF-I or IGF-II, IGF binding protein-3 or -5, and an acid-labile subunit (ALS). Circulation of IGF-I and IGF-II in 150-kDa complexes leads to their retention in the vascular system and promotes their endocrine actions. This review focuses on recent progress on the biology of ALS, the most important factor driving the formation of the 150-kDa complex in plasma. In a variety of animals, including sheep, the single-copy ALS gene spans approximately 3.3 kb and is composed of two exons and one intron. Transcription of the ALS gene produces a mRNA of about 2.2 kb, which encodes proteins of 603 amino acids in mice and 611 amino acids in sheep. Mature ALS circulates in plasma as a glycosylated protein of 84 to 86 kDa and is organized by repeating leucine-rich domains of 24 amino acids into a donut-shaped protein. In all species studied so far, the ALS gene is expressed at high levels only in liver. In sheep, weak expression is first detected at d 130 of fetal life, increases suddenly during the 1st wk after birth, and changes little thereafter.

After birth, growth hormone increases ALS synthesis by activating transcription of the gene. Analysis of sheep, mouse, and human ALS promoters reveals conservation of a growth hormone response element. This element mediates the effects of growth hormone by binding signal transducer and activator of transcription-5a and -5b, two related transcription factors. To define the role of ALS in the circulating IGF system, studies have been performed in ALS-null mice, which are devoid of both ALS and 150-kDa complexes. ALS null mice grow at a slower rate after birth than wild-type mice. This growth depression is associated with 65 to 90% decreases in the plasma concentrations of IGF-I and IGF binding protein-3, indicating that ALS is needed to maintain plasma concentrations of both proteins. In conclusion, ALS plays a critical role in regulating the plasma concentration of IGF-I and IGF-II and their access to target tissues. In view of this important role, ALS must be considered in future studies if animal scientists are to understand the roles of plasma IGF-I and -II in regulating important productive functions such as growth, reproduction, and lactation.

Key Words: Insulin-like Growth Factor, Endocrinology, Liver, Somatotropin, Transcription

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J. Anim. Sci. 79(E. Suppl.):E41-E47

## Introduction

Insulin-like growth factors-I and -II are involved in the regulation of cellular processes such as proliferation, differentiation, and the prevention of apoptosis (Jones and Clemmons, 1995; Stewart and Rotwein, 1996). These actions are conveyed by IGF-I and insulin receptors during fetal life and exclusively by IGF-I receptors after birth (Baker et al., 1993; Louvi et al.,

1997). The functional importance of the IGF system was confirmed by the growth retardation and developmental defects observed in mice with targeted inactivation of the IGF-I, IGF-II, and IGF-I receptor genes (Baker et al., 1993; Liu et al., 1993). Before birth, most of the effects of IGF result from autocrine/paracrine action (Jones and Clemmons, 1995; Stewart and Rotwein, 1996). After birth, however, the endocrine arm of the IGF system is thought to become increasingly important, with IGF-I mediating many of the effects of GH and linking anabolic processes to nutrient availability (Thissen et al., 1994; Jones and Clemmons, 1995).

Before birth, circulating IGF form binary complexes of 40 to 50 kDa with members of a family of IGF-binding proteins (IGFBP-1 to -6). After birth, however, synthesis of the acid-labile subunit (ALS) by liver sequesters most IGF into ternary complexes of 150 kDa consisting of one molecule each of IGF, IGFBP-3, or IGFBP-5 and

<sup>1</sup>We thank J. Parsons for her help in preparing this manuscript. This work was supported in part by NIH grant DK-51624 and by the Cornell Univ. Exp. Sta.

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Received July 25, 2000.

Accepted March 20, 2001.

ALS (Rechler, 1993; Baxter, 1994; Ooi and Boisclair, 1999). Despite evidence that ALS plays an important role in the biology of circulating IGF, it has received only scant attention, particularly in domestic animals. Herein we provide an overview of the biology of ALS, with emphasis on recent work.

### *Structure of the ALS Gene and cDNA*

The ALS gene was first cloned in 1996, in mice (Boisclair et al., 1996). The murine gene covers approximately 3.3 kb of chromosomal DNA and is composed of two exons separated by a 1,126-bp intron. Exon 1 encodes the first five amino acids of the signal peptide, and exon 2 encodes the remaining 22 amino acids of the signal peptide and the 576 amino acid residues of the mature protein. This chromosomal structure is conserved across species, as shown by the subsequent descriptions of the gene in rats, humans, and sheep (Delhanty and Baxter, 1997; Rhoads et al., 2000b; Suwanichkul et al., 2000). The ALS is a single-copy gene and was mapped to bands A2–A3 of mouse chromosome 17 and to the short arm of human chromosome 16 at p13.3 (Boisclair et al., 1996; Suwanichkul et al., 2000).

The ALS gene is devoid of a TATA box. Transcription of the gene produces mRNA of ~ 2.2 kb in primates (Leong et al., 1992; Delhanty and Baxter, 1996), rodents (Boisclair et al., 1996; Dai and Baxter, 1992, 1994), sheep (Rhoads et al., 2000b), and humans (Leong et al., 1992). They encode proteins ranging in size from 603 amino acids in the mouse to 611 residues in the sheep. Identity of mature ALS between mouse and human is 79%, and it is 73% between mouse and sheep. Structural features almost completely conserved across species include the presence of 12 to 13 cysteine residues, 6 to 7 asparagine-linked glycosylation sites, and 18 to 20 repeating leucine-rich domains of 24 amino acids. These leucine rich-domains account for approximately 75% of the mature protein and identify ALS as a member of the superfamily of leucine-rich repeat proteins. A general attribute of members of this superfamily is their ability to participate in protein–protein interactions. The leucine-rich motifs organize ALS into a donut-shaped structure (Janosi et al., 1999b).

### *Biochemical Properties of ALS*

Serum ALS has an apparent molecular weight of 84 to 86 kDa after purification and of 66 kDa after enzymatic deglycosylation (Baxter and Martin, 1989; Baxter and Dai, 1994). The ALS has no affinity for free IGF-I or IGF-II and very low affinity for uncomplexed IGFBP-3 but readily binds to binary complexes of IGF and IGFBP-3 (Baxter et al., 1989; Twigg and Baxter, 1998). The affinity of ALS for these binary complexes is considerably less than that of IGFBP-3 for IGF-I or IGF-II (Holman and Baxter, 1996).

The IGFBP-1, -2, -4, or -6 cannot substitute for IGFBP-3 in forming the ternary complex with ALS. In

contrast, IGFBP-5, the member of the IGFBP family most closely related to IGFBP-3, is able to form ternary complexes with ALS and comigrate with ALS in human serum (Twigg and Baxter, 1998). The physiological significance of these 150-kDa complexes containing IGFBP-5 remains unclear. Because the concentration of IGFBP-5 is low in serum, they account at best for ~10% of the 150-kDa complexes in serum (Mohan et al., 1995). In addition, unlike IGFBP-3, IGFBP-5 is able to associate weakly with ALS in the absence of IGF, raising the possibility that a large fraction of the 150-kDa complexes containing IGFBP-5 do not carry any IGF (Twigg et al., 1998).

Structurally, IGFBP-1 to -6 share homologous amino and carboxyl terminal domains but have unique central domains (Rechler, 1993; Ooi and Boisclair, 1999). Domain swapping experiments with IGFBP that are unable to form ternary complexes (i.e., IGFBP-2 and IGFBP-6) have demonstrated that the carboxyl terminal domains of IGFBP-3 and IGFBP-5 are important for binding ALS (Hashimoto et al., 1997; Twigg et al., 1998). Binding ability was further mapped to a conserved region of 18 amino acid residues corresponding to residues 201 to 218 in IGFBP-3 and residues 215 to 232 in IGFBP-5 (Firth et al., 1998; Twigg et al., 1998). This region is composed of mostly basic and positively charged amino acid residues. More recently, the central domain of IGFBP-5 was also shown to bind ALS even in the absence of the carboxyl terminal domain (Twigg et al., 2000).

In the case of ALS, recent studies have sought to determine the role played by the sugar residues. Removal of the negatively charged sialic acid from the glycan chains of ALS reduces the affinity of ALS for the IGF-I and IGF-II binary complexes, but it does not eliminate complex formation (Janosi et al., 1999a). Independent mutations of each of the seven N-linked glycan attachment sites of human ALS do not eliminate its ability to form ternary complexes with IGFBP-3, but complete deglycosylation does. Overall, these data are consistent with a model in which the positively charged, conserved domain of 18 amino acids present in IGFBP-3 and IGFBP-5 interact with negatively charged regions of ALS. This is supported by molecular modeling of ALS, which predicts two densely negatively charged regions, the first one created by the clustering of six of the seven N-linked sugar chains and the second one by the amino acids present at the internal surface of the donut-shaped protein (Janosi et al., 1999b).

### *Regulation of ALS Synthesis*

The ALS is found in high concentration almost exclusively in postnatal serum (Baxter, 1990a; Khosravi et al., 1997). Typical concentrations in human and rat serum are 300 and 570 nM, respectively. The ALS circulates in excess over the other components of the ternary complex, with 50 to 60% of serum ALS found in free form (Baxter, 1990a; Baxter and Dai, 1994; Khosravi

et al., 1997). When total RNA is analyzed by Northern blot in rats, primates, and sheep, ALS gene expression can only be detected in liver (Dai and Baxter, 1994; Delhanty and Baxter, 1996; Rhoads et al., 2000b). Synthesis in liver is confined to parenchymal cells (Chin et al., 1994).

Immunoreactive ALS is also present in very low concentration in cerebrospinal fluid, amniotic fluid, milk, and lymph, and in low to medium concentrations in peritoneal, synovial, ovarian, and blister fluid (Baxter, 1990a; Xu et al., 1995; Cwyfan-Hughes et al., 1997; Khosravi et al., 1997; Labarta et al., 1997). Serum is likely the source of most of this extravascular ALS, although local synthesis may occur in some tissues. For example, ALS mRNA has been detected in adult mouse and rat kidney and in theca and granulosa cells from porcine ovary (Chin et al., 1994; Wandji et al., 2000). Extravascular ALS may be particularly significant in the ovary because IGF are found almost exclusively in 150-kDa complexes in human follicular fluid (Cwyfan-Hughes et al., 1997). Irrespective of its origin, extravascular ALS can modulate local IGF action, as recently shown by the ability of ALS to potentiate the inhibitory effects of IGFBP-5 on thyroidal cell proliferation (Twigg et al., 1999).

Onset of ALS synthesis is one of the last events in the development of the circulating IGF system. In humans, ALS is undetectable in fetal serum at 27 wk of gestation, but it is present at term (Lewitt et al., 1995). Serum levels of ALS increase fivefold from birth to puberty and decline somewhat in older individuals (Baxter, 1990a). Studies in rats have shown that an induction of ALS gene expression in liver is responsible for this increase in plasma ALS in early life (Baxter and Dai, 1994; Dai and Baxter, 1994; Frystyk et al., 1998). In sheep, abundance of ALS mRNA is also low before birth, but it increases abruptly within 7 d of postnatal life (Rhoads et al., 2000b). The functional consequence of this pattern of ALS expression in the sheep is that IGF circulate primarily in 50-kDa complexes before birth and in 150-kDa complexes 1 wk after birth (Butler and Gluckman, 1986).

Growth hormone is by far the most potent inducer of ALS mRNA in liver and of ALS in plasma (Baxter, 1990a; Baxter and Dai, 1994; Ooi et al., 1997; Olivecrona et al., 1999). The importance of this regulation is underlined by the nearly complete absence of ALS in GH-deficient states (Zapf et al., 1989; Gargosky et al., 1994; Aguiar-Oliveira et al., 1999) and by the temporal correlation between appearance of ALS mRNA and functional GH receptor in liver from sheep and rats (Gluckman et al., 1983; Tiong and Herington, 1992). These effects of GH in liver are direct and occur at the level of ALS gene transcription (Ooi et al., 1997, 1998).

A variety of conditions have been shown to reduce serum ALS in rats and humans. They include feed deprivation, undernutrition, and catabolic diseases such as diabetes, burn injury, and cirrhosis (Dai and Baxter, 1994; Bereket et al., 1996; Oster et al., 1996; Fukuda

et al., 1999; Lang et al., 1996, 2000; Moller et al., 2000). Negative regulation of ALS synthesis occurs at both transcriptional and posttranscriptional levels. Dexamethasone, cAMP, and epidermal growth factor decrease secretion of ALS in primary rat hepatocytes, primarily by reducing the abundance of ALS mRNA (Dai et al., 1994; Delhanty and Baxter, 1998). Increase in factors such as glucocorticoid and in cellular cAMP could explain the marked decrease in ALS synthesis observed during thermal injury and liver failure (Lang et al., 2000; Moller et al., 2000). In contrast, insulin deficiency may be the primary defect causing reduced concentration of serum ALS during feed deprivation, undernutrition, and diabetes. This effect of insulin occurs posttranslationally; insulin increases ALS secretion in the absence of any change in ALS mRNA abundance in primary hepatocytes (Dai et al., 1994). Finally, decreased ALS synthesis could also occur secondarily to the development of GH resistance in liver. Recent studies have shown that most of the negative actions of cAMP and the inflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ) on ALS synthesis occur via the induction of a GH-resistant state in liver cells (Delhanty, 1998; Boisclair et al., 2000).

#### *Regulation of ALS Gene Transcription in Liver*

The ALS gene offers opportunities to understand the basis for the temporal and GH regulation of gene expression in liver. So far, most studies have been devoted to the GH regulation of ALS gene transcription, a focus justified by the importance of the IGF system in mediating the effects of GH (Etherton and Bauman, 1998). Using rat liver cells, a GH-responsive promoter was identified in the genomic fragment corresponding to nt -2001 to nt -49 of the mouse ALS gene (relative to A<sub>1</sub>TG) (Ooi et al., 1997). Deletion and mutational analysis of this promoter located the GH-response element to ALSGAS1, a 9-bp sequence located between nt -633 and -625 of the mouse gene that resembles the consensus sequence for the  $\gamma$ -interferon activated sequence (GAS) (Ooi et al., 1998). The effects of GH on the ALS gene are mediated by the JAK-STAT pathway; the tyrosine kinase JAK2 is recruited to the activated GH receptor complex and phosphorylates signal transducers and activators of transcription (STAT)-5a and STAT-5b (Carter-Su et al., 1996). These STAT-5 isomers then translocate to the nucleus and activate ALS gene transcription by binding to the ALSGAS1 element (Ooi et al., 1998).

Further validation of this mechanism of GH activation of the ALS gene was obtained from studies of the human and sheep ALS genes. Despite limited homology between their proximal sequence, the mouse, sheep, and human genes share complete conservation in sequence and position of the ALSGAS1 element (Rhoads et al., 2000b; Suwanichkul et al., 2000). Sheep and human ALS promoters are also GH-responsive when transfected in liver cells, and this responsiveness is

dependent on the presence of the ALSGAS1 element (Rhoads et al., 2000b; Suwanichkul et al., 2000). Overall, these observations indicate that GH stimulates transcription of the ALS gene by similar mechanisms across species.

Inflammatory cytokine interleukin-1 $\beta$  has the ability to block the GH-dependent induction of ALS and IGF-I mRNA in primary hepatocytes (Wolf et al., 1996; Thissen and Verniers, 1997; Delhanty, 1998). Some of these effects have been attributed to the down-regulation of the GH receptor by IL-1 $\beta$  (Wolf et al., 1996; Thissen and Verniers, 1997). Using ALS as a model of GH-regulated gene transcription, IL-1 $\beta$  was shown also to interfere with the activation of STAT-5 (Boisclair et al., 2000). Interleukin-1 $\beta$  exerts this interference by inducing expression of the intracellular suppressor of cytokine signaling (SOCS)-3, an inhibitor of the JAK-STAT pathway. This mechanism may be relevant to other GH-resistant states.

### *Functional Role of ALS*

In most adult animals, serum IGF reach levels that are ~1,000-fold that of insulin. The ALS contributes to the development of this large reservoir by extending the half-lives of IGF from 10 min when in free form to over 12 h when in ternary complexes (Guler et al., 1989; Zapf et al., 1995). In the context of such a large reservoir of bioactive IGF, a second important role of ALS must be the prevention of their nonspecific metabolic effects (Zapf et al., 1995). They occur because free IGF and IGFBP:IGF complexes readily traverse capillary endothelia and activate the insulin receptor. Incorporation of IGF into ternary complexes completely blocks these insulin-like effects (Zapf et al., 1995). These observations imply that mechanisms must exist to release IGF from ternary complexes for their actions on target cells. Proteolytic attack of IGFBP-3 and interactions of the ternary complex with proteoglycans have been shown to release IGF (Baxter, 1990b; Lee and Rechler, 1996). It is also possible that much of the released IGF is the product of the equilibration between the ternary complex and its individual components in serum.

These roles of ALS have been inferred mostly from short-term studies of GH-deficient animals (Zapf et al., 1989; Gargosky et al., 1994). In these animals, the concentration of all the components of the ternary complex are decreased, making it difficult to delineate the roles of ALS from those of IGF-I and IGFBP-3. Moreover, longitudinal studies covering the entire life of GH-deficient animals are usually not feasible. For these reasons, the generation of an ALS-null mouse model, in which ALS and ternary complexes are absent, is an important development (Ueki et al., 2000). Compared with their wild-type counterparts, null ALS mice have reductions in serum IGF-I and IGFBP-3 of 62 and 88%, respectively. These changes occurred in the absence of any reduction in the synthesis of IGF-I or IGFBP-3, indicating that ALS is absolutely necessary for their

accumulation in serum. These findings suggest that without ALS, induction of IGF-I and IGFBP-3 synthesis after birth would only cause a modest increase in their plasma concentrations (Albiston and Herington, 1992; Kikuchi et al., 1992).

Under normal circumstances, ALS is usually not considered to play a role in regulating serum IGF because it circulates in large excess over the concentrations of IGF and IGFBP-3. This notion needs to be reconsidered in view of the low association constant of ALS for the binary complexes of IGFBP-3 and IGF (Holman and Baxter, 1996). Mice with a single null ALS allele provide an example of this phenomenon. They secrete less ALS and have significant reductions in serum IGF-I and IGFBP-3 (Ueki et al., 2000). Another example is provided by GH treatment of normal animals. Higher concentration of serum IGF-I likely represents the combined effect of increased hepatic synthesis of IGF-I and ALS, whereas higher concentration of IGFBP-3 and IGFBP-5 must reflect primarily stabilization by ALS (Cohick et al., 1992; Powell et al., 1999).

Despite these disturbances in the circulating IGF system, null ALS animals suffered only a 13% growth deficit by adulthood. This modest effect is surprising given the central role postulated for plasma IGF-I in regulating postnatal growth (Etherton and Bauman, 1998). However, it is consistent with the observation that abrogation of IGF-I synthesis only in liver, which results in reduction in plasma IGF-I similar to that of the null ALS mice, does not alter postnatal growth (Sjogren et al., 1999; Yakar et al., 1999). A modified somatomedin hypothesis that would accommodate these findings is one in which the primary function of liver is to supply the IGF-I needed to respond to various challenges such as GH treatment. In this model, ALS plays a critical role by capturing liver-derived IGF-I into long-lived ternary complexes. This hypothesis can now be tested using null ALS mice.

The null ALS mice have normal concentrations of plasma glucose, NEFA, and insulin, even though ternary complexes cannot form. This is in contrast to the chronic hypoglycemia and hypoinsulinemia in humans suffering from non-islet tumor-induced hypoglycemia, a condition associated with a depression of ternary complex formation and high concentration of circulating IGF-II (Baxter et al., 1995). This discrepancy is explained by the near absence, in mice, of serum IGF-II (Wolf et al., 1994), a much more potent insulin receptor agonist than IGF-I (Frasca et al., 1999). In mammals such as humans, ruminants, and pigs, with high concentration of serum IGF-II, the presence of ALS and formation of ternary complex are probably essential for containing the metabolic effects of IGF-II.

### **Implications**

Plasma insulin-like growth factor-I and -II play critical roles in the regulation of productive functions such as reproduction, growth, and lactation. However, we

still have an incomplete understanding of factors regulating their concentrations and their access to target tissues. One factor known to regulate both of these processes is the acid-labile subunit. Despite this important role, the acid-labile subunit has generally not been considered when studying changes in plasma insulin-like growth factors that occur in domestic animals during development, nutrition, and physiological states. This omission must be corrected before animal scientists can understand and take advantage of the insulin-like growth factor system to improve efficiency of productive functions.

### Literature Cited

- Aguiar-Oliveira, M. H., M. S. Gill, A. B. E. S. de, M. R. Alcantara, F. Miraki-Moud, C. A. Menezes, A. H. Souza, C. E. Martinelli, F. A. Pereira, R. Salvatori, M. A. Levine, S. M. Shalet, C. Camacho-Hubner, and P. E. Clayton. 1999. Effect of severe growth hormone (GH) deficiency due to a mutation in the GH-releasing hormone receptor on insulin-like growth factors (IGFs), IGF-binding proteins, and ternary complex formation throughout life. *J. Clin. Endocrinol. Metab.* 84:4118–4126.
- Albiston, A. L., and A. C. Herington. 1992. Tissue distribution and regulation of insulin-like growth factor (IGF)-binding protein-3 messenger ribonucleic acid (mRNA) in the rat: Comparison with IGF-I mRNA expression. *Endocrinology* 130:497–502.
- Baker, J., J.-P. Liu, E. J. Robertson, and A. Efstratiadis. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 75:73–82.
- Baxter, R. C. 1990a. Circulating levels and molecular distribution of the acid-labile subunit of the high molecular weight insulin-like growth factor-binding protein complex. *J. Clin. Endocrinol. Metab.* 70:1347–1353.
- Baxter, R. C. 1990b. Glycosaminoglycans inhibit formation of the 140 kDa insulin-like growth factor-binding protein complex. *Biochem. J.* 271:773–777.
- Baxter, R. C. 1994. Insulin-like growth factor binding proteins in the human circulation: A review. *Horm. Res.* 42:140–144.
- Baxter, R. C., and J. Dai. 1994. Purification and characterization of the acid-labile subunit of rat serum insulin-like growth factor binding protein complex. *Endocrinology* 134:848–852.
- Baxter, R. C., S. R. Holman, A. Corbould, S. Stranks, P. J. Ho, and W. Braund. 1995. Regulation of the insulin-like growth factors and their binding proteins by glucocorticoid and growth hormone in nonislet cell tumor hypoglycemia. *J. Clin. Endocrinol. Metab.* 80:2700–2708.
- Baxter, R. C., and J. L. Martin. 1989. Structure of the Mr 140,000 growth hormone-dependent insulin-like growth factor binding protein complex: Determination by reconstitution and affinity-labeling. *Proc. Natl. Acad. Sci. USA* 86:6898–6902.
- Baxter, R. C., J. L. Martin, and V. A. Beniac. 1989. High molecular weight insulin-like growth factor binding protein complex. *J. Biol. Chem.* 264:11843–11848.
- Bereket, A., T. A. Wilson, S. L. Blethen, Y. Sakurai, D. N. Herndon, R. R. Wolfe, and C. H. Lang. 1996. Regulation of the acid-labile subunit of the insulin-like growth factor ternary complex in patients with insulin-dependent diabetes mellitus and severe burns. *Clin. Endocrinol.* 44:525–532.
- Boisclair, Y. R., D. Seto, S. Hsieh, K. R. Hurst, and G. T. Ooi. 1996. Organization and chromosomal localization of the gene encoding the mouse acid labile subunit of the insulin-like growth factor binding complex. *Proc. Natl. Acad. Sci. USA* 93:10028–10033.
- Boisclair, Y. R., J. Wang, J. Shi, K. R. Hurst, and G. T. Ooi. 2000. Role of the suppressor of cytokine signaling-3 (SOCS3) in mediating the inhibitory effects of interleukin-1 $\beta$  on the growth hormone-dependent transcription of the acid-labile subunit gene in liver cells. *J. Biol. Chem.* 275:3841–3847.
- Butler, J. H., and P. D. Gluckman. 1986. Circulating insulin-like growth factor-binding proteins in fetal, neonatal and adult sheep. *J. Endocrinol.* 109:333–338.
- Carter-Su, C., J. Schwartz, and L. S. Smit. 1996. Molecular mechanism of growth hormone action. *Annu. Rev. Physiol.* 58:187–207.
- Chin, E., J. Zhou, J. Dai, R. C. Baxter, and C. A. Bondy. 1994. Cellular localization and regulation of gene expression for components of the insulin-like growth factor ternary binding protein complex. *Endocrinology* 134:2498–2504.
- Cohick, W. S., M. A. McGuire, D. R. Clemmons, and D. E. Bauman. 1992. Regulation of insulin-like growth factor-binding proteins in serum and lymph of lactating cows by somatotropin. *Endocrinology* 130:1508–1514.
- Cwyfan-Hughes, S. C., H. D. Mason, S. Franks, and J. M. P. Holly. 1997. The insulin-like growth factors (IGFs) in follicular fluid are predominantly bound in the ternary complex. *J. Endocrinol.* 155:R1–R4.
- Dai, J., and R. C. Baxter. 1992. Molecular cloning of the acid-labile subunit of the rat insulin-like growth factor binding protein complex. *Biochem. Biophys. Res. Commun.* 188:304–309.
- Dai, J., and R. C. Baxter. 1994. Regulation in vivo of the acid-labile subunit of the rat serum insulin-like growth factor-binding protein complex. *Endocrinology* 135:2335–2341.
- Dai, J., C. D. Scott, and R. C. Baxter. 1994. Regulation of the acid-labile subunit of the insulin-like growth factor complex in cultured rat hepatocytes. *Endocrinology* 135:1066–1072.
- Delhanty, P., and R. C. Baxter. 1996. The cloning and expression of the baboon acid-labile subunit of the insulin-like growth factor binding protein complex. *Biochem. Biophys. Res. Commun.* 227:897–902.
- Delhanty, P. J. D. 1998. Interleukin-1 $\beta$  suppresses growth hormone-induced acid-labile subunit mRNA levels and secretion in primary hepatocytes. *Biochem. Biophys. Res. Commun.* 243:269–272.
- Delhanty, P. J. D., and R. C. Baxter. 1997. Cloning and characterization of the rat gene for the acid-labile subunit of the insulin-like growth factor binding protein complex. *J. Mol. Endocrinol.* 19:267–277.
- Delhanty, P. J. D., and R. C. Baxter. 1998. The regulation of acid-labile subunit gene expression and secretion by cyclic adenosine 3',5'-monophosphate. *Endocrinology* 139:260–265.
- Etherton, T. D., and D. E. Bauman. 1998. Biology of somatotropin in growth and lactation of domestic animals. *Physiol. Rev.* 78:745–761.
- Firth, S. M., U. Ganeshprasad, and R. C. Baxter. 1998. Structural determinants of ligand and cell surface binding of insulin-like growth factor-binding protein-3. *J. Biol. Chem.* 273:2631–2638.
- Frasca, F., G. Pandini, P. Scalia, L. Sciacca, R. Mineo, A. Costantino, I. D. Goldfine, A. Belfiore, and R. Vigneri. 1999. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol. Cell. Biol.* 19:3278–3288.
- Frystyk, J., H. Gronbaek, C. Skjaerbaek, A. Flyvbjerg, H. Orskov, and R. C. Baxter. 1998. Developmental changes in serum levels of free and total insulin-like growth factor I (IGF-I), IGF-binding proteins-1 and -3, and the acid labile subunit in rats. *Endocrinology* 139:4286–4292.
- Fukuda, I., M. Hotta, N. Hizuka, K. Takano, Y. Ishikawa, K. Asakawa-Yasumoto, E. Tagami, and H. Demura. 1999. Decreased serum levels of acid-labile subunit in patients with anorexia nervosa. *J. Clin. Endocrinol. Metab.* 84:2034–2036.
- Gargosky, S. E., P. Tapanainen, and R. G. Rosenfeld. 1994. Administration of growth hormone (GH), but not insulin-like growth factor-I (IGF-I), by continuous infusion can induce the formation of the 150-kilodalton IGF-binding protein-3 complex in GH-deficient rats. *Endocrinology* 134:2267–2276.
- Gluckman, P. D., J. H. Butler, and T. B. Elliott. 1983. The ontogeny of somatotrophic binding sites in ovine hepatic membranes. *Endocrinology* 112:1607–1612.
- Guler, H.-P., J. Zapf, C. Schmid, and E. R. Froesch. 1989. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinol.* 121:753–758.

- Hashimoto, R., M. Ono, H. Fujiwara, N. Higashihashi, M. Yoshida, T. Enjoh-Kimura, and K. Sakano. 1997. Binding sites and binding properties of binary and ternary complexes of insulin-like growth factor-II (IGF-II), IGF-binding protein-3, and acid-labile subunit. *J. Biol. Chem.* 272:27936–27942.
- Holman, S. R., and R. C. Baxter. 1996. Insulin-like growth factor binding protein-3: Factors affecting binary and ternary complex formation. *Growth Reg.* 6:42–47.
- Janosi, J. B., S. M. Firth, J. J. Bond, R. C. Baxter, and P. J. Delhanty. 1999a. N-Linked glycosylation and sialylation of the acid-labile subunit. Role in complex formation with insulin-like growth factor (IGF)-binding protein-3 and the IGFs. *J. Biol. Chem.* 274:5292–5298.
- Janosi, J. B., P. A. Ramsland, M. R. Mott, S. M. Firth, R. C. Baxter, and P. J. Delhanty. 1999b. The acid-labile subunit of the serum insulin-like growth factor-binding protein complexes: Structural determination by molecular modeling and electron microscopy. *J. Biol. Chem.* 274:23328–23332.
- Jones, J. I., and D. R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: Biological actions. *Endocrine. Rev.* 16:3–34.
- Khosravi, M. J., A. Diamandi, J. Mistry, R. G. Krishna, and A. Khare. 1997. Acid-labile subunit of human insulin-like growth factor-binding protein complex: Measurement, molecular, and clinical evaluation. *J. Clin. Endocrinol. Metab.* 82:3944–3951.
- Kikuchi, K., D. P. Bichell, and P. Rotwein. 1992. Chromatin changes accompany the developmental activation of insulin-like growth factor I gene transcription. *J. Biol. Chem.* 267:21505–21511.
- Labarta, J. I., S. E. Gargosky, D. M. Simpson, P. D. Lee, J. Argente, J. Guevara-Aguirre, and R. G. Rosenfeld. 1997. Immunoblot studies of the acid-labile subunit (ALS) in biological fluids, normal human serum and in children with GH deficiency and GH receptor deficiency before and after long-term therapy with GH or IGF-I respectively. *Clin. Endocrinol.* 47:657–666.
- Lang, C. H., J. Fan, R. A. Frost, M. C. Gelato, Y. Sakurai, D. N. Herndon, and R. R. Wolfe. 1996. Regulation of the insulin-like growth factor system by insulin in burn patients. *J. Clin. Endocrinol. Metab.* 81:2474–2480.
- Lang, C. H., X. Liu, G. J. Nystrom, and R. A. Frost. 2000. Acute response of IGF-I and IGF binding proteins induced by thermal injury. *Am. J. Physiol.* 278:E1087–1096.
- Lee, C. Y., and M. M. Rechler. 1996. Proteolysis of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in 150-kilodalton IGFBP complexes by a cation-dependent protease activity in adult rat serum promotes the release of bound IGF-I. *Endocrinology* 137:2051–2058.
- Leong, S. R., R. C. Baxter, T. Camerato, J. Dai, and W. I. Wood. 1992. Structure and functional expression of the acid-labile subunit of the insulin-like growth factor binding protein complex. *Mol. Endocrinol.* 6:870–876.
- Lewitt, M. S., F. P. Scott, N. M. Clarke, and R. C. Baxter. 1995. Developmental regulation of circulating insulin-like growth factor-binding proteins in normal pregnancies and in pre-eclampsia. *Prog. Growth Factor Res.* 6:475–480.
- Liu, J.-P., J. Baker, A. S. Perkins, E. J. Robertson, and A. Efstratiadis. 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I (*Igf-1*) and type 1 IGF receptor (*Igf1r*). *Cell* 75:59–72.
- Louvi, A., D. Accili, and A. Efstratiadis. 1997. Growth-promoting interaction of IGF-II with the insulin receptor during mouse embryonic development. *Dev. Biol.* 189:33–48.
- Mohan, S., C. Libanati, C. Dony, K. Lang, N. Srinivasan, and D. J. Baylink. 1995. Development, validation, and application of a radioimmunoassay for insulin-like growth factor binding protein-5 in human serum and other biological fluids. *J. Clin. Endocrinol. Metab.* 80:2638–2645.
- Moller, S., A. Juul, U. Becker, and J. H. Henriksen. 2000. The acid-labile subunit of the ternary insulin-like growth factor complex in cirrhosis: relation to liver dysfunction. *J. Hepatol.* 32:441–446.
- Olivecrona, H., A. Hilding, C. Ekstrom, H. Barle, B. Nyberg, C. Moller, P. J. Delhanty, R. C. Baxter, B. Angelin, J. Ekstrom, and M. Tally. 1999. Acute and short-term effects of growth hormone on insulin-like growth factors and their binding proteins: Serum levels and hepatic messenger ribonucleic acid responses in humans. *J. Clin. Endocrinol. Metab.* 84:553–560.
- Ooi, G. T., and Y. R. Boisclair. 1999. Molecular biology of the insulin-like growth factor binding proteins. In: R. Rosenfeld and J. Roberts (ed.) *Contemporary Endocrinology: The IGF System*. p 111–139. Humana Press, Totowa, NJ.
- Ooi, G. T., F. J. Cohen, L. Y.-H. Tseng, M. M. Rechler, and Y. R. Boisclair. 1997. Growth hormone stimulates transcription of the gene encoding the acid-labile subunit (ALS) of the circulating insulin-like growth factor-binding protein complex and ALS promoter activity in rat liver. *Mol. Endocrinol.* 11:997–1007.
- Ooi, G. T., K. R. Hurst, M. N. Poy, M. M. Rechler, and Y. R. Boisclair. 1998. Binding of STAT5a and STAT5b to a single element resembling a  $\gamma$ -interferon activated sequence mediates the growth hormone induction of the mouse acid-labile subunit promoter in liver cells. *Mol. Endocrinol.* 12:675–687.
- Oster, M. H., N. Levin, P. J. Fielder, I. C. Robinson, R. C. Baxter, and M. J. Cronin. 1996. Developmental differences in the IGF-I system response to severe and chronic calorie malnutrition. *Am. J. Physiol.* 270:E646–653.
- Powell, D. R., S. K. Durham, E. D. Brewer, J. W. Frane, S. L. Watkins, R. J. Hogg, and S. Mohan. 1999. Effects of chronic renal failure and growth hormone on serum levels of insulin-like growth factor-binding protein-4 (IGFBP-4) and IGFBP-5 in children: a report of the Southwest Pediatric Nephrology Study Group. *J. Clin. Endocrinol. Metab.* 84:596–601.
- Rechler, M. M. 1993. Insulin-like growth factor binding proteins. *Vitam. Horm.* 47:1–114.
- Rhoads, R. P., P. L. Greenwood, A. W. Bell, and Y. R. Boisclair. 2000a. Nutritional regulation of the genes encoding the acid labile subunit and other components of the circulating insulin-like growth factor system in the sheep. *J. Anim. Sci.* 78:2681–2689.
- Rhoads, R. P., P. L. Greenwood, A. W. Bell, and Y. R. Boisclair. 2000b. Organization and regulation of the gene encoding the sheep acid labile subunit of the 150 kDa-binding protein complex. *Endocrinology* 141:1425–1433.
- Sjogren, K., J. L. Liu, K. Blad, S. Skrtic, O. Vidal, V. Wallenius, D. LeRoith, J. Tornell, O. G. Isaksson, J. O. Jansson, and C. Ohlsson. 1999. Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *Proc. Natl. Acad. Sci. USA* 96:7088–7092.
- Stewart, C. E. H., and P. Rotwein. 1996. Growth, differentiation, and survival: Multiple physiological functions for insulin-like growth factors. *Physiol. Rev.* 76:1005–1026.
- Suwanchikul, A., Y. R. Boisclair, R. C. Olney, S. K. Durham, and D. R. Powell. 2000. Conservation of a growth hormone-responsive promoter element in the human and mouse acid-labile subunit genes. *Endocrinology* 141:833–838.
- Thissen, J.-P., J.-M. Ketelslegers, and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. *Endocrine Rev.* 15:80–101.
- Thissen, J.-P., and J. Verniers. 1997. Inhibition by interleukin-1 $\beta$  and tumor necrosis factor- $\alpha$  of the insulin-like growth factor I messenger ribonucleic response to growth hormone in rat hepatocyte primary culture. *Endocrinology* 138:1078–1084.
- Tiong, T. S., and A. C. Herington. 1992. Ontogeny of messenger RNA for the rat growth hormone receptor and serum binding protein. *Mol. Cell. Endocrinol.* 83:133–141.
- Twigg, S. M., and R. C. Baxter. 1998. Insulin-like growth factor (IGF)-binding protein 5 forms an alternative ternary complex with IGFs and the acid-labile subunit. *J. Biol. Chem.* 273:6074–6079.
- Twigg, S. M., M. C. Kiefer, J. Zapf, and R. C. Baxter. 1998. Insulin-like growth factor-binding protein 5 complexes with the acid-labile subunit. Role of the carboxyl-terminal domain. *J. Biol. Chem.* 273:28791–28798.
- Twigg, S. M., M. C. Kiefer, J. Zapf, and R. C. Baxter. 2000. A central domain binding site in insulin-like growth factor binding protein-5 for the acid-labile subunit. *Endocrinology* 141:454–457.

- Twigg, S. M., B. G. Robinson, and R. C. Baxter. 1999. The acid-labile subunit potentiates the inhibitory effect of insulin-like growth factor (IGF) binding protein-5 on IGF-I induced cell proliferation on thyroidal cells *in vitro*. In: Proc. 81st Annu. Mtg. Endocr. Soc., San Diego, CA. p 170.
- Ueki, I., G. T. Ooi, M. L. Tremblay, K. R. Hurst, L. A. Bach, and Y. R. Boisclair. 2000. Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. Proc. Natl. Acad. Sci. USA 97:6868–6873.
- Wandji, S. A., J. E. Gadsby, F. A. Simmen, J. A. Barber, and J. M. Hammond. 2000. Porcine ovarian cells express messenger ribonucleic acids for the acid-labile subunit and insulin-like growth factor binding protein-3 during follicular and luteal phases of the estrous cycle. Endocrinology 141:2638–2647.
- Wolf, E., R. Kramer, W. F. Blum, J. Fll, and G. Brem. 1994. Consequences of postnatally elevated insulin-like growth factor-II in transgenic mice: Endocrine changes and effects on body and organ growth. Endocrinology 135:1877–1886.
- Wolf, M., S. Bohm, M. Brand, and G. Kreymann. 1996. Proinflammatory cytokines interleukin 1 beta and tumor necrosis factor alpha inhibit growth hormone stimulation of insulin-like growth factor I synthesis and growth hormone receptor mRNA levels in cultured rat liver cells. Eur. J. Endocrinol. 135:729–737.
- Xu, S., S. C. Cwyfan-Hughes, J. W. J. Van der Stappen, J. Sansom, J. L. Burton, M. Donnelly, and J. M. P. Holly. 1995. Insulin-like growth factors (IGFs) and IGF-binding proteins in human skin interstitial fluid. J. Clin. Endocrinol. Metab. 80:2940–2945.
- Yakar, S., J. L. Liu, B. Stannard, A. Butler, D. Accili, B. Sauer, and D. LeRoith. 1999. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc. Natl. Acad. Sci. USA 96:7324–7329.
- Zapf, J., C. Hauri, E. Futo, M. Hussain, J. Rutishauser, C. A. Maack, and E. R. Froesch. 1995. Intravenously injected insulin-like growth factor (IGF) I/IGF binding protein-3 complex exerts insulin-like effects in hypophysectomized, but not in normal rats. J. Clin. Invest. 95:179–186.
- Zapf, J., C. Hauri, M. Waldvogel, E. Futo, H. Hasler, K. Binz, H. P. Guler, C. Schmid, and E. R. Froesch. 1989. Recombinant human insulin-like growth factor I induces its own specific carrier protein in hypophysectomized and diabetic rats. Proc. Natl. Acad. Sci. USA 86:3813–3817.