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Title: Factors Affecting Circulating Growth Hormone Binding Protein in Chickens

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Steven L. Davis

Growth hormone binding protein (GHBP) may be an important factor in the regulation of growth as well as an indirect, less invasive way of predicting the status of growth hormone receptors. Several factors (age, nutritional status, sex, and glucocorticoid administration) have been reported to influence circulating growth hormone (GH) levels, growth hormone receptor (GHR) activity and/or GHBP in mammalian species. Therefore, the studies conducted in this research were designed to determine if these factors have any affect on serum GHBP in the young broiler chicken. Serum GHBP activity was expressed as a percent specifically bound ^{125}I-hGH (%SB), as measured by a dextran-coated charcoal assay. Serum GHBP activity was highest (mean %SB= 14.6 ± 1.2) at hatch and decreased linearly (r= -.9516) to 4 wk of age (mean %SB= 4.1 ± 0.6). Sex had no significant affect on serum GHBP activity from hatch to 4 wk of age. Short term

nutrient deprivation (24 h fast) of 4 wk old birds had no significant affect on serum GHBP activity, nor did refeeding. Feeding birds nutrient poor diets (low energy, low protein or low energy and low protein) did not significantly affect serum GHBP activity when compared to birds fed a commercial broiler diet. Pulsatile delivery of cortisone acetate (1, 5 and 10 mg/d/b) had no affect on serum GHBP activity at any dose. These results suggest that serum GHBP activity in the chicken is not affected by many factors which do influence GHBP in mammalian species. The lack of response to nutrient deprivation and cortisone acetate may be a factor related to the age of the birds used in these studies.

Factors Affecting Circulating Growth Hormone Binding Protein in Chickens

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Eric T. Tobar-Dupres

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Redacted for Privacy

Head of Department of Animal Sciences

Redacted for Privacy

Dean of Graduate School

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Typed by Eric T. Tobar-Dupres

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Factors Affecting Circulating Growth Hormone Binding Protein in Chickens

I. Introduction

Circulating proteins that specifically bind growth hormone (GH) were originally reported in the rabbit (Ymer and Herrington, 1985) and human (Baumann et al., 1986). Recent efforts have led to the identification of GHBP in domestic species including: sheep and horses (Davis et al., 1992); pigs (Daughaday et al., 1987a; Mullins and Davis, 1992); and poultry (Vasilatos-Younken et al., 1991; Davis et al., 1992; Anderson et al., 1992).

Sequencing of rabbit (Leung et al., 1987; Spencer et al., 1988) mouse (Smith et al., 1989) and rat GHBP (Mathews et al., 1989) have led to the observation that GHBP is identical to the extracellular portion of the GHR. The relationship between GHBP and GHR was further demonstrated in studies that showed positive correlations between serum GHBP and hepatic GHR activity (Hochberg et al., 1990, Bick et al., 1990, Massa et al., 1990). In addition, GH deficient humans (Laron-type dwarfism) have been reported to have no detectable high affinity GHBP (Baumann et al., 1987b; Daughaday and Trivedi, 1987). It has been suggested that circulating GHBP is the product of the proteolytic cleavage of the GHR (Baumann et al., 1986; Herrington et al., 1986). Others have identified a unique mRNA encoding GHBP in mice and rats (Smith et al., 1989; Baumbach et al., 1989).

With the exception of a short hydrophobic tail, this unique mRNA is identical to mRNA encoding the extracellular domain of the GHR. It has been suggested that this unique GHBP mRNA is an alternately spliced GHR transcript. Each hypothesis would suggest that GHBP and GHR are under the same transcriptional controls. It is, therefore, reasonable to assume that serum GHBP levels may be an acceptable indicator of GHR status in some animals.

Evidence for biologic roles for the GHBP has recently been reported. The growth promoting properties of GH were enhanced by recombinant GHBP in vivo (Clark et al., 1991; Cook et al., 1991). The half-life of GH is extended by the vascular confinement of the GH-GHBP complex (Baumann et al., 1987a). In vitro GHBP has been shown to inhibit GH dependent adipogenesis (Lim et al., 1990). Thus, the presence of circulating GHBP appears to be relevant to growth.

Avian GHBP has been identified and partially characterized (Vasilatos-Younken et al., 1991; Anderson et al., 1992; Davis et al., 1992). However, quantitative evidence of how various factors affect avian GHBP has not been reported. This study was undertaken to determine whether differing physiological states which affect GH, GHR, and or GHBP in mammals also affect serum GHBP activity in the chicken.

II. Literature Review

Molecular Aspects of Growth Hormone

Growth Hormone (GH) is a protein secreted from the somatotroph cells in the anterior caudal lobe of the anterior pituitary gland. It has long been recognized that GH promotes growth in numerous tissues in mammalian species. Growth hormone contains approximately 190 amino acids and is a single-chain polypeptide with 2 intrachain disulfide bonds (Niall et al., 1973). Growth hormone belongs to a family of related proteins including prolactin (PRL, Niall et al., 1973) and placental lactogen (PL), which is also known as chorionic somatomamotropin (Niall et al., 1971 and 1973; Catt et al., 1967). The GH, PRL, and PL genes all code for roughly 200 amino acids and the structural organization of their exons and introns are identical (Parks et al., 1989). Sequence homology and other evolutionary aspects of this family of proteins have been discussed by Wallis et al. (1975) and Parks et. al. (1989). Some evidence suggests that GH and PRL descended from a common ancestral gene and that these genes have been modified to encode PL.

Amino acid sequences of GH have been determined for a number of animals. These include the rat (Seeburb et al., 1977), pig (Seeburg et al., 1983), sheep, horse (Wallis,

1978), whale (Tsubokawa and Kawauchi, 1985), bullfrog (Pan and Chang, 1988), rhesus monkey (Li et al., 1986) and human (Li et al., 1969). Avian pituitary GH has been purified from the chicken (Farmer et al., 1974; Harvey and Scanes, 1977a; Leung et al., 1984); duck; pigeon; turkey (Farmer et al., 1974); and ostrich (Papkoff et al., 1982). Recombinant chicken GH (cGH) has also been produced (Souza et al., 1984). Pituitary-derived cGH preparations have been sequenced (Lai et al., 1984; Leung et al., 1984) and the amino acid composition has also been derived from the nucleotide sequences of avian GH cDNA (Lamb et al., 1988 and Chen, et al., 1988). Scanes et al. (1986) has compared the sequences of rat, bovine, human, and chicken GH. Sequence homology for chicken and rat GH (81%) and chicken and bovine GH (77%) are considerably greater than chicken and human GH (58%).

Growth hormone is heterogenic in mass and charge. Human growth hormone, for example, exists in a 22 kDa form and a 20 kDa form. This difference in mass has been shown to be the result of differential mRNA splicing (DeNoto et al., 1981 and Cooke et al., 1987). GH heterogeneity is also influenced by multiple genes encoding GH variants, potential sites for glycosylation and proteolytic cleavage events co/postranslationaly, dimerization and a tendency to form large aggregates, carrier/binding-protein interactions,

fragment formation (by way of harsh extraction procedures), N-acylation, deamidation and phosphorylation (Baumann, 1987).

GH heterogeneity is common to many classes of vertebrates, including mammals (Hart et al., 1984; Chawla et al., 1983; Liberti et al., 1985), reptiles (Yasuda et al., 1989), fish (Kawauchi et al., 1986) and chicken (Houston and Goddard, 1988). Houston and Goddard (1988) described 10 different protein bands after isoelectrofocusing and immunoblot analysis of pituitary derived chicken GH (cGH). Isoelectric points ranged from 8.45 to 6.0, with 7.85 being the predominant variant. SDS-PAGE and western blot analysis of purified pituitary cGH and pituitary extracts showed four immunoreactive band of 16, 22, 26 and 52 kDa (Aramburo et al., 1990a). Further studies confirmed the existence of 4 GH variants in chicken serum identical to those found in the pituitary gland (Montiel and Aramburo, 1992). Aramburo et al. (1990a), also demonstrated that 2 of three 3 charge variants, isolated by Non-denaturing-PAGE, possessed different bioactivities. One form conveyed lipolytic activity while the other conveyed an antilypolytic response. Heterogeneity of cGH has been associated with differential glycosylation (Berghman et al., 1987) and phosphorylation (Aramburo et al., 1990b).

The cGH most commonly occurs as a monomer of 22-23 kDa with an isoelectric point of 7.5 (Farmer et al., 1974; Harvey and Scanes., 1977; Burke and Papkoff, 1980). It has been shown, through sequencing of its cDNA, that cGH consists of a 191 amino acid protein in addition to a 25 amino acid segment which is not expressed in the mature, circulating chicken GH (Lamb et al., 1988; Zhvirblis et al., 1987).

The Regulation of Growth Hormone Secretion

The release of GH is episodic in mammals, including rats (Tannenbaum and Martin, 1976), cattle (Anfinson et al., 1975), sheep (Davis et al., 1977), primates (Stewart et al., 1981) and birds (Shaw et al., 1987; Vasilatos-Younken and Leach, 1986; Buonomo, et al., 1984; Vasilatos-Younken and Zarkower, 1987). The release of GH is under the control of hypothalamic neuropeptides, of which two (thyrotropin releasing hormone, TRH and growth hormone releasing factor, GRF) are stimulatory and one (somatostatin or somatotropin release inhibiting factor, SRIF) is inhibitory (Harvey et al., 1978; Tannenbaum, 1991). The release of these neuropeptides is under the control of neurotransmitters within the CNS that innervate the hypothalamus and transfer neural information to hormone-secreting hypothalamic neurons (peptidergic neurons). Neurons containing the biogenic amines, dopamine, norepinephrine and epinephrine, are, in

turn, thought to be the primary regulators of hypothalamic neuropeptide release (Terry et al., 1982; Eden et al., 1981). Therefore, control of GH release is ultimately dependant on CNS responses to external and internal stimuli. Self-regulation of GRF and SRIF occurs via ultrashort loop negative feedbacks at the hypothalamic level.

A number of other factors have also been shown to influence GH secretion and have been reviewed by McCann (1988) and Scanes (1986). Growth hormone regulates its own release at the hypothalamic and pituitary level via short loop feedbacks. Insulin-like growth factor-1 also exerts negative feedback on the release of GH (McCann, 1988). Serotonin, prostaglandin (E_1, E_2 and $F_{2\alpha}$), high blood glucose levels and androgens have been implicated in the inhibition of GH release (Scanes, 1986). On the other hand, nutrient deprivation, goitrogen administration, autoimmune thyroiditis and iodothyronine-deficient dwarfism elevate plasma GH concentrations (Scanes, 1986). Hypothalamic defects can cause aberrant GH levels, as is the case in hypopituitarism and acromegaly (McCann, 1988).

A general pattern of GH release has been recognized in birds. Plasma concentrations of GH are high in the early, post-hatch broiler chickens, where they peak (175 ng/mL plasma) at about 2 wk of age. After this peak, GH levels decline and remain relatively low (25 ng/mL plasma). This pattern has been observed in chickens (Harvey et al, 1979;

Vasilatos-Younken and Zarkower, 1987); turkeys (Harvey et al., 1977; Proudman and Wentworth, 1980; Bacon et al., 1989); doves (Scanes and Balthazart, 1981) and geese (Scanes et al., 1979), although the age at which the GH peak was observed varies between avian species and breeds. Evidence also exists that GH secretion is sexually dimorphic in birds (Harvey et al., 1979; Harvey et al., 1977b; Shaw et al., 1987; Bacon et al., 1989).

The Action of Growth Hormone

GH has long been known to have an important role in the regulation of somatic growth. It appears that GH has both direct and indirect actions in the regulation of nutrient utilization. The indirect action is mediated through insulin-like growth factors (IGFs), also known as somatomedins, which are produced in many tissues including the intestine, heart, liver, kidney, lung, brain and fibroblasts (reviewed by Luskey, 1988).

<u>In vivo</u> GH has been reported to promote growth in many tissues including cartilage, adipose tissue, smooth and skeletal muscle and lymphoid organs. (Isaksson, 1985). Exogenous mammalian GH administration has been reported to increase growth rate in many intact vertebrates including mammals, reptiles, amphibians, and fish (Scanes et al., 1986).

In contrast, administration of mammalian GH in normal, intact birds has been ineffective in stimulating growth (Scanes et al., 1984). However, mammalian GH did stimulate growth in hypophysectomized chickens, while control hypophysectomized birds showed a reduction in growth rate (King and Scanes, 1985). Meyers and Peterson (1974) reported that administration of a tryptic digest of bovine GH (bGH) could stimulate growth in chickens. It has been suggested that the tryptic digest of bGH was able to stimulate growth because some especially antigenic bGH component was removed by the digestive treatment (Vasilatos-Younken and Scanes, 1991). In a study conducted by Scanes et al., (1975) bGH caused increased growth of the liver and epiphyseal cartilage and increase spleen RNA content in chickens. Also, ovine GH was able to stimulate plasma levels of free fatty acids in pigeons (John, et al., 1973). A recombinant bGH was able to stimulate a growth response in the chicken after one wk of treatment (Buonomo and Baile, 1988). However, after a two wk treatment, the response was lost and high antibody titres against the recombinant bGH were detected. It has, therefore, been suggested that it is the avian antigenic response that has been responsible for lack of mammalian GH activity in birds. If that theory is correct, only short term treatment and response studies

using mammalian GH would give a response to any foreign GH, as is the case with the mammalian GH studies mentioned above (Vasilatos-Younken and Scanes, 1991).

Surprisingly, administration of pituitary-purified or recombinant cGH has not been shown to substantially or consistently enhance growth in early posthatch birds when administered by injections or continuous infusion (Vasilatos-Younken and Scanes, 1991). Treatment regimes (pulsatile versus continuous cGH administration) and age of birds, late posthatch versus early posthatch, have been reported to play an important role in determining the metabolic response to GH administration (Vasilatos-Younken and Scanes, 1991). In their review, Vasilatos-Younken and Scanes (1991) suggest that pulsatile administration of cGH in late posthatch chickens improved growth rate and feed efficiency and reduced lipogenesis. Continuous administration of cGH, on the other hand, actually reduced growth rate and feed efficiency and had no effect on lipogenesis.

Molecular Aspects of the Growth Hormone Receptor

The GH receptor (GHR) is a membrane-bound protein molecule that, upon binding GH, leads to a variety of biological responses. The mechanism of the signal transduction is not well understood.

The evidence for and the identification of second messengers for GHR is conflicting. Some observations have suggested that GHRs are associated with guanylate cyclase activity, tyrosine kinase activity, protein kinase C and the production of diacylglycerol and inositol triphosphate (Kelly et al., 1991). Crystals of the human GH-GHR complex have revealed that GH binds to the extracellular portion of the GHR in a stoichiometry of one GH molecule to two molecules of GHR (Cunningham et al., 1991; de Vos, et al., 1992). This dimerization process occurs sequentially and is thought to be relevant to the signal transduction mechanism.

Complementary DNA sequences of the GHR from rabbit (Leung et al., 1987), sheep (Adams et al., 1990), mouse (Smith et al., 1989), rat (Baumbach et al., 1989; Mathews et al., 1989), human (Leung et al., 1987), and cow (Hauser et al, 1990) encode a protein with a calculated molecular weight (MW) of approximately 70 kD. However, analysis of mammalian GHRs identified by western blots and SDS-PAGE indicate the existence of MW variants ranging from 130 kD to 50 kD (Hocquette et al., 1990; Leung et al., 1987; Spencer et al., 1988 and Yamada and Donner, 1984). According to Hocquette et al. (1990) and Yamada and Donner (1984) discrepancy in apparent MW of GHR is associated with glycosylation and the binding of ubiquitin and limited proteolysis. The MW heterogeneity of the GHR has also been

associated with multiple subtypes of the GHR (Barnard et al., 1985) and possible associations with other membrane-bound proteins (Taga et al., 1989). The GHR contains an extracellular domain of 242 amino acids, a single transmembrane-spanning domain of 24 amino acids, and a cytoplasmic domain of 350 amino acids.

The chicken GHR (cGHR) has also been cloned, but it shares only a 58% overall homology with reported sequences of mammalian GHR (Burnside et al., 1991). Chicken GHR cDNA encoded for a mature peptide of 592 amino acids with a predicted MW close to that reported for mammalian GHR. Burnside et al. (1991) found that cGHR was expressed in many tissue types, including liver; skin; heart; lung; bursa; kidney; muscle; cerebellum; cerebrum; testes; and adrenals.

The Regulation of Growth Hormone Receptors

The biological response to a given amount of GH is dependant on the number of membrane bound GHR (Landron et al., 1989; and Straus and Takemoto, 1990). Thus, receptor turnover may play an important role in the regulation of the tissue responsiveness to GH. The half-life of membrane bound GHR is about 30 min (Gorin et al., 1984). A higher proportion of GHR are localized within the endosomal compartment of hepatic cells than are associated

with hepatic plasma membrane (Picard and Postel-Vinay, 1984 and Hocquette et al., 1989), suggesting that GHR are internalized. More direct evidence of receptor internalization has been reported as well (Roupas and Herrington, 1988). Treatment of adipocytes with cycloheximide or puromycin (inhibitors of protein synthesis) decreased GH binding activity with increasing exposure to either inhibitor (Gorin and Goodman, 1985), suggesting that GHR are not recycled as is typically observed in receptor-mediated endocytosis (Brown et al., 1983). Therefore, mechanisms involved in the de novo protein synthesis of GHR may play an important role in GHR capacity and the type of response GH target cells produce.

Many factors have been implicated in the regulation of GHR. Nutritional status is important to membrane-bound GHR capacity. Several days of fasting in rats resulted in over a 50% decrease in the number of hepatic GHR and refeeding restored GHR numbers (Postel-Vinay et al., 1982; Baxter et al., 1981). Addition of glucose prevented a decrease in GH binding activity observed in glucose-poor media cultures of rat hepatocytes (Niimi et al., 1991). Levels of circulating GH are also implicated in the regulation of its own receptor. Adipose cells from hypopsectomized rats had low GH binding activity that was increased 2-fold by treatment with bovine GH (Grichting and Goodman, 1986).

In vivo studies have complicated the picture of GHR regulation by GH. Apparently, the pattern of exogenous GH administration is an important factor in the determining GH-related responses in the turkey (Vasilatos-Younken et al, 1988; Cravener et al., 1990). Thus, continuous and pulsatile administration of GH in birds produce different responses that may relate directly or indirectly to the regulation of GHR. Administration of GH caused an induction in hepatic GHR in pigs (Ambler et al., 1990) and rats (Baxter and Zaltsman, 1984). Other factors that have been reported to affect GHR response include estrogen; insulin; sex; age; pregnancy; and dwarfism (see Kelly et al., 1991 for review).

Few studies have examined the regulation of the avian GHR. The ontogeny of hepatic GHR activity in chickens has been examined by Vanderpooten et al. (1991). Relatively high hepatic GHR activity was found in chicken embryos and adult chickens (2 yr old). A dramatic decrease in GHR activity was observed at hatch and relatively low GHR activity was maintained through 8 wk of age. Post-hatch ontogeny of hepatic GHR activity in turkeys remained relatively low from 2 wk of age to 15 wk of age, while older turkeys (24 wk of age) showed much higher GHR activity (Vasilatos-Younken et al., 1990). A recent study found that chicken hepatic GHR mRNA transcription was developmentally regulated in such a way that low levels were transcribed in

young birds, while transcription increased significantly in birds which were 6 to 8 wk of age (Oldham et al., 1992).

The effect of sex and endogenous plasma GH on turkey GHR activity was examined by Vasilatos-Younken et al. (1990) as well. Sexual dimorphism was significant only in adult turkeys, where females exhibited more GHR binding activity that males. Correlations between plasma GH and GHR binding activity were significant only in adult turkeys (r=-.55). Leung et al. (1986) gave daily injections of GH to chickens and observed down-regulation of GHR binding activity as well. Correlations between low GHR binding activity and dwarfism (Vanderpooten et al., 1991) have been observed. In addition, a restriction fragment length polymorphism was found in sex-linked dwarf chicken GHR DNA (Burnside et al., 1991), and it was reasoned that this deletion was responsible for biologically defective GHR.

The Identification and Characterization of Circulating Growth Hormone Binding Proteins

Observations of a GH plasma binding protein was considered as early as the 1960's (Hadden and Prout, 1964; Collipp et al., 1964). These reports were dismissed as artifacts of protein purification/iodination procedures and went against current dogma of the status of circulating peptide hormones. It was not until the 1980's that a

definitive GHBP was reported in the rabbit (Ymer and Herrington, 1985) and human by two independent researchers (Baumann, et al., 1986; Herrington et al., 1986). Researchers have since discovered GHBP in mice (Sadeghi et al., 1990; Cramer et al., 1992); poultry (Vasilatos-Younken et al., 1991; Davis et al., 1992); pigs (Daughaday et al., 1987b; Mullins and Davis, 1992; Ambler et al., 1992); rats (Bick et al., 1990; Massa et al., 1990; Amit et al., 1990; Tiong and Herrington, 1991a); cattle; sheep and horses (Davis et al., 1992); and dogs (Daughaday et al., 1987a).

Baumann and Shaw (1990) observed two circulating human forms of GHBP, one of low affinity (K_a, 10^5 M^{-1}), with a MW of 100 kD, a maximum binding capacity (B_{max}) of 15 mg hGH/L plasma and an isoelectric point (pI) of 7 when complexed to hGH. The other higher affinity (K_a= 3 X 10^8 M^{-1}) GHBP had a MW of 60 kD, a B_{max} of 20 μg hGH/L plasma and a pI of 5 when complexed to hGH. The lower K_a GHBP was shown to carry about 10-15% of the bound circulating GH (4-7% of total circulating GH). Rat GHBP also has two variants, one at 200 kD GHBP and another at 90 kD GHBP (Massa et al., 1990). Although these two rat GHBP were not characterized individually, the collective K_a (2 x 10^8 M^{-1}) was not sexually dimorphic, but the B_{max} was higher in females (6.4 x 10^{-8} mol bGH/L) than in males (1.6 x10^{-8} mol bGH/L).

Rabbit GHBP has been characterized both in recombinant form (Leung et al., 1987) and as a serum purified product (Spencer et al., 1988). Both research groups observed a 51 kD rabbit GHBP with an affinity of 6×10^9 M^{-1} for hGH. Davis et al., (1992) has characterized serum GHBP purified from the pigs and sheep. Porcine GHBP MW was approximated at 50-60 kD with an affinity of 1.55×10^9 L/mol and B_{max} of 1.7×10^{-10} mol/mg GHBP for hGH. Sheep GHBP had an affinity of 2.3×10^8 L/mol and a B_{max} of 6.0×10^{-10} mol/mg GHBP for hGH. Competitive binding experiments have revealed that GHBP specifically binds GH except in one case where ovine PL competed with porcine GH binding to porcine GHBP (Davis et al., 1992). In almost all cases, GH binding to GHBP is reversible as well as temperature and time dependant.

Identification of avian GHBP was first reported by Vasilatos-Younken et al. (1991) and Davis et al. (1992). Vasilatos-Younken's group determined that at least two cGHBP of approximately 70 kD and 30 kD exist in chickens and turkeys through SDS-PAGE, western blotting and autoradiographic techniques using serum and plasma. Only the larger MW cGHBP was shown to be glycosylated (cGHBP of 62 kD after enzymatic hydrolysis). Davis' group have characterized partially purified serum binding proteins from 7 wk old broilers. The affinity was 1.55×10^9 L serum/mol

hGH and 2.07 x 10^7 L serum/mol cGH and the B_{max} was 1.7 x 10^{-10} mol cGHBP/mg hGH and 1.5 x 10^{-10} mol cGHBP/mg protein, respectively.

The Amino Acid Sequence of Growth Hormone Binding Protein

The amino acid sequence of rabbit GHBP purified from serum is identical to the extracellular portion (N-terminal) of the rabbit GHR derived from a cDNA clone (Leung et al., 1987) and purified from hepatic membranes (Spencer et al., 1988). The mouse GHBP (Smith et al., 1989) and rat GHBP (Baumbach et al., 1989; Mathews et al., 1989) have been cloned and the amino acid sequences derived were equivalent to the extracellular portion of the GHR with the exception of a hydrophilic tail (17 amino acids in the rat and 8 amino acids in the mouse) apparently in place of the hydrophobic transmembrane domain of the GHR.

Although work is in progress, the chicken GHBP has not been sequenced. A suspect chicken GHBP cDNA has been cloned and expressed in COS-7 cells, but no definitive evidence has yet been produce to confirm it as a cGHBP (Dr. L. A. Cogburn, personal communications).

The Origin of Growth Hormone Binding Protein

The origin of GHBP has been controversial. Through sequence analysis of purified GHBP and comparisons to the GHR, a relationship of the GHBP to the hydrophilic extracellular domain of the GHR has been recognized. It has been suggested that GHBP is a product of the proteolytic cleavage and extracellular release of the growth hormone receptor (Baumann et al., 1986; Herrington et al., 1986). Many studies have shown positive correlations between levels of GHBP and the number of GHR. Trivedi and Daughaday (1988) have induced human IM-9 cells to shed GHBP from their GHRs in vitro. Thus, it is speculated that GHR and GHBP are similarly regulated.

In mice and rats a unique mRNA has been shown to encode a GHBP (Smith et al., 1989; Baumbach et al., 1989). Sadeghi et al. (1990) has reported that the rat mRNA that encodes GHBP (1.2 kb) contains an alternately spliced exon that codes for the short, hydrophobic, C-terminal tail that takes the place of the transmembrane and intracellular domain of the GHR. Tiong and Herrington (1991b) have also identified a 2.6 kb GHBP mRNA in the rat that is expressed only in hepatic tissue. The 2.6 kb tissue specific mRNA differs from the 1.2 kb mRNA primarily with respect to the length of the 3' untranslated portion of the mRNA. The untranslated 2.6 kb mRNA may help researchers identify a

second GHR-related gene, although the 2.6 kb mRNA could certainly be the result of alternate splicing of the GHR.

The Biologic Role of Circulating Growth Hormone Binding Protein

The role of GHBP is unknown, but evidence exists that suggests some possible functions. The GHBP/GHR is expressed in most tissues (Mathews et al., 1989). The localization of GHBP to specific tissues/subcellular components has led to many hypotheses. The rat GHBP gene was transcribed in all tissues in which GHR mRNA was detected (liver, spleen, heart, muscle, tongue, skin, kidney, adrenal and intestine), although the major source of GHBP was hepatic tissue (Carlsson et al., 1990). Thus, it was proposed that GHBP may represent an additional control mechanism involved in the balance between GH and GHR at a cellularly localized level. Rat GHBP was found distributed throughout the male and female reproductive system and it was suggested that GHBP may exert a direct effect on reproductive function (Lobie et al., 1990). A nuclear GHBP has been reported in rabbit hepatic tissue (Lobie et al., 1991). Therefore, Lobie et al. (1991) proposed that GHBP may be involved in GH action at the nuclear level. Frick and Goodman (1992) observed that GHBP was associated with particulate fractions of rat adipocytes. This observation was unusual because of the hydrophilic properties of GHBP. It was, therefore,

suggested that GHBP may be associated with a membrane bound protein involved in signal generation or transduction.

The growth promoting effects of recombinant human GH (body weight gain, bone growth, liver weight gain and serum IGF-1 stimulation) were enhanced by recombinant human GHBP in a dose dependant manner in hypophysectomized rats (Clark et al., 1991). Cook et al. (1991) got similar results using dwarf rats as a model, but it was noted that although IGF-1 levels were increased, the levels of circulating IFG binding proteins were not induced by the addition of recombinant human GHBP. Consequently, it was proposed that the enhanced growth response to GH induced by GHBP may be related to the ability of the GHBP to alter the ratio between IGF-1 and its binding protein (see De Mellow and Baxter, 1988 for a discussion of IGF binding proteins).

Baumann et al. (1987a) used a partially purified human GHBP preparation and ^{125}I-hGH to test the metabolic clearance rate, distribution volumes and degradation of hGH as affected by GHBP in mice. It was concluded that GHBP was associated with the confinement of hGH to the vascular compartment. It was reasoned that, due to its large molecular size, complexed hGH was prevented from being filtered and excreted by the kidney and, therefore, was protected from degradation, thus extending its half-life. However, this proposed role of GHBP has been discounted by some reports (Holl et al., 1991 and Paul et al., 1991).

The GH-dependent stimulation of adipogenesis was inhibited by serum purified human GHBP *in vitro* (Lim et al., 1990). Lim and coworkers also found that human GHBP was able to block ^{125}I-hGH binding to IM-9 lymphocytes in a dose-dependant manner. Thus, it was speculated that GHBP may dampen the biological effect of pulsatile GH by reducing free GH during secretory pulses, with the effect of flattening the biologically active GH secretory profile.

The Ontogeny of Growth Hormone Binding Protein

The ontogeny of GHBP may play an important role in developmental regulation of the response to GH and may be an indicator of the ontogeny of the GHR as well. Human circulating GHBP activity is low at early ages and increase in a linear fashion ($r=0.55$, $n=96$) from a half yr old infant to 40 yr of age (Tar et al., 1990). Human serum from premature infants and adults over 60 yr of age had relatively low GHBP activity compared to full term infants and adults between the ages of 20-46 yr of age, respectively (Daughaday et al., 1987b).

In the rat, serum GHBP activity increases steadily from 1 to 12 wk of age with a 5-10 fold increase in measured GHBP activity (Mulumba et al., 1991). An ontological study in rat from 19 d of gestation to 110 d of age revealed mRNA

expression of GHBP and serum GHBP activity increased steadily up to 40 d of age where adult plateau levels were reached (Tiong and Herrington, 1992). Interestingly, Tiong's group observed that, although reasonable correlations existed between GHBP patterns and GHR mRNA expression, a strong correlation existed between GHBP and IGF-1 mRNA expression. However, Tiong and Herrington (1991b) observed no change in the abundance of 2.6 GHBP mRNA in from fetal age to 80 d old, but the 1.2 kb GHBP mRNA increased with age during this same developmental period.

The ontogeny of GHBP in chickens has recently come under scrutiny. Only qualitative information (via SD-PAGE and western blotting of plasma proteins and visualization using autoradiographic techniques with ^{125}I-rcGH) exists for determining the presence of cGHBP (Anderson et al., 1992). Embryonic appearance of the 70 kD and 30 kD cGHBP (see Vasilatos-Younken et al., 1991) did not occur until d 12 and d 20 of incubation, respectively. Each cGHBP increased progressively from the age at which it was first observed to 1 d post-hatch. Although prominent at hatching, the 70 kD cGHBP was greatly diminished and the 30 kD cGHBP was not evident at 1 and 2 wk post-hatch, but both reappeared at 4 wk post-hatch and remained prominent at 6, 8, 12, and 18 wk post-hatch. Interestingly, circulating GH followed an inverse pattern to the observed patter of cGHBP expression.

Growth Hormone Binding Protein and Sex

In the human, no sex differences have been observed from infants to adults when using gel chromatography to measure serum GHBP (Tar et al., 1990 and Snow et al., 1990). However, techniques employing immunoprecipitation of GHBP and ^{125}I-hGH binding to GHBP were used together with corrections made for endogenous hGH measured by radioimmunoassay to give a more reliable estimate of human GHBP capacity. Using this methodology, Barnard et al. (1989) observed a sex related difference in GHBP capacity in adult humans (female= 804 pmol/L; males= 505 pmol/L; $P<0.02$). Rats have been reported to have sex related differences in serum GHBP activity, but these difference were not significant from 1 to 3 wk of age (Mulumba et al., 1991). Female rats had significantly higher serum GHBP activity at 6 ($P<0.01$) and 12 ($P<.005$) wk of age. Serum from adult rats also showed significant sexually dimorphic serum GHBP activity (Massa et al., 1990 and Amit et al., 1990). However no sex differences were observed in the abundance of the tissue specific 2.6 kb GHBP mRNA in rats (Tiong and Herrington, 1991b). No sex effect on GHBP has been reported for any avian species.

Growth Hormone Binding Protein and Nutritional Status

Few studies have reported the effects of nutritional status on circulating growth hormone binding protein. Rats fasted for 3 d had significantly lower serum GHBP ($P<0.001$) and upon refeeding serum GHBP activity returned to control levels (Mulumba et al., 1991). The high affinity human GHBP which is elevated in adipose children (Silbergeld et al., 1989) was decreased after a 6 wk weight reduction program (mean weight lost= 7.7 kg) and weight loss correlated with high affinity GHBP reduction ($r= 0.37$, $P<0.05$; Holl et al., 1992). However, the low affinity human GHBP increased significantly ($P<0.005$) after the weight reduction program. No cGHBP studies related to nutritional status have been reported.

Growth Hormone Binding Protein and Stature

Perhaps the best example of the relationship between the GHBP and the GHR can be observed as the result of a growth hormone deficiency or biologically inactive GHR. The short stature that is the phenotypic expression of Laron-type dwarfism (LTD) is also characterized by low levels of IGF and normal to high levels of GH (Laron et al., 1966). There is evidence of a defective GHR in LTD patients (Eshet et al., 1984). The high affinity serum GHBP in humans is absent in some LTD patients (Baumann et al., 1987b; Daughaday and Trivedi, 1987).

The LTD phenotype has also been associated with variable levels of high affinity serum GHBP (Rosenbloom et al., 1990). However, the LTD phenotype is heterogenous and the GHR gene shows variations (deletions, point mutations, or no demonstrable abnormality) in the gene encoding the extracellular domain of the GHR (Amselem et al., 1989 and Godowski et al., 1989). Thus the variation in high affinity serum GHBP in LTD patients is not unexpected.

An isolated population of receptor deficient patients in Ecuador (Fielder et al., 1992) and African pygmies (Baumann et al., 1989) had very low level of GHBP and IGF. The Mountain Ok people of Papua New Guinea have characteristic short statue with normal levels of IGF-I and GH, yet the level of serum GHBP is half that of control sera (Baumann et al., 1991). No data on GHBP in dwarf chickens has been reported.

Growth Hormone Binding Protein and Pregnancy

Pregnancy in the rat caused a significant ($P<0.02$) increase in the serum GHBP binding capacity in comparison to rats in estrus, but no significant difference was observed in GHBP activity (Amit et al., 1990). Tiong and Herrington (1991a and 1991b) observed a pregnancy related increase in the abundance of rat 1.2 kb GHR mRNA but no change was seen in the 1.2 kb GHR mRNA. However, it was also observed that

serum GHBP activity was significantly higher in pregnant rats (female vs pregnant, P=0.005; Tiong and Herrington 1991a).

The mouse serum GHBP measured by a radioimmunoassay was found to steadily increase from 5 to 15 d of gestation and was 32-fold higher than non-pregnant mice at 17 d of gestation (Cramer et al., 1992). Sanchez-Jimenez et al. (1990) reported that pregnant mice has significantly more serum GHBP activity than virgin mice and hypophysectomized pregnant mice experienced a reduced level of serum GHBP activity that was not significantly different from non-pregnant mice.

Growth Hormone Binding Protein and Hormones

In rats serum GHBP fluctuated, with a 60 min lag, with naturally occurring GH pulses over a 4 h period (Bick et al., 1990). Continuous delivery of hGH to hypophysectomized rats caused a dose dependant increase in serum GHBP (Bick et al., 1990). In another paper Bick et al. (1991) observed that continuous delivery of human GH precipitated increased serum GHBP in the rat to a greater degree than pulsatile administration.

Daily administration of a recombinant porcine GH over a 12 d period caused elevated pig serum GHBP (P=0.03) levels (Ambler et al., 1992). In another lab, long term treatment (7 wk) with daily injections of recombinant porcine growth

hormone increased serum GHBP levels ($p<0.05$), however periodic treatment of recombinant porcine GH (daily, every second d or every fourth d for two wk) did not cause a significant difference in serum GHBP levels (Mullins and Davis, 1992). Paradoxically, it appears that plasma cGHBP levels have an inverse relationship to GH levels throughout embryonic and post-hatch stages (Anderson et al., 1992).

III. Factors Affecting Circulating Growth Hormone Binding Protein in Chickens[1]

E. T. Tobar-Dupres[2], D. Froman and S. L. Davis[3]

Dept. of Animal Science

Oregon State University

Corvallis, Oregon 97331

Abstract

Growth hormone binding protein (GHBP) may be an important factor in the regulation of growth as well as an indirect, less invasive way of predicting the status of growth hormone receptors. Several factors have been reported to influence GH, GHR, and or GHBP. Therefore, these studies were conducted to test how the factors of age, sex, nutritional status and cortisone acetate, (CA) affected serum levels of chicken GHBP. Serum GHBP activity was highest (mean %SB= 14.6 ± 1.2) at hatch and decreased linearly (r= -.9516) to 4 wk of age (mean %SB= 4.1 ± 0.6). Sex had no significant affect on serum GHBP activity from hatch to 4 wk of age.

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[1]Oregon Agricultural Experimental Station Publication No._____.

[2]Present address: Department of Poultry Science, The Pennsylvania State University, University Park, Pennsylvania 16802.

[3]Corresponding author.

Short term nutrient deprivation (24 h fast) of 4 wk old birds had no significant affect on serum GHBP activity, nor did refeeding. Feeding birds nutrient poor diets (low energy; low protein; or low energy and low protein) did not significantly affect serum GHBP activity when compared to birds fed a commercial broiler diet (23-21% crude protein and 3151-3111 Kcal/Kg ME). Pulsatile delivery of cortisone acetate (1, 5 and 10 mg/d) had no affect on serum GHBP activity at any dose. These results suggest that serum GHBP activity in the chicken is not affected by many factors which do influence GHBP in mammalian species. The lack of response to the treatments used may be a factor related to the age (hatch to 7 wk) of the birds used in these studies.

Key Word: Chicken, Growth Hormone Binding Protein, Growth.

Introduction

Circulating proteins that specifically bind growth hormone (GH) were originally identified in the rabbit (Ymer and Herrington, 1985) and human (Baumann et al., 1986). Recent efforts have led to the identification of GHBP in domestic species (Davis et al., 1992; Daughaday et al., 1987; Mullins and Davis, 1992). The following evidence suggest the importance of growth hormone binding protein (GHBP) as a potential non-invasive indicator of growth hormone receptors (GHR) status .

Sequencing of rabbit (Leung et al., 1987; Spencer et al., 1988) mouse (Smith et al., 1989) and rat GHBP (Mathews et al., 1989) led to the observation that GHBP was identical to the extracellular portion of the GHR. The relationship between GHBP and GHR was further demonstrated in studies that showed positive correlations between serum GHBP and hepatic GHR activity (Hochberg et al., 1990; Bick et al., 1990; Massa et al., 1990). In addition, GH deficient humans (Laron-type dwarfism) have been reported with no detectable high affinity GHBP (Baumann et al., 1987; Daughaday and Trivedi, 1987). It has been suggested that circulating GHBP is the product of the proteolytic cleavage of the GHR (Baumann et al., 1986; Herrington et al., 1986; Leung et al., 1987). However, unique GHBP mRNA in mice and rats are similar to mRNA encoding the extracellular domain of the GHR (Smith et al., 1989; Baumbach et al., 1989).

It has been suggested that this unique GHBP mRNA is an alternately spliced GHR transcript. Thus, each hypothesis suggests that GHBP and GHR are, at least, under the same transcriptional controls. It is, therefore, reasonable to assume that serum GHBP levels may be an acceptable indicator of GHR status in some animals.

The ontogeny of avian growth hormone receptors depict a developmental regulation that confers relatively high levels of GHR activity during embryonic stages and at the initiation of sexual maturity through adulthood, while young birds experience relatively low GHR activity (Vasilatos-Younken et al., 1990.; Vandepooten et al, 1991). Circulating GH profiles appear to have an inverse relationship to the developmental pattern seen for GHR (Vasilatos-Younken et al., 1990; Vasilatos-Younken and Zarkower, 1987; Proudman and Wentworth, 1980), while reported hepatic GHR activity (Vanderpooten et al., 1991) appears to correlated positively with cGHBP (Anderson et al., 1992). Avian GHBP has been identified and partially characterized (Vasilatios-Younken et al., 1991; Davis et al., 1992).

The objective of this study was to measure relative circulating GHBP activity in chickens of various physiologic states.

Materials and Methods

Commercial broiler chickens (Keith Smith Farm, Hot Springs, AK) were feather-sexed at hatch. Birds were fed a starter ration (23% crude protein, CP and 3151 Kcal/Kg metabolizable energy, ME; Table 1) from hatch to 4 wk of age at which time a finisher ration (21% CP and 3111 Kcal/Kg ME; Table 1) was fed through the remainder of each study unless otherwise specified. In each study, birds were maintained in a 24 h light photoperiod and given feed and water ad libitum, unless otherwise specified.

Birds were weighed and wing banded prior to each blood collection. Blood was collected by severing the cutaneous ulnar vein in birds younger than 3 wk of age. In older birds blood was collected from the cutaneous ulnar with 4 mL vacutainer tubes. Blood was drawn from each bird one time only. Blood samples were allowed to clot for 2 h at room temperature. Clots were separated from serum by centrifugation at 2000 x g at 4° C for 10 min. All serum was stored in microfuge tubes between 9 to 12 mo at -20° C until assayed.

Experiment 1. Male and female birds were housed in separate pens, 76 of each sex per pen. Blood samples were collected from 15 birds of each sex at hatch, 1, 2, 3, and 4 wk of age.

Experiment 2. Male birds were randomly assigned to control (n=60) or treatment (n=60) groups. When chickens reached 4 wk of age feed was removed from the treatment group for 24 h. After the fast, birds were fed ad libitum. Blood samples were drawn 24 h after fasting and 12, 24 and 48 h after refeeding.

Experiment 3. Male birds were divided into a control and three treatment groups. Each group contained 65 birds. When the chickens reached 13 d of age, the starter ration was removed from each treatment group and replaced with one of the following: a low protein ration (12% CP and 3154 Kcal/Kg ME; Table 2), low energy ration (21% CP and 2664 Kcal/Kg ME; Table 2), or a low protein-low energy ration (12% CP and 2582 Kcal/Kg ME; Table 2). Blood collection began at 14 d of age and was performed once each wk through 7 wk of age.

Experiment 4. A total of 190 male birds were randomly assigned to a control and three treatment groups. All birds received daily injections in the breast muscle beginning at 5 wk of age and continuing for a wk. Treatment birds were injected once a d with a 1, 5 or 10 mg suspension of cortisone acetate (CA; Sigma Chemical Co., St. Louis, MO, U.S.A.) in 0.5 mL of corn oil. Control birds were injected with 0.5 mL of corn oil alone.

Blood was collected 24 h, 8 d and 2 wk after the first injection was administered. On d when injections were administered, blood was drawn prior to any injection.

DCC Assay. Pituitary human growth hormone (hGH; NIDDK-hGH-I-1, 2.2 IU/mg) was radiolabled with ^{125}I (Amersham Corp., Arlington Heights, IL) using a modified chloramine-T method (Hunter and Greenwood, 1962). Specific activity ranged from 90 to 120 μCi/μg. Free iodine was separated from ^{125}I-hGH by anion exchange chromatography using a Biorad AG1-X8 resin (50-100 mesh). The monomeric ^{125}I-hGH was separated from aggregated ^{125}I-hGH by size exclusion chromatography using a 1 x 50 cm Biogel-P100 (Biorad, Hercules, CA) column eluted at 4° C with PBS-0.5% BSA. The assay described by Davis et al. (1992) was modified to measure ^{125}I-hGH binding activity in chicken serum as follows: Sample binding (SB) tubes were prepared by adding 100 μL of approximately 20 K cpm [diluted with PBS (pH 7.4)] to assay tubes (10 X 75 mm, polypropylene) containing 350 μL assay buffer (PBS containing 0.5% BSA and 75 mM $MgCl_2$) and 50 μL chicken serum. Non-specific binding (NSB) tubes were identical to SB tubes with the exception that 10 μl of 0.1 μg/μL

unlabeled recombinant hGH[4] (in expt. 1 and 2) or pituitary hGH[5] (in expt. 3 and 4) was substituted for 10 μL of assay buffer. Total count (TC) tubes consisted of 400 μL of assay buffer to which 100 μL of ^{125}I-hGH was added. All tubes were vortexed vigorously for 3 s and allowed to incubate for 2 h at 23° C. Bound and unbound ^{125}I-hGH were separated by adding 500 μL of ice cold DCC (0.2% dextran, Sigma; 2% activated charcoal, Sigma; in PBS) to each assay tube. Tubes were vortexed vigorously for 3 s, and then centrifuged at 2000 x g for 20 min at 4° C. Supernatants were carefully removed from TC tubes and the remaining pellet was counted. A 500 μL portion of the supernatant from SB and NSB tubes was withdrawn from SB and NSB tubes and counted. Radioactive emissions were counted for 2 min per tube in a Beckman model 5500 Gamma Counter.

Specific binding was calculated as a % by subtracting the respective NSB from the SB and dividing by the TC [%SB=((SB-NSB)/TC)*100]. All tubes were run in duplicate, and averages were used for data analysis. The %SB is, therefore, a measurement of relative cGHBP activity in these studies. In order to prevent an assay effect from confounding treatment effects, equal numbers of samples from each group and sampling period were divided among the assays necessary to complete all the samples in an experiment.

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[4]Recombinant hGH (Novatropin®) kindly provided by Genentech

[5]Pituitary purified hGH kindly provided by NIDDK.

The last tubes in an assay may incubate for slightly longer than the first tubes. To avoid an assay effect associated with tube placement within an assay, all samples within each assay were randomly assigned their placement by random computer generated numbers. Intraassay variation was measured by running 10 replicates of a pooled serum sample (PSS; blood collected from 10 7-wk-old broilers at slaughter) per assay. The PSS was used in duplicate to measure the interassay variation and as an internal assay control in all assays. Within any one assay, serum from individual samples within the same treatment group were pooled in order to obtain that groups' respective NSB value. Although rare, occasionally very low to negative values were obtained for samples. Most of these occurred in one particular assay (the first assay we ran for experiment 1) and so that entire assay was left out of the results. We felt that any values $\leq$ 1% SB should be discarded and not be used for any calculations. Low %SB almost always occurred along with very high NSB for the respective sample, indicating that the problem was one inherent in the way in which NSB's were obtained.

The inability of chicken prolactin[6] (cPRL) to inhibit

[6]Chicken PRL kindly provided by Dr. A. F. Parlow, Harbor-UCLA Medical Center, Torrance, CA.

125I-hGH binding to cGHBP in concentrations up to 5 μg cPRL/50 μL pooled chicken serum, confirms the specificity of the assay. The intraassay and interassay coefficient of variation was 5.9% (n=5) and 15.0% (n=6), respectively.

Statistical Analysis. Data were analyzed by single classification ANOVA using the General Linear Models procedure of the Statistical Analysis System (SAS Institute, 1982).

Results and Discussion

Experiment 1. No significant difference (P>.05) in cGHBP binding activity was detected between male and female birds between hatch and 4 wk of age (Table 3). An interaction between sex and age was evident in a comparison of body weights at hatch (P>.05) and 4 wk of age (P<.0001). Therefore, the difference in growth rate between sexes appears to be independent of any action of biologically active cGHBP in young broilers. The cGHBP activity in chickens was highest (mean %SB= 14.6 ± 1.2) at hatch and then decreased linearly (r= -.9516) to (mean %SB= 4.1 ± 0.6) 4 wk of age (Figure 1). Of the 107 samples assayed, 19 gave %SB ≤ 1% and thus were eliminated from this study.

Several reports have indicated that sex is not a factor that influences GHBP activity (Silbergeld et al., 1989; Snow et al., 1990; Baumann et al., 1989; Mullins and Davis, 1992). However, females have been reported with higher GHBP activity than their male counterparts in rats (Mulumba et al, 1991; Sadeghi et al., 1990) and humans (Barnard et al., 1989; Massa et al., 1991). Where sexually dimorphic GHBP activity has been observed, scatchard analysis has revealed these differences to be a function of serum GHBP binding capacity as opposed to GHBP affinity (Massa et al., 1990; Amit et al., 1990). Massa et al. (1990) also showed that

the high levels of GHBP found in females and the low levels in males correlated positively with respective hepatic GHR binding levels.

Secretion of GH is sexually dimorphic in the chicken (Johnson, 1988; Harvey et al., 1979) and turkey (Bacon et al., 1989; Harvey et al., 1977; Shaw et al., 1987) but GH binding activity of hepatic tissue was not significantly different between male and female turkeys until sexual maturity had been reached (Vasilatos-Younken et al., 1990). GH secretion in chickens increases from hatch until about 4 wk of age, at which point plasma GH levels decline (Vasilatos-Younken and Zarkower, 1987; Harvey et al., 1979). The same pattern has been shown in turkeys, with a GH peak from 4 to 7 wk of age (Harvey et al., 1977). In the chicken, hepatic GHR binding activity was low from hatch to 8 wk of age when compared to embryo and adult (2 yr old) birds (Vanderpooten et al., 1991) Similar findings were reported for the turkey (Vasilatos-Younken et al., 1990). Vanderpooten et al. (1991) also found that differences between dwarf and normal chicken hepatic GHR binding activity were significant in embryo and adult birds, but less so in young birds. In an initial study (E. Tobar-Dupres, unpublished data), a line of dwarf leghorns had very low to no detectable serum GHBP activity (n= 4, range= 0 to 2.5 %SB), while normal leghorns of the same age (2 yr old) had an average %SB = 11.0 (n=4, range= 6.4 to 19.9).

This apparent lack of serum GHBP in dwarf birds may be the result of a deletion of the GHR gene (Burnside et al., 1991) and may be comparable to the Laron Type Dwarfism in humans. These data suggest that the lack of a sex effect on cGHBP may be a factor of the age of the birds. Hepatic GHR activity observed in chickens correlate with our findings as well.

In addition, a qualitative study has described two variants of cGHBP in an ontological study (Anderson et al., 1992). Embryonic appearance of the 70 kD and 30 kD cGHBP did not occur until d 12 and d 20 of incubation, respectively. Each cGHBP increased progressively from the age at which it was first observed to 1 d post-hatch. Although prominent at hatching, the 70 kD cGHBP was greatly diminished and the 30 kD cGHBP was not evident at 1 and 2 wk post-hatch, but both reappeared at 4 wk post-hatch and remained prominent at 6, 8, 12, and 18 wk post-hatch. These findings appear to correlate with our data.

Experiment 2 and 3. Neither fasting nor subsequent refeeding caused a detectable difference ($P>.05$) in cGHBP activity at any sampling point (Table 4). In addition, no difference ($P>.05$) in cGHBP activity was observed in response to feeding broiler chickens suboptimal diets (Table 5). However, ad libitum dietary treatments had a profound effect on protein intake (51% of control CP consumption) and

a moderate effect on energy consumption (81% of control ME consumption) after 15 d of being fed nutrient poor diets. Therefore, cGHBP activity appears to be independent of nutritional status under the conditions of these studies. Out of 114 samples assayed in experiment 2 and 233 samples assayed in experiment 3, only 10 and 9 samples had %SB $\leq$ 1% in experiment 2 and 3, respectively.

Serum GHBP is low in humans under poor nutritional conditions (Hizuka, 1991). Similarly, 50% reduction in serum GHBP was seen in rats after a 3 d fast and normal levels of GHBP were restored upon refeeding (Mulumba et al., 1991). This reduction in GHBP was also shown to correlate positively with hepatic GH receptor binding capacity. Fasting in the rat has also been shown to diminish hepatic GHR and GHBP mRNA levels (Straus and Takemoto, 1990). In vitro glucose was shown to counteract the downregulation of GH binding sites in primary cultured rat hepatocytes (Niimi et al., 1991). Based on these studies, we had expected fasting and protein/energy deprivation to modulate cGHBP activity.

Experiment 4. As shown in Table 6, CA administration had no effect (P>.05) on cGHBP activity at any dose in samples taken 24 h after the last CA injection. Blood serum drawn at other periods, therefore, were not analyzed.

In contrast, CA was very effective ($P<.0001$) at inhibiting normal body weight gain in the group given 10 mg CA daily. Controls gained 0.25 ± .01 kg over the 1 wk CA administration interval, whereas birds given 10 mg CA daily gained only 0.14 ± .01 kg. Thus the growth of the CA treated birds was only 56% of the controls.

Based on data obtained from radioimmunoassays (Harvey et al., 1986) basal peripheral corticosterone are approximately 10 ng/mL of avian plasma. Assuming total blood volume to be 7% of body weight, a 1.42 kg (mean weight of all birds on d of first CA treatment) broiler chicken would contain a total of 0.8 μg of circulating corticosterone. Therefore, daily injection of 10 mg CA provided 12,500 times the total amount of the broilers principle glucocorticoid. Even so, such a dosage was without effect ($P>.05$) on cGHBP (Table 6). No samples assayed in this experiment had to be eliminated due to low %SB.

Glucocorticoids have been shown to inhibit body weight gain in chickens (Glick, 1960; Harvey and Scanes, 1979) and rats (Silience and Etherton, 1991). Evidence suggests that glucocorticoids induce GH transcription via binding to glucocorticoid response elements on the GH gene (Rousseau et al., 1987; Evans et al., 1982). Plasma GH levels rise in chickens treated with glucocorticoids (Harvey and Scanes, 1979).

Thus, in addition to the factors of sex, age and diet, we suspected that cGHBP activity would be modulated by glucocorticoids.

In summary, of the factors evaluated, only age was found to affect GHBP in broiler chickens (Table 3). Paradoxically, cGHBP levels fell during a period of rapid growth (Figure 1), were essentially inverse to reported levels of circulating cGH, and were independent of factors (sex, nutrient deprivation and CA administration) that had profound effects on growth in young broiler chicks in these studies. However, serum GHBP activity did appear to correlate with reported GHR activity found in young birds. The role of cGHBP in the growth process of the young broiler is enigmatic. Due to the relatively high levels of cGHBP at hatch and its subsequent decline, an argument could be advanced that its primary role may be played during embryonic development. It is also noteworthy that, in a preliminary analysis, birds beginning 6 wk and up to 2 yr of age had regained much of the cGHBP activity seen in birds a hatch (Tobar-Dupres, unpublished data). Thus, a role for GHBP could also be argued in older birds as well.

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VI. APPENDIX

Table 1. Broiler starter/finisher feed rations.

Ingredients	% in Starter[1]	% in Finisher[2]
Corn	59.00	64.00
Animal Fat	2.00	2.00
Soybean Meal	32.50	27.75
Meat & Bone Meal	5.00	5.00
Monodical (16% Ca, 21 % P)	0.35	0.25
Limestone	0.52	0.40
Salt	0.25	0.25
Trace Mineral Mix - 65	0.05	0.50
Vitamin Premix	0.20	0.20
DL-Methionine	0.13	0.10
Amproline	0.05	0.05
Baciform	0.05	0.05
Total	100.00	100.00

[1]Calculated: Crude Protein (CP)= 23%
Metabolizable Energy (ME)= 3151 Kcal/Kg

[2]Calculated: CP= 21%
ME= 3111 Kcal/Kg

Table 2. Low Energy (LE), Low Protein (LP), and LE and LP broiler feed rations.

Ingredients	LE[1]	LP[2]	LELP[3]
	% in Feed		
Barley	30.00	0.00	50.00
Corn	28.00	85.50	30.00
Solka Floc	1.95	1.10	5.60
Soybean Meal 47.5%	27.75	8.00	9.00
Meat & Bone Meal	5.00	1.50	1.50
Monodical (16% Ca, 21 % P)	0.25	1.25	1.25
Limestone	0.40	1.25	1.25
Salt	0.25	0.25	0.25
Trace Mineral Mix - 65	0.05	0.05	0.05
Vitamin Premix 1-75	0.20	0.20	0.20
DL-Methionine 98%	0.10	0.25	0.25
Amproline	0.05	0.05	0.05
L-Lysine 86%	0.00	0.60	0.60
Total	100.00	100.00	100.00

[1]Calculated: Crude Protein (CP)= 21%
Metabolizable Energy (ME)= 2664 Kcal/Kg

[2]Calculated: CP= 12%
ME= 3154 Kcal/Kg

[3]Calculated: CP= 12%
ME= 2582 Kcal/Kg

Table 3. Effect of sex on the level[1] of chicken growth hormone binding protein (cGHBP) in the serum of broiler chickens.

Age[2] (wk)	Sex	Replicates (n)	cGHBP[3] (%SB)
0	M	10	16.1 ± 1.6
	F	10	13.0 ± 1.7
1	M	14	9.8 ± 1.6
	F	6	9.8 ± 2.1
2	M	11	6.8 ± 1.0
	F	7	7.4 ± 1.1
3	M	7	7.6 ± 1.3
	F	7	5.6 ± 1.2
4	M	8	4.0 ± 0.9
	F	8	4.1 ± 0.8

[1]cGHBP level denotes a percentage of ^{125}I-hGH bound to chicken serum protein following removal of unbound ^{125}I-hGH with dextran-coated charcoal.

[2]"0" wk denotes birds at hatch.

[3]Each value denotes a Mean ± SEM.

Table 4. The effect of fasting and refeeding on the level[1] of chicken growth hormone binding protein (CGHBP) in the serum of male broiler chickens[2].

Sampling Period[3] (h)	Group	Replicates (n)	GHBP[4] (%SB)
0	Treatment	6	5.6 ± 1.8
	Control	13	6.8 ± 0.7
12	Treatment	15	5.7 ± 0.6
	Control	15	4.3 ± 0.5
24	Treatment	14	4.5 ± 0.4
	Control	15	4.4 ± 0.5
48	Treatment	14	4.0 ± 0.4
	Control	12	4.9 ± 0.7

[1]cGHBP level denotes a percentage of ^{125}I-hGH bound to chicken serum protein following removal of unbound ^{125}I-hGH with dextran-coated charcoal.

[2]All birds in this study were fasted at 4 wk of age.

[3]The "0" denotes a time point immediately after a 24 h fast and just before refeeding. All other time points denote h after refeeding.

[4]Each value denotes a Mean ± SEM.

Table 5. The effect of a low protein[1](LP), low energy[2](LE), and low protein/low energy[3](LELP) diet compared to a control[4](CTRL) diet on the level[5] of chicken growth hormone binding protein (cGHBP) in the serum of male broiler chickens.

Days on Treatment[6] (d)	Treatment	Chickens (n)	cGHBP[7] (%SB)
1	LE	9	4.03 ± 1.03
	LP	10	6.03 ± 0.84
	LELP	10	4.52 ± 0.78
	CTRL	9	5.08 ± 0.78
7	LE	12	5.63 ± 0.36
	LP	12	5.94 ± 0.47
	LELP	10	5.60 ± 0.85
	CTRL	12	5.99 ± 0.59
14	LE	12	5.66 ± 0.58
	LP	11	5.12 ± 0.34
	LELP	11	5.82 ± 0.87
	CTRL	12	5.69 ± 0.55
21	LE	15	5.93 ± 0.79
	LP	12	5.09 ± 0.59
	LELP	11	6.26 ± 0.87
	CTRL	12	6.75 ± 0.55
28	LE	11	5.34 ± 0.37
	LP	12	6.30 ± 0.59
	LELP	11	5.60 ± 0.64
	CTRL	10	6.36 ± 0.62

[1]12% crude protein.

[2]2664 Kcal/Kg ME.

[3]12% crude protein; 2582 Kcal/Kg ME.

[4]21-23% crude protein; 3151-3111 Kcal/Kg ME.

[5]cGHBP level denotes a percentage of ^{125}I-hGH bound to chicken serum protein followed removal of unbound ^{125}I-hGH with dextran-coated charcoal.

[6]Treatment diets began when birds reached 14 d of age

[7]Each value denotes a Mean ± SEM.

Table 6. Effect of daily cortisone acetate (CA) Administration[1] on the level[2] of chicken growth hormone binding protein (cGHBP) in the serum of male broiler chickens.

Treatment (mg/d)	Replicates (n)	cGHBP[3] (%SB)
None	14	9.6 ± 0.7
1 mg	15	10.1 ± 1.0
5 mg	13	11.4 ± 1.1
10 mg	15	10.5 ± 0.6

[1]Each bird received 0.5 ml of corn oil, with or without CA, per day. Injections were administered in the breast muscle at 24 h intervals for seven consecutive days. Blood samples were collected 24 h after the last CA injection.

[2]cGHBP level denotes a percentage of ^{125}I-hGH bound to chicken serum protein following removal of unbound ^{125}I-hGH with dextran-coated charcoal.

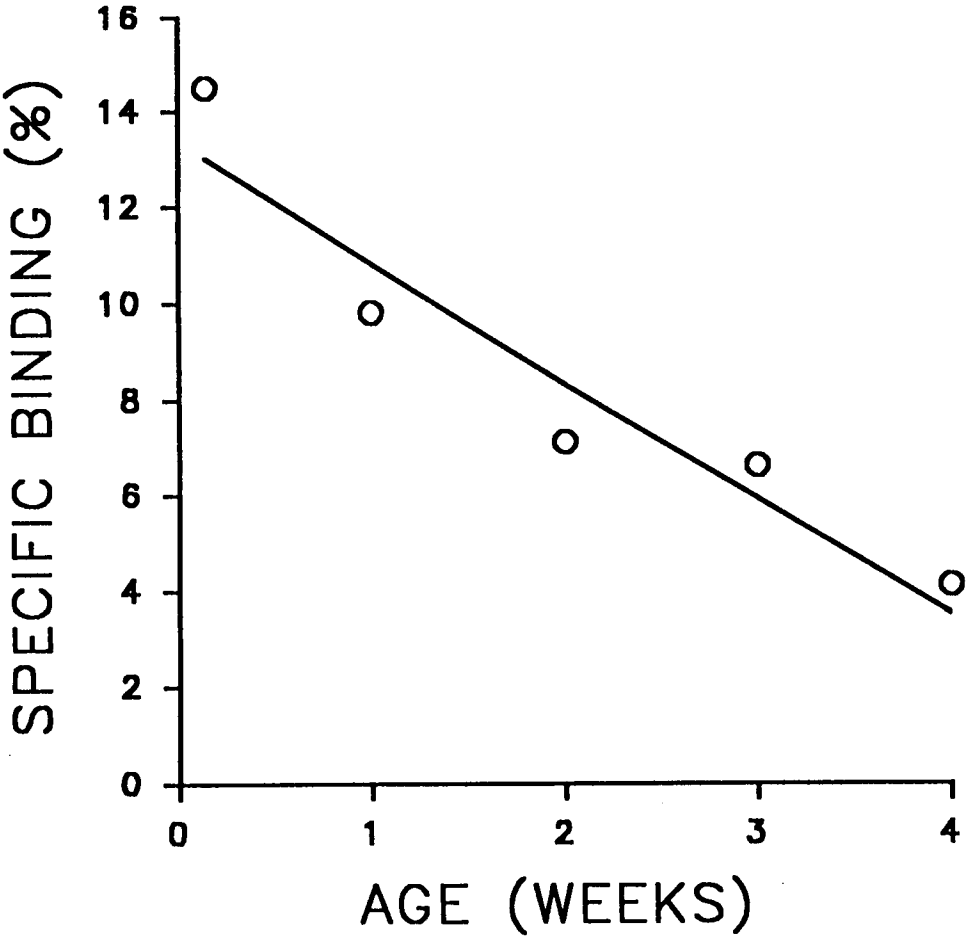
[3]Each value denotes a Mean ± SEM.

Legend to Figure

Figure 1. The specific binding of ^{125}I-hGH to chicken serum growth hormone binding protein as a function of time. Data points represent means. The solid line represents the function:

$$y(x) = 13.3 - 2.46(x),$$

where 13.1 and -2.46 are estimates of the parameters α and β, respectively. The correlation coefficient for the predicted line was -.9516.


SPECIFIC BINDING (%)
16
14
12
10
8
6
4
2
0
0
1
2
3
4
AGE (WEEKS)