Ontogeny of growth hormone receptor gene expression in tissue of growth-selected strains of broiler chickens

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Abstract

The purpose of this study was to determine the relationship between genetic selection for growth traits and tissue expression of the chicken growth hormone receptor (cGHR) gene. Two different populations of broiler chickens were studied. One population consisted of strain (S) 80, selected for 14 generations for high 9-week body weight (BW), and its progenitor, S90 (a 1950's strain). The second population consisted of S21, selected for 10 generations for high 4-week BW and low abdominal fat, and its progenitor S20 (a 1970's strain). Tissue (liver, fat, breast and leg muscle) and blood samples were collected from six birds/strain at 2-week intervals between 1 and 11 weeks of age. An RNase protection assay was developed to measure mRNA levels of full-length cGHR (3.2 and 4.3 kb) transcripts and chicken glyceraldehyde 3-phosphate dehydrogenase (for normalization) in total RNA prepared from tissue. Analysis of the area-under-curve (AUC) was used for strain comparisons of certain developmental profiles (BW, plasma hormones and tissue cGHR mRNA). The BW AUC showed that the growth rates are different ($P<0.05$) among the four strains (S21>S20>S80>S90). Both slow-growing strains (S90 and S80) had a higher

($P<0.05$) plasma GH AUC than the two fast-growing strains (S20 and S21). The plasma T₃ AUC was highest ($P<0.05$) in S90 due to maintenance of higher T₃ levels after 3 weeks of age. At 11 weeks of age, hepatic and plasma GH-binding activities were positively related to growth rate (S21>S20>S80>S90). However, the developmental increase in cGHR mRNA in liver and fat was similar among these different populations of growth-selected broiler chickens. Steady-state levels of cGHR mRNA increased in a developmental manner in the liver (5-fold at 9 weeks of age) and abdominal fat (4.5-fold at 11 weeks of age) of all strains. In contrast, there was no developmental increase or strain difference in cGHR mRNA levels in breast and leg muscle. There is a discrepancy between GH-binding activity in liver and plasma, which is different among strains, and steady-state levels of tissue cGHR mRNA which are similar among strains. These observations suggest that the cGHR is under translational or post-translational regulation which would determine the amount of cGHR protein available for GH binding.

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Introduction

As a potential growth enhancer, biosynthetic growth hormone (GH) has been under commercial development to improve meat quality, productive efficiency and/or milk production in domestic animals. The GH-mediated improvements in productive efficiency of livestock are usually associated with up-regulation of hepatic GH receptor (GHR) (Chung & Etherton 1986, Gluckman & Breier 1988, Sauerwein *et al.* 1991), dose-dependent increases in plasma insulin-like growth factor-I (IGF-I) levels (Etherton *et al.* 1987), and inhibition of lipogenesis

(Magri *et al.* 1990, Wolverson *et al.* 1992, Clarke 1993, Harris *et al.* 1993, Liu *et al.* 1994). In contrast, chronic treatment of broiler chickens with chicken GH (cGH) usually leads to a greater accumulation of body fat (Leung *et al.* 1986, Burke *et al.* 1987, Cogburn *et al.* 1989), down-regulation of hepatic cGHR and a failure to enhance hepatic IGF-I synthesis (Cogburn 1991). These differences in regulation of the somatotrophic axis in domestic birds point to the need for a better understanding of the basic mechanism of GH action in target tissue.

Strains of broiler chickens selected for different traits provide experimental models for study of regulation of

somatic growth. Generally, selection for rapid growth alone results in fatter chickens, while selection for improved feed efficiency as well results in leaner, albeit slightly slower-growing chickens. Fast-growing lines of broiler chickens have lower circulating GH levels than slow-growing lines (Scanes *et al.* 1980, Burke & Marks 1982, Goddard *et al.* 1988, Leenstra *et al.* 1991). Plasma GH concentrations are higher in chickens selected for food conversion (slow-growing) than in chickens selected for body weight (BW) (fast-growing) (Decuyper *et al.* 1991). However, no consistent difference was found in plasma GH concentrations among other lines selected for weight gain, food consumption, or efficiency of food conversion (Tomas *et al.* 1991). There was no significant difference in plasma GH levels in chickens divergently selected for either low or a high abdominal fat content although hepatic GH-binding activity was higher in the fat line than in the lean line (Buyse *et al.* 1994). Hepatic GH-binding activity is also higher in fast-growing chickens than in slow-growing chickens (Decuyper *et al.* 1991). Higher plasma GH concentrations in chickens selected for feed efficiency causes down-regulation of hepatic GH-binding activity (Vanderpooten *et al.* 1993). An apparent inverse relationship between plasma GH levels and expression of cGHR mRNA is also evident from ontogeny studies of commercial broiler chickens (Burnside & Cogburn 1992).

The relationship between growth rate and cGHR mRNA level in target tissues of growth-selected strains has not been studied so far. This paper presents data on hormone profiles, hepatic and serum GH-binding activity, and tissue cGHR mRNA levels in response to genetic selection for different growth traits. The area-under-curve (AUC) was calculated to enable comparisons for strain differences in development profiles (from 1 to 11 weeks of age) of BW, hormones (GH, IGF-I and tri-iodothyronine (T₃)) and cGHR mRNA levels. We also determined if expression of the cGHR mRNA in liver is related to hepatic GH-binding activity (a measure of cGHR protein) and the soluble cGH-binding protein (cGHBP) levels at 11 weeks of age.

Materials and Methods

Animals

Two populations of chickens used in this study were from the strains maintained by the Center for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada. Strain (S) 90 has not been selected since 1955 (the 1950's population). From this parent population, S80 was selected for 14 generations for high 9-week BW and has been unselected since 1974 (Somes 1988, Sabour *et al.* 1992). The BW of S80 chickens is 40% higher at 28 days and about 50% higher at 47 days (Chambers *et al.* 1989), while abdominal fat of S80 birds at 47 days is

2.3-fold higher than that of unselected S90 chickens. The sire (Cornish) population (S20) has not been artificially selected since 1978 (the 1970's population) (Somes 1988). Strain 21, derived from S20, was selected for 10 generations from S20 for high 28-day BW (16% higher than S20) and low abdominal fatness (31% lower at 28 days) of sibs at 47 days of age. Day-old cockerels were shipped by air freight to the University of Delaware and maintained with a ratio of 20 h light:4 h darkness in a controlled-environment room in a heated-battery brooder until 3 weeks of age. At 3 weeks of age, cockerels were transferred to wire cages held in two identical controlled-environment rooms and maintained under the same 20 h light:4 h darkness cycle until conclusion of the experiment. The birds were provided with water and allowed to feed *ad libitum*. A commercial broiler-starter ration was fed until 3 weeks of age and afterwards a grower/finisher ration was fed until 11 weeks of age. Six randomly selected birds from each strain were weighed every week, bled (4 ml sample) and killed for collection of tissue (liver, breast muscle, leg muscle and abdominal fat) every other week. The tissues were immediately immersed in liquid nitrogen and stored at -70°C for isolation of RNA.

Animals were handled and cared for in accordance with the principles and procedures outlined by the University's Animal Care and Use Committee.

RNase protection assay

To validate the RNase protection assay (RPA), increasing amounts of the sense-strand RNA (from 1.56 to 200 pg for the cGHR and 0.0625 to 16 ng for cGAPDH), 20 000 c.p.m. cGHR riboprobe and 20 000 c.p.m. cGAPDH riboprobe were hybridized and digested with RNase T1 (0.5 U/ μl). Protected fragments were separated by electrophoresis in a 6% polyacrylamide/8.3 M urea gel. Following autoradiography, protected fragments were excised and the radioactivity measured in a β -scintillation counter. Under optimized conditions, the radioactivity of the cGHR and chicken glyceraldehyde 3-phosphate dehydrogenase (cGAPDH) protected fragments was linearly related ($P<0.01$) to the amount of sense-strand RNA added (from 1.56 to 200 pg for the cGHR and 0.0625 to 16 ng for cGAPDH) in the hybridization reaction (data not shown). Duplicate RNA samples from four chickens of each strain were assayed at each age. Figure 1 shows the negative and positive RNase controls in the cGHR RPA and duplicate 25 μg samples of total RNA from liver of a 1-week-old chicken. Duplicate standards of 12.5 pg sense-strand cGHR RNA and 500 pg sense-strand cGAPDH RNA, were included in each assay so that data could be pooled from different gels. The ratio of radioactivity of full-length cGHR/cGAPDH in each chicken was first normalized with the sense-strand standards on each gel and then data from all gels were combined for statistical analysis.

Figure 1 RNase protection assay of cGHR and cGAPDH. Twenty-five micrograms total RNA were hybridized to a mixture of cGAPDH and cGHR riboprobes, digested with RNase and protected fragments were separated by electrophoresis on a 6% denaturing polyacrylamide gel. The gel was exposed to film for 16 h. The 227, 177 and 96 bp protected fragments correspond to the cGAPDH, full-length (4.3 and 3.2 kb) and 0.8 kb cGHR transcripts respectively. Lane 1 represents undigested cGHR and cGAPDH riboprobes while lane 2 represents riboprobes digested with RNase. Lanes 3 and 4 represent protected fragments in 25 µg total RNA from a liver sample.

Radioimmunoassay (RIA), radioreceptor assay and GH-binding protein (GHBP) assay

Double-antibody RIA procedures were used to quantitate plasma cGH (Proudman 1984) and cIGF-I (McGuinness & Cogburn 1990) levels. A mixture of ethanol:acetone:acetic acid (60:30:10 by volume) (Enright *et al.* 1989) was used to extract cIGF-I from plasma to eliminate interference of the IGF-I-binding proteins in the RIA. A commercial RIA kit was used to determine plasma T_3 levels (ICN Biomedicals, Inc., Costa Mesa, CA, USA). Hepatic GH-binding activity was determined by GH radioreceptor assay (RRA) (Krishnan *et al.* 1989) which we have slightly modified (Burnside *et al.* 1992, Moellers & Cogburn 1995). Plasma membranes for the hepatic GH-binding assay were prepared from 11-week-old chickens according to the above method and pretreated with or without 3.8 M $MgCl_2$ to remove bound endogenous ligand (Kelly *et al.* 1979). An HPLC method, originally developed for measurement of the human GHBP (Hocquette *et al.* 1990, Tar *et al.* 1990), was used to determine levels of the cGHBP in duplicate 25 µl samples of plasma from 11-week-old birds. Human GH was used as the radioligand because the affinity of hGH for the cGHBP is about two orders of magnitude greater than that

of cGH for the serum cGHBP (Davis *et al.* 1992). The specific GH-binding is calculated as radioactivity of total binding minus nonspecific binding divided by total radioactivity added times 100.

Statistical analysis

This experiment had a factorial design (4×6) with four different genetic strains and six different ages for collection of samples (1, 3, 5, 7, 9 and 11 weeks of age). Chickens were assigned to cages according to a randomized-complete-block design with cage level from the floor and environmental rooms as the blocking factors. The developmental profiles of cGHR mRNA, cGH, cIGF-I and T_3 of each strain were used for calculation of AUC from 1 to 11 weeks of age using the trapezoidal rule. The developmental expression of cGHR mRNA in each tissue is represented by the AUC (GHR/GAPDH ratio $\times$ weeks). Plasma concentrations of GH, IGF-I and T_3 were transformed into the natural logarithm (to account for heterogeneity of error variances) for the AUC calculation and statistical analysis. Significant differences ($P < 0.05$) due to strain or age were determined by the protected Fisher's least significance difference test (Steel & Torrie 1960).

Results

Growth rate of broiler strains

The BW of broiler strains selected for different growth traits begins to diverge after 3 weeks of age (Fig. 2). The difference in BW among strains becomes greater with age. Comparison of the BW AUC shows that growth rate (BW) is significantly ($P < 0.05$) different for each strain. The BW AUC of Ottawa strains was 19, 16.6, 10.4 and 7.7 kg $\times$ weeks for S21, S20, S80 and S90 respectively.

Developmental profiles of plasma hormones in broiler strains

The developmental profile of plasma GH in growth-selected strains shows that plasma GH levels were highest at 3 weeks of age and then gradually declined until a low basal level was reached at 11 weeks of age (Fig. 3A). The GH AUC of S90 was 49% higher ($P < 0.05$) than S20 and 19% higher ($P < 0.05$) than S80. Although the GH AUC was not different between S20 and S21, the GH AUC of S80 was 38% higher than S21.

Plasma IGF-I levels were highest at 5 weeks of age in S90 and S80 while that of S21 was highest at 3 weeks of age (Fig. 3B). The plasma IGF-I AUC of S90 (unselected 1950's population) was higher ($P < 0.05$) than that of S20 (unselected 1970's population). It appears that the decline

Figure 2 Growth rate of growth-selected broiler chickens from Ottawa Research Center strains (S90, S80, S20, and S21). Each value represents the average body weight $\pm$ S.E.M. of six birds. Body weight area-under-curve (AUC) (kg x weeks) values possessing a different superscript are significantly ($P < 0.05$) different.

Figure 3 Developmental profiles of plasma (A) GH, (B) IGF-I and (C) T₃ in growth-selected broiler strains. Values represent means $\pm$ S.E.M. of six birds. The area-under-curve (AUC) (ng/ml x weeks) values of the hormone profiles are presented for each strain. Plasma hormone AUC without the same superscript(s) are significantly ($P < 0.05$) different.

in plasma IGF-I lags considerably behind the developmental decline of plasma GH.

Plasma T₃ levels were high in all strains at 1 week of age then sharply declined to a low basal level at 3 weeks of age

which was maintained in S80, S20 and S21 (Fig. 3C). However, the plasma T₃ AUC was higher ($P < 0.05$) in S90 due to maintenance of higher T₃ levels after 3 weeks of age.

Hepatic and serum GH-binding activity

Specific GH-binding activity was determined for liver membranes and serum from different broiler strains at 11 weeks of age (Fig. 4). Hepatic cGH-binding activity increases with genetic selection for higher growth rate (Fig. 4A). There is a significant difference ($P < 0.05$) in total hepatic GH-binding sites (with MgCl₂ treatment) in S80 (45% increase) when compared with S90. Strain 21 has higher total hepatic GH-binding sites than S20 although the difference is not significant. There is a significant difference ($P < 0.05$) in the unoccupied hepatic GH-binding sites (without MgCl₂ treatment) in S21 (37% increase) when compared with S20, although there is no difference between S90 and S80 (Fig. 4A). Similarly, the plasma GH-binding activity of S21 was 44% higher ($P < 0.05$) than the average (45.1% specific-binding) of slower-growing birds (S90 and S80), although there was no significant difference in plasma GH-binding activity between S20 (51% specific-binding) and the other strains (Fig. 4B).

Quantitation of cGHR mRNA levels in tissues

The GHR mRNA levels in liver of different broiler strains were examined using RPA. Hepatic cGHR mRNA levels in all four strains show a developmental increase from 1 to 9 weeks of age then slightly declined at 11 weeks of age (Fig. 5A). The developmental increase in cGHR mRNA levels reaches a maximum of 5-fold by 9 weeks of age. There is also a developmental increase in the cGHR mRNA levels in abdominal fat of all four strains from 1 to 11 weeks of age (Fig. 5B). The cGHR mRNA levels in fat at 11 weeks of age is about 4.5-fold higher than that at 1 week of age. The effect of age on the cGHR mRNA levels in both liver and fat is significant. There is no significant difference in the cGHR mRNA AUC among strains for either liver or fat.

Unlike liver and fat, both breast and leg muscle did not show a developmental increase in the expression of cGHR mRNA. There is no significant difference among the cGHR mRNA AUC of strains (Fig. 5C and D). At 11 weeks of age, the average amount of cGHR mRNA (in 25 μ g total RNA) across all strains was 21.2 pg for liver, 19.1 pg for fat, 15 pg for breast muscle, and 5.9 pg for leg muscle. The average amount of cGAPDH mRNA across all strains at 11 weeks of age was 10.6 ng for breast muscle, 6.1 ng for leg muscle, 1.17 ng for liver, and 0.32 ng for fat. Thus, the order of higher abundance of cGHR mRNA (in pg) was liver and fat > breast muscle > and leg muscle, while that of cGAPDH mRNA (in ng) was breast

A. Hepatic GH-binding activity **B. Plasma GH-binding (GHBP) activity**

Figure 4 GH-binding activity in hepatic membranes and in plasma of growth-selected chickens. (A) Specific ^{125}I -cGH-binding activity of hepatic membranes with or without 3.8 M MgCl_2 treatment from different broiler strains at 11 weeks of age. (B) Specific ^{125}I -hGH-binding activity of plasma (25 μl) from different strains of broiler chickens at 11 weeks of age. Values represent means $\pm$ S.E.M. of four birds. Means without the same superscript(s) are significantly ($P<0.05$) different.

muscle>leg muscle>liver>fat. Consequently, the ratio cGHR/cGAPDH varied greatly among the tissues examined (Fig. 5).

Figure 5 Developmental profiles of cGHR mRNA in (A) liver, (B) fat, (C) breast muscle and (D) leg muscle of broiler strains. The data represent the average of normalized 177 bp (full-length cGHR) in duplicate samples from four chickens at each age. The AUC (cGHR/cGAPDH ratio $\times$ weeks) represents the developmental profile of tissue cGHR mRNA for each strain. Strain AUC values within each tissue without the same superscript(s) are significantly different ($P<0.05$).

Discussion

In this study, we examined the effect of genetic selection for different growth traits on cGHR gene expression by determining the ontogeny of cGHR mRNA in target tissue. Despite distinct strain differences in growth rate and GH-binding activity in liver and plasma, the developmental increase in concentration of cGHR mRNA in liver and fat was similar across strains. The present discrepancy between cGHR mRNA in liver and hepatic GH-binding activity has also been observed in other animals. For example, GH treatment in hypoxic rats increased GH-binding activity by 2-fold without increasing the abundance of GHR mRNA which suggests post-transcriptional regulation of the GHR gene (Maiter *et al.* 1992). Plasma GH levels appear to be inversely related to selection for increased growth rate in the chicken, which suggests that GH could act at either the translational or post-translational level to regulate cGHR binding. In the 1950's population (S90), plasma GH levels could

determine cGHR turnover and degradation since total GH-binding activity was higher in S80 which has lower plasma GH levels. Efficient internalization of the trans-membrane receptor requires a signal sequence in the cytoplasmic tail of the protein (Vaux *et al.* 1992). Several di-leucine residues found in the intracellular domain of the cGHR appear to be important for ligand-induced endocytosis of the cGHR. The intracellular domain of several membrane receptors contains a di-leucine-based internalization motif (leucine-leucine or leucine-isoleucine) which is important for internalization and/or lysosomal targeting (Shin *et al.* 1991, Johnson & Kornfeld 1992, Letourneur & Klausner 1992, Chen *et al.* 1993, Farrar & Schreiber 1993, Dittrich *et al.* 1996). In addition, phenylalanine 346 in the cytoplasmic domain of the rat GHR is essential for internalization and ligand-mediated downregulation (Allevato *et al.* 1995). The increase of GH-binding in liver and plasma of growth selected chickens without a coordinated increase in cGHR mRNA levels suggests that the cGHR is under translational or post-translational regulation.

In addition to hormonal factors or genetic selection, developmental factors appear to play a dominant role in regulating expression of cGHR mRNA in liver and fat of chickens. Regardless of strain, there was a developmental increase of 4- to 5-fold in cGHR mRNA (AUC) in liver and fat between 1 and 11 weeks of age. A similar developmental increase in cGHR mRNA was found during this period in liver of a commercial broiler strain (Burnside & Cogburn 1992). Even in the absence of a functional cGHR, there is a developmental increase in expression of a truncated (0.8 kb) cGHR transcript in the sex-linked dwarf chicken which is similar to the age-related increase in expression of full-length and truncated cGHR transcripts in normal chickens (Mao *et al.* 1997). In contrast, steady-state levels of cGHR mRNA in breast and leg muscle were not affected by either age or strain. We have recently proposed that a single cGHR promoter is expressed in target tissue (liver, fat and muscle) of the broiler chicken (Mao *et al.* 1997). Assuming that all tissues utilize the same cGHR promoter, the developmental increase in abundance of cGHR mRNA in liver and fat would depend upon the presence of a developmental factor in these tissues.

The absence of a developmental increase of cGHR mRNA in breast and leg muscle also suggests that other factors, such as IGF-I, could regulate muscle growth in a local manner. The paracrine production of IGF-I in extrahepatic tissue of the chicken appears to be GH independent (Tanaka *et al.* 1996). Indeed, type-I IGF receptor transcripts are found in the muscle of chickens (Armstrong & Hogg 1994) and turkeys (Minshall *et al.* 1990, McFarland *et al.* 1992). Furthermore, exogenous IGF-I has been shown to increase the proliferation of muscle cells isolated from the chicken (Duclos *et al.* 1991) and the turkey (Sun *et al.* 1992).

Similar to the strain differences found in hepatic GH-binding activity, levels of the soluble cGHBP appear to be related to genetic selection for growth traits (Fig. 4B). In our study, GH-binding activity in plasma and liver membrane at 11 weeks of age were positively related to each other (Fig. 4) which implies coordinated regulation of cGHBP and cGHR binding in the chicken. The GHBP found in the circulation corresponds to the extracellular ligand-binding domain of the membrane-bound GHR (Leung *et al.* 1987, Spencer *et al.* 1988, Baumbach *et al.* 1989, Smith *et al.* 1989, Carlsson *et al.* 1990, Sadeghi *et al.* 1990). In rodents, the serum GHBP is produced from alternative splicing of the GHR gene (Baumbach *et al.* 1989, Smith *et al.* 1989). In the rat, the developmental profile of serum GHBP does not follow that of hepatic GH-binding activity (Tiong & Herington 1992). In the mouse, GHR and GHBP mRNA with alternative 5'-UTRs are differentially expressed in mouse liver and placenta (Southard *et al.* 1995). In humans and rabbits, the circulating GHBP probably arises from the proteolytic cleavage of the membrane receptors (Leung *et al.* 1987, Trivedi & Daughaday 1988, Sotiropoulos *et al.* 1993, Bingham *et al.* 1994). In the chicken, it appears that the serum GHBP is produced from proteolytic cleavage of the membrane receptors rather than from alternative splicing of the cGHR gene (Cogburn *et al.* 1997). Apparently, the cGHBP serves as a reliable indicator of the functional activity of the cGHR protein found in liver membranes.

Selection for higher BW results in lower plasma GH levels (S20 and S21) and higher hepatic and plasma GH-binding activity in liver membrane and plasma which is consistent with other studies (Burke & Marks 1982, Goddard *et al.* 1988, Leenstra *et al.* 1991, Vanderpooten *et al.* 1993). The slowest growing strain (S90) appears to be hyperpituitary since these birds have higher plasma GH, IGF-I, and T_3 AUCs than the parent unselected 1970's strain (S20). The developmental hyperpituitarism found in S90 could contribute to their lower hepatic and plasma GH-binding activity. The treatment of broiler chickens with either GH or T_3 alone reduces hepatic GH-binding activity while the combination of GH and T_3 treatments further reduces GH-binding (Cogburn *et al.* 1995). Developmental regulation of the hepatic cGHR and GH-binding activity in liver and plasma appear to be down-regulated by the high endogenous GH levels found in early development, especially in the slower-growing strains (S90 and S80). Several studies have shown that exogenous GH down-regulates hepatic GH-binding activity in normal chickens (Leung *et al.* 1986, Cogburn 1991, Moellers & Cogburn 1995). In a previous ontogeny study, we observed an apparent inverse relationship between plasma GH levels and expression of hepatic cGHR mRNA in a commercial strain of broiler chickens (Burnside & Cogburn 1992). In the cGHR-deficient dwarf chicken, the absence of GH down-regulation results in over expression of the truncated cGHR transcript in

liver (Huang *et al.* 1993). In addition to GH down-regulation, the expression of the cGHR gene in liver and fat appears to be up-regulated by some as yet unidentified developmental factor since the greatest abundance of cGHR mRNA and the highest hepatic GH-binding activity is found in older birds.

A hyperthyroid status in chickens is associated with a decreased fat content (Decuypere *et al.* 1987) whereas in hypothyroid chickens, fat deposition is greatly enhanced (Cogburn 1991). The higher plasma T_3 levels observed in slow-growing S90 is unexpected since most studies have shown an inhibiting effect of T_3 on circulating plasma GH levels (Harvey *et al.* 1991) which could be due to reduced cGH mRNA and hence cGH synthesis (Radecki *et al.* 1994). Chickens selected for low abdominal fat content have higher plasma T_3 concentrations (Buyse *et al.* 1994). Theoretically, chickens selected for increased growth rate and reduced abdominal fat content should have higher plasma T_3 and lower plasma GH. The reason why the high plasma T_3 levels found in S90 birds does not inhibit their enhanced GH secretion is presently unknown.

There is an apparent dissociation between the higher plasma IGF-I levels in S90 and low GH-binding activity in liver and plasma. If extrahepatic production of IGF-I is GHR-independent in the chicken (Tanaka *et al.* 1996), then autocrine/paracrine activity in tissue could account for strain differences in muscle and fat deposition. Extrahepatic IGF-I production in the GHR-deficient dwarf chicken could account for the maintenance of low plasma IGF-I levels and their continued growth at two-thirds the normal rate (Cogburn *et al.* 1995). Selection for increased growth rate in S80 (from S90) and in S21 (from S20) does not affect plasma IGF-I levels. Numerous studies have shown that genetic selection of broiler chickens for improved growth traits does not affect plasma IGF-I levels (Cahaner 1988, Goddard *et al.* 1988, Leclercq 1988, Sorensen 1988, Whitehead 1988, Leenstra *et al.* 1991, Tomas *et al.* 1991). However, plasma IGF-I levels were significantly lower in two fast-growing strains than in a slow-growing strain (Lee *et al.* 1989). The plasma IGF-I was higher in a faster-growing turkey line and positively correlated with growth rate (Bacon *et al.* 1993). The abundance of type-I IGF receptor mRNA of liver and muscle in unselected chickens does not differ from that in growth-selected chickens (Armstrong & Hogg 1994). In the chicken, plasma IGF-I levels do not appear to be strictly GH-dependent since developmental changes in plasma IGF-I do not necessarily reflect plasma cGH levels or cGHR status.

In conclusion, there is a developmental increase in the abundance of cGHR mRNA in liver and fat. However, the absence of a developmental increase in breast and leg muscle suggests that a tissue-specific developmental factor(s) could be involved in the regulation of cGHR expression. Selection for increased growth rate alone (S80 from S90) or increased growth rate and lower abdominal

fat (S21 from S20) results in higher hepatic and plasma GH-binding activity, which appears to be independent of cGHR mRNA levels in tissue. The discrepancy between higher GH-binding activity and unaffected levels of cGHR mRNA suggests that, in these strains of growth selected chickens, the cGHR could be under translational or post-translational regulation.

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