Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate

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Abstract

Insulin-like growth factors (IGFs) stimulate growth rate in a number of animal species and are likely to contribute to genetic variations of growth potential. The present study was designed to link levels of IGF-I and IGF-II mRNA and peptides with growth rate in divergently selected genotypes of chickens with high (HG) or low (LG) growth rates. Circulating IGF-I and -II and hepatic mRNA levels were measured under *ad libitum* feeding conditions from 1 to 12 weeks of age, and at 6 weeks of age under three different nutritional conditions (fed, fasted for 16 or 48 h, re-fed for 4 or 24 h after a 48-h fast). IGF binding proteins (IGFBPs) were also measured. Circulating IGFs increased with age and were higher in HG chickens from 1 to 6 weeks. They decreased with fasting and only IGF-II was fully restored after 24 h of re-feeding, while IGF-I remained low. A significant decrease in steady state IGF-I

mRNA levels was also observed with fasting. Across the nutritional study, hepatic IGF-I mRNAs were significantly higher in HG chickens. Variations of IGF-II mRNA levels with nutritional state or genotype exhibited a similar trend. IGFBP (28, 34 and 40 kDa) levels increased with age, while only faint differences were observed between genotypes. IGFBP-28 transiently increased with fasting and was inversely related to blood glucose and insulin levels, suggesting that it is equivalent to mammalian IGFBP-1. In HG chickens, IGFBP-28 and IGFBP-34 levels decreased markedly following re-feeding. Therefore, high and low growth rates were respectively associated with high and low IGF-I and -II levels, supporting the hypothesis of a stimulatory role for both IGFs during post-hatching growth of chickens.

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Introduction

Genetic models exhibiting extreme (high or low) growth potential are valuable tools for studying the regulatory roles of hormones and growth factors. Alterations in the concentrations of circulating growth hormone (GH), the central player in the somatotroph axis, have explained a number of genetic variations of growth in the mouse (Efstratiadis 1998). Surprisingly, higher GH levels in chickens are consistently associated with low growth rates, whether in unrelated genotypes (such as broiler vs layer) (Harvey *et al.* 1979, Burke & Marks 1982, Goddard *et al.* 1988) or in divergently selected genotypes (Nir *et al.* 1987). This suggests that GH levels do not directly explain growth rate in the chicken and implies the need for studies on the factors acting downstream of GH, namely the insulin-like growth factors (IGFs). The physiology of the IGF system in birds has been described in recent reviews (McMurtry *et al.* 1997, Duclos *et al.* 1999).

The possible involvement of the IGF system in explaining differences between chickens divergently selected for

high (HG) or low (LG) growth rates has already been considered. On the one hand, adult myoblasts isolated from HG chicks were more responsive to serum and IGF-I than those from LG chicks (Duclos *et al.* 1996). The pathways accounting for this remains to be determined. On the other hand, no difference was found in either the number, the affinity or the tyrosine kinase activity of solubilized and purified IGF-I receptors from muscles of HG and LG chickens at 1 or 7 weeks of age (Oudin *et al.* 1998). Data on circulating IGF-I, IGF-II and IGFBPs, and their corresponding mRNAs are missing for a full interpretation of the involvement of the IGF system. The objective of the present study was to assess those parameters in HG and LG chickens during the early period of post-hatch growth (1–12 weeks of age). Responses to extreme nutritional states (*ad libitum* feeding, prolonged fasting and re-feeding), which in mammals largely alter the concentrations of circulating IGF peptides and IGFBPs, were also compared. The IGF system (mainly IGF-I) is significantly altered by genotype, suggesting a key role in the control of growth rate in broiler chickens.

Materials and Methods

Animals

These studies were performed at the Institut National de la Recherche Agronomique, Research Center of Nouzilly, France using experimental strains of chickens. All animal procedures and care were performed in accordance with Authorization 006621 issued by the French Ministry of Agriculture and Fishery to M J Duclos. Male chickens were from the 37th and the 38th generation of high (HG) and low (LG) growth rate lines which have been divergently selected for high or low body weights at 9 weeks of age (Ricard 1975). In the first experiment, chickens from the 37th generation were raised in floor pens and given free access to a regular and balanced diet under 14 h light : 10 h darkness cycles. Blood samples were obtained at 1 and 6 weeks of age; after clotting at room temperature and centrifugation, sera were collected, aliquoted and stored at -20°C . After sacrifice, livers were collected, frozen, ground in liquid nitrogen using a mortar and pestle and kept at -80°C ($n=8$ per age and genotype). For the longitudinal study of the second experiment, birds from the 38th generation were raised on the floor under the same conditions, serum samples were obtained at 1, 2, 4, 6 and 12 weeks of age ($n=8$ per age and genotype). In order to examine the effect of the nutritional status, birds of the same flock were raised in cages and fed *ad libitum* until 6 weeks of age when they were submitted to one of the following regimens: *ad libitum* feeding, fasting for 16 or 48 h or *ad libitum* re-feeding for 4 or 24 h after a 48 h fast ($n=8$ chickens per nutritional state and genotype). Blood samples were collected and chilled on ice using heparin as anticoagulant. After centrifugation, plasma were collected, aliquoted and stored at -20°C . After sacrifice, livers were collected and treated as above.

IGF-I and -II RIA

Recombinant DNA-derived human IGF-I (hIGF-I) and hIGF-II were kindly provided by Ciba-Geigy (Basle, Switzerland), recombinant chicken IGF-I and IGF-II (cIGF-I and cIGF-II) were obtained from GroPep Pty Ltd (Adelaide, Australia). Human IGF-I and hIGF-II were iodinated as described previously (Duclos *et al.* 1994). IGFs were extracted from serum or plasma following acidification and ultrafiltration using Centricon 30 membranes (Amicon, Millipore Corp., Saint-Quentin en Yvelines, France), and assayed by heterologous RIAs as validated previously (Beccavin *et al.* 1999). Samples from experiment 1 or experiment 2, longitudinal and nutritional study, were assayed independently in three different assays and processed as one dilution in triplicate or two different dilutions in duplicate depending upon age and nutritional state.

Determination of glucose and insulin circulating concentrations

Plasma concentrations of glucose and insulin were measured only in the nutritional study (experiment 2). Plasma glucose levels were determined using an automated analyser (Glucose Beckman Analyser 2, Palo Alto, CA, USA). Plasma insulin concentrations were measured by RIA using a guinea pig anti-porcine insulin serum (Ab 27–6, provided by Dr G Rosselin, Hôpital Saint-Antoine, Paris, France) using iodinated porcine insulin as a tracer and chicken insulin (Litron Laboratories, Rochester, NY, USA) as a standard, as previously described (Simon *et al.* 1974). All samples were processed in one assay at one dilution in triplicate or at two or three different dilutions in duplicate, depending on the nutritional state.

IGFBP analysis

Serum or plasma samples were subjected to SDS polyacrylamide gel electrophoresis and Western ligand blotting (WLB) using ^{125}I -hIGF-I as previously described (Beccavin *et al.* 1999). Each experimental parameter (age, genotype or nutritional state) was tested within a single experiment running two gels in parallel and splitting individual samples from each experimental group (6 or 8) in half on each gel. Following transfer, the two membranes were incubated with the same batch of ^{125}I -hIGF-I. Quantifications were performed using a Phosphorimager (Storm 840, Molecular Dynamics, Bondoufle, France).

RNA isolation and analysis

Total RNA was extracted from liver (Chomczynski & Sacchi 1987) using a commercial kit (RNA Instapure, obtained from Eurogentec France s.a., Angers, France) and their integrity was checked by denaturing agarose gel electrophoresis (not shown). The cIGF-I cDNA (224 bp) and chicken IGF type 1 receptor (cIGFR) cDNA (274 bp) were previously described (Armstrong & Hogg 1992, 1996). The cIGF-II cDNA (406 bp) obtained from Dr M Holzenberger and Dr C Ayer-LeLievre (Institut d'Embryologie, Nogent sur Marne, France) corresponded to nt -20 to $+386$ of the published cIGF-II sequence (Darling & Brickell 1996) cloned in the sense orientation into pGEM-4Z (Promega, Charbonnières, France) between the EcoRI and SacI restriction sites. A cDNA fragment including 170 bp of the chicken 18S cDNA (nt $+54$ to $+223$) and 53 bp of plasmid vector was amplified by PCR ($5'$ -GTGCGATCGGCTCGAGGTT- $3'$; $5'$ -GCCGCTCGAGCATGCATCTAGA- $3'$) from a c18S cDNA cloned in antisense position (obtained from Dr A L Johnson, University of Notre Dame, Notre Dame, IN, USA; Johnson *et al.* 1998). A T7 promoter was subsequently added by PCR as described in RPA III instruction manual (Ambion, Clinisciences, Montrouge, France). An

extended forward primer including a core T7 sequence was used (5'-TAATACGACTCACTATAG GGTGC GATCGGCTCGAGGTTA-3'). Labelled antisense riboprobes were prepared from the linearized cDNA clones or the PCR c18S product using [32 P]CTP (ICN Pharmaceuticals France, Orsay, France) and a Riboprobe Combination System (Promega, Charbonnières, France). About 2×10^8 (IGF-I, IGF-II and IGFR) or 2×10^5 (18S) d.p.m. of labelled RNA probes were obtained from 1 μ g of cDNA. These were used to quantify mRNA levels by ribonuclease protection assay using the RPA III kit (Ambion, Clinisciences, Montrouge, France). Two RPA experiments were run in parallel. In one, the RNA extract (20 μ g) was hybridized with a mixture of labelled antisense RNA corresponding to IGF-II, IGFR (200 000 d.p.m.) and c18S (2000 d.p.m.). In the other it was hybridized with 200 000 d.p.m. of the labelled antisense RNA corresponding to IGF-I alone. Following RNase digestion, the protected hybrids were submitted to electrophoresis on an 8% polyacrylamide/8 M urea denaturing gel which was subsequently dried before exposure to phosphor screen imager or autoradiography. Quantification of protected fragments was performed using a Phosphorimager (Storm 840, Molecular Dynamics, Bondoufle, France). To account for possible inter-sample variability and to normalize data, the radioactivity of each band was expressed as a ratio of the band corresponding to c18S rRNA. However, variability in specific activities of the different probes does not permit comparisons between different experiments.

Statistical analysis

All results were presented as the mean ($\pm$ S.E.M.) and analysed by one- or two-way ANOVA as appropriate, followed by least square mean comparisons (LSM) between means using the SuperAnova Software (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Growth and blood parameters in the longitudinal studies

The growth curves of HG and LG chickens of the 38th generation between 1 and 12 weeks of age are shown in Fig. 1. Body weights recorded at 1 and 6 weeks of age in the 37th generation are also indicated by bars. Growth performance was largely different between the two genotypes and fairly stable from generation to generation.

Circulating concentrations of IGF-I and IGF-II in animals from the 38th and the 37th generation are shown in Fig. 2A and B respectively. IGF-I gradually increased from 1 to 6 weeks of age in HG chickens and from 1 to 12 weeks in LG chickens (Fig. 2A). Across the 1–6 weeks period the concentrations were consistently ($P < 0.001$ at

Figure 1 Body weight of HG and LG chickens. At the 37th generation, birds were weighed at 1 and 6 weeks only; these results are shown as bars. Data are means $\pm$ S.E.M.s (37th generation: HG, open bars and LG, hatched bars; 38th generation: HG, (○) and LG, (●). Error bars are hidden by the symbols.

each age) higher in HG chickens than in LG chickens. An increase in circulating IGF-II concentrations with age was also observed across lines (Fig. 2A), averages at 6 weeks of age were significantly higher than at 1 week of age ($P < 0.01$, Fig. 2). During the 1–6 weeks of age period, IGF-II concentrations were higher ($P < 0.01$) in HG chickens at 2, 4 and 6 weeks of age ($P < 0.05$). At 12 weeks of age, IGF-I and IGF-II concentrations were very similar between the two lines. At 1 and 6 weeks of age, birds from the 37th generation (Fig. 2B) were qualitatively close, with similar differences observed between genotypes across age. However, in birds from this earlier generation, the differences for IGF-I and IGF-II concentrations between the two genotypes were only significant at 6 weeks of age.

A typical WLB using chicken serum samples from both genotypes at 1 and 6 weeks of age is shown in Fig. 3. Three major IGF binding proteins, designated as IGFBP-28, -34 and -40 according to their molecular weights, were identified (Beccavin *et al.* 1999). Their radio-labelling obviously increased with age, which was further substantiated in a larger number of individual chickens from the 37th and the 38th generation (data not shown). The effect of genotype was then considered independently, in all available samples age by age. IGFBP-34 labelling was higher ($P < 0.01$) in HG than in LG chickens at 6 weeks of age only, in both generations ($35 \pm 11\%$ and $77 \pm 19\%$ increases respectively).

Effect of the nutritional state on blood parameters

Circulating glucose and insulin concentrations were measured as indicators of the metabolic status. Indeed,

Figure 2 Effects of age and genotype on circulating IGF-I (closed symbols) and IGF-II (open symbols) concentrations at the 38th (A) or 37th generation (B). Individual serum samples ($n=8$ in each category) were collected from HG (squares) and LG (circles) chickens between 1 and 12 weeks of age. Circulating IGF-I and IGF-II levels were measured by RIA. IGF-I and IGF-II values correspond to means $\pm$ S.E.M.s. The statistical analysis of these data is presented in the Results section.

both parameters (Fig. 4) were significantly altered by nutritional state ($P<0.001$). Insulin levels, but not glucose, were also altered by the genotype ($P<0.001$). No significant interaction was found between genotype and nutritional status for either parameter, suggesting that both genotypes responded similarly to feed intake. Glucose and insulin levels decreased after 16 h fasting, as expected from earlier studies (Simon & Leclercq 1982), and both returned to near normal after 48 h fasting. Re-feeding chickens for 4 h after a 48 h fast increased glucose and insulin levels over those observed after 48 h. After 24 h re-feeding, both glucose and insulin returned to levels similar to those of fed animals in both lines. For insulin, the difference between genotypes mainly resulted from the

re-fed states, where circulating insulin levels were significantly higher in HG than LG chickens (LSM comparisons, 4 h re-feeding: $P<0.001$, 24 h re-feeding: $P<0.05$).

Circulating IGF-I and IGF-II concentrations (Fig. 4) were altered ($P<0.001$) by nutritional state and genotype. A significant interaction between the two factors was, however, observed for the two IGFs ($P<0.05$ for IGF-I and $P<0.001$ for IGF-II). For IGF-I, the interaction disappeared if fasting and re-feeding conditions were considered separately; for IGF-II, the interaction was still apparent when fasting alone was considered. Circulating levels of the two IGFs were significantly lower in fasted (16 h and 48 h) than in fed animals in both lines (LSM comparisons, $P<0.01$). The amplitude of the fasting-induced decrease of IGF-I was the greatest at 48 h fasting in the two genotypes, while for IGF-II it was the greatest at 16 h in LG chickens and at 48 h in HG chickens. Re-feeding (4 h and 24 h) after the 48 h fast had no significant effect on IGF-I concentrations as compared with those observed in 48 h-fasted chickens. Conversely, after 24 h of re-feeding, IGF-II levels were restored to the levels observed in fed animals in both genotypes, this recovery started at 4 h in HG chickens. Circulating IGF-I concentrations were always significantly higher in HG than in LG chickens, whatever the nutritional state ($P<0.01$ or higher). As a whole, IGF-II levels were also higher ($P<0.001$) in HG than in LG chickens, although mainly in the 16 h-fasted ($P<0.001$) and 4 h re-fed ($P<0.05$) states.

Figure 3 Western ligand blotting (WLB) of sera from 1- and 6-week-old HG and LG chickens. For each lane, 8 μ l of a serum from individual HG and LG chickens were submitted to WLB using 125 I-hIGF-I ligand.

Figure 4 Effect of nutritional state and genotype on circulating levels of glucose, insulin, IGF-I and IGF-II. Individual plasma samples ($n=7$ in each category) were obtained from 6-week-old HG (open bars) and LG (closed bars) chickens in the fed, fasted (16 h or 48 h) or re-fed state (4 or 24 h following the 48 h fasting). Values are means $\pm$ S.E.M.S. The statistical analysis of these data is presented in the Results section.

The data on circulating IGFBPs are reported in Table 1. IGFBP-28 labelling was significantly altered by fasting ($P<0.02$) in both genotypes, exhibiting a transient increase

at 16 h-fasting followed by a return to the fed level after 48 h. Labelling of IGFBP-28 was significantly altered by re-feeding ($P<0.001$) but only in HG chickens, with a significant decrease at both 4 and 24 h re-feeding (compared with 48 h fasting). Variations of IGFBP-34 and -40 labelling were less consistent, except for a large decrease of IGFBP-34 observed upon re-feeding, in the HG chickens. In subsequent experiments, the effect of genotype was examined within each nutritional state: the intensity of IGFBP-28 labelling was higher in HG than in LG chickens (a 72% increase, $P<0.05$) in the 48 h-fasted state. The labelling of IGFBP-34 was also significantly higher in HG chickens, in fed (+48%) and 48 h-fasted (+36%) states (both $P<0.05$), consistent with the data obtained in the longitudinal study.

Steady state levels of hepatic IGF-I and IGF-II mRNA

Total RNA was extracted from HG and LG chicken livers of the different experimental conditions ($n=6$ per group). For illustration, the result of a solution hybridization/RPA experiment performed with samples from 1- and 6-week-old chickens using the four different antisense riboprobes (IGF-I, IGF-II, IGFR and 18S) is presented in Fig. 5. By reference to the RNA markers, the protected fragments were of the expected sizes: 226 bp for IGF-I and 274 bp for IGF receptor mRNAs, as previously described (Armstrong and Hogg 1996) and 406 bp for IGF-II and 170 bp for c18S, as expected from the riboprobes. The figure suggests that IGF-I and IGF-II mRNAs increased with age. Quantitative results expressed as ratios over 18S RNA levels (in arbitrary units) are shown in Table 2 for IGF-I and IGF-II mRNA. Although IGFR mRNA were detected in all samples, variations were less consistent and are therefore not reported. IGF-I mRNA levels clearly increased ($P<0.001$) between 1 and 6 weeks of age in both genotypes, and also tended to be higher in the HG line, but not significantly ($P=0.12$) different. IGF-II mRNA levels were also significantly altered by age ($P<0.001$), but not by genotype ($P=0.08$), exhibiting a significant interaction between age and genotype. Indeed, when each factor was analysed separately, IGF-II mRNA levels increased significantly with age in the HG genotype only ($P<0.01$), leading to significantly higher levels in HG than in LG chickens at 6 weeks of age ($P<0.05$).

The effect of nutritional status on hepatic mRNA levels in 6-week-old HG and LG chickens of the 38th generation is summarized in Fig. 6A (effect of fasting) and 6B (effect of re-feeding). IGF-I mRNA levels were significantly ($P<0.001$) altered by fasting. They were already decreased at 16 h of fasting and remained low at 48 h in both lines (LSM comparisons, $P<0.01$). Re-feeding (Fig. 6B) tended to increase IGF-I mRNA ($P=0.10$), at least in the HG genotype. IGF-II mRNA was not significantly altered by fasting or re-feeding, although a trend for a decrease was noticed during fasting ($P=0.06$). A

Table 1 Effect of nutritional state on plasma IGFBPs

Genotype	Experiment	Nutritional state	IGFBP-28 (aU)	IGFBP-34 (aU)	IGFBP-40 (aU)
HG	Fasting	Fed	0.9 ± 0.2 ^a	2.6 ± 0.2	1.3 ± 0.2
		Fasted (16 h)	1.8 ± 0.2 ^b	3.1 ± 0.4	2.0 ± 0.2
		Fasted (48 h)	1.1 ± 0.2 ^a	2.6 ± 0.2	1.3 ± 0.3
		ANOVA	<i>P</i> <0.05	NS	NS
HG	Re-feeding	Fasted (48 h)	2.4 ± 0.5 ^a	4.6 ± 0.5 ^a	0.9 ± 0.1
		Re-fed (4 h)	0.5 ± 0.1 ^b	2.0 ± 0.4 ^b	1.2 ± 0.1
		Re-fed (24 h)	0.7 ± 0.2 ^b	1.8 ± 0.2 ^b	1.4 ± 0.3
		ANOVA	<i>P</i> <0.001	<i>P</i> <0.001	NS
LG	Fasting	Fed	0.9 ± 0.2 ^a	3.8 ± 0.5	1.6 ± 0.2 ^a
		Fasted (16 h)	3.3 ± 0.8 ^b	2.9 ± 0.5	1.6 ± 0.4 ^a
		Fasted (48 h)	0.5 ± 0.1 ^a	2.4 ± 0.2	0.7 ± 0.1 ^b
		ANOVA	<i>P</i> <0.001	NS	<i>P</i> <0.05
LG	Re-feeding	Fasted (48 h)	0.8 ± 0.1	2.7 ± 0.2	1.3 ± 0.3
		Re-fed (4 h)	0.9 ± 0.1	3.3 ± 0.3	1.6 ± 0.2
		Re-fed (24 h)	0.9 ± 0.3	3.3 ± 0.4	1.7 ± 0.3
		ANOVA	NS	NS	NS

For each genotype, the effect of fasting (fed vs fasted 16 or 48 h) or the effect of re-feeding (fasted 48 h vs re-fed 4 or 24 h following 48 h fasting) were tested independently. Individual plasma samples (*n*=6 in each category) were submitted to Western ligand blotting using ¹²⁵I-hIGF-I. The radioactivity in each band was quantitated using a Phosphorimager and expressed as arbitrary units (aU). Values are means ± S.E.M.s and were analysed by one-way ANOVA and subsequent least square means comparisons were performed between groups for each IGFBP moiety. Different superscripts indicate a significant difference between two means at the *P*<0.05 or higher level, NS=non significant.

significant difference between genotypes was observed for both the IGF-I and IGF-II transcripts during the fasting and the re-feeding period (*P*<0.001 in each case). IGF-I

mRNA levels were significantly higher in HG chickens than in LG chickens in each nutritional condition (LSM comparisons, *P*<0.05). As a whole, the levels of the IGF-II transcripts were also higher in the HG chickens than in LG chickens across the fasting and re-feeding experiments.

Figure 5 Hepatic IGF-I, IGF-II, IGF-1R and c18S RNA expression in 1- and 6-week-old HG and LG chickens. In each lane, 20 µg of a liver RNA from individual HG and LG chickens aged 1 or 6 weeks (*n*=3) were submitted to solution hybridization/RPA using antisense RNA probes as described in Materials and Methods. The two extreme right lanes contain unhybridized probes (P) and RNA size markers (M, from top to bottom of the picture: 500, 400, 300, 200 bp). The quantitative results of six chickens per category are presented in Table 2.

Discussion

The objective of the present study was to compare the components of the IGF system between two genotypes of chickens divergently selected for high (HG) or low (LG) growth rate. This has been done with two generations, at different ages and in different nutritional states. Circulating IGF-I concentrations markedly increased with age, until chickens of both genotypes reached a body weight of about 1 kg (6 weeks in HG and 12 weeks in LG). This is consistent with a number of previous reports, in which a decline was observed following a plateau in older (or heavier) birds (Johnson *et al.* 1990, McGuinness & Cogburn 1990, McMurtry *et al.* 1994, Radecki *et al.* 1997). Circulating IGF-II levels also increased with age (1 week vs 6 weeks of age) although with less contrast. In the chicken, previous data vary from the absence of post-hatch variations (Radecki *et al.* 1997) to a fall from hatch up to at least 50 days of age (McMurtry *et al.* 1998). In mammalian species, during the postnatal period, results differ widely between species with almost undetectable

Table 2 Effect of age and genotype on hepatic expression of IGF-I, IGF-II and IGF-1R mRNAs

Genotype	Age (weeks)	IGF-I mRNA (aU)	IGF-II mRNA (aU)
HG	1	53.0 ± 13.6 ^a	8.5 ± 0.9 ^a
	6	246.2 ± 34.4 ^b	23.1 ± 3.9 ^b
LG	1	32.2 ± 6.8 ^a	10.8 ± 0.8 ^a
	6	189.4 ± 30.7 ^b	13.0 ± 1.1 ^a
Statistics (two-way ANOVA)	Line	NS	NS
	Age	**	**
	Interaction	NS	**

Total hepatic RNA ($n=6$ in each category) was obtained from HG and LG chickens of the 37th generation at 1 or 6 weeks of age. IGF-I and IGF-II mRNA levels were detected by solution hybridization/RNase protection assay and the radioactivity in each band was quantitated using a Phosphorimager and expressed in arbitrary units (aU) as a ratio of the band corresponding to c18S rRNA. The data (mean ± S.E.M.) were analysed by two-way ANOVA (NS=non significant, * $P<0.05$; ** $P<0.01$). Least square means comparisons were subsequently performed between groups within a column. Significant differences between two means at the $P<0.05$ level or higher are indicated by different superscripts.

circulating IGF-II in rodents (Moses *et al.* 1980, Daughaday *et al.* 1982) or increasing IGF-II levels in the pig (Lee *et al.* 1993). The large increase in circulating IGF-I concentrations observed in both genotypes between 1 and 6 weeks was accompanied by a marked increase in

Figure 6 Effect of nutritional state and genotype on the expression of hepatic IGF-I and IGF-II RNA. The effect of fasting (A: fed vs fasted 16 or 48 h) and re-feeding (B: fasted 48 vs re-fed 4 h or 24 h following the 48 h fasting) were tested in independent experiments. RNA extracts were obtained from 6-week-old HG (open bars) and LG (closed bars) chickens ($n=6$ in each category) and were submitted to solution hybridization/RPA. The radioactivity in each band was quantitated using a Phosphorimager. The expression of each specific mRNA was calculated as a ratio of the radioactivity in the c18S protected fragment with results expressed as arbitrary units (aU). Values are means ± S.E.M.s. The statistical analysis of these data is presented in the Results section.

hepatic IGF-I mRNA levels (about fivefold), consistent with previous observations in the chicken (Kajimoto & Rotwein 1989, Kikuchi *et al.* 1991, Burnside & Cogburn 1992). To our knowledge, the present study is the first one to consider possible developmental changes in IGF-II mRNA levels in the chicken, showing an increase between 1 and 6 weeks of age, at least in HG chickens.

At 6 weeks of age, circulating concentrations of IGF-I and IGF-II fell during the 48 h fast as previously observed in another genotype (Beccavin *et al.* 1999). In the present study, this decrease was largely engaged at 16 h of fasting for both peptides and in both genotypes. Following re-feeding (4 or 24 h following a 48 h fasting period) circulating levels of IGF-II returned to levels found in the fed state, whereas IGF-I remained low. It has previously been observed that 4 days of food restriction (50% of *ad libitum* intake) in 3-week-old broiler chickens lowered circulating IGF-I and hepatic IGF-I mRNA levels and that 3 days of *ad libitum* intake returned it to normal (Kita *et al.* 1996), but these variations were not unambiguously associated with specific changes in liver IGF-I mRNA (Kita *et al.* 1996, 1998). Presently, liver IGF-I mRNA levels in both HG and LG genotypes (expressed as a ratio of c18S rRNA) were greatly decreased by fasting, as early as 16 h, most likely accounting for the gradual decrease in circulating IGF-I levels. Liver IGF-I mRNA was not restored by re-feeding up to 24 h (only a small but not significant increase was suggested) which would account for the lack of increase in IGF-I levels after 24 h re-feeding. This slow rate of recovery for liver IGF-I mRNA is in agreement with observations in the rat where, after 3 days of fasting, IGF-I levels remain suppressed for at least the first 24 h after re-feeding (Frystyk *et al.* 1999 and references herein). Circulating IGF-II levels exhibited a rapid decrease during fasting with a

return initiated at 4 h and completed with 24 h of re-feeding. Surprisingly, this pattern was observed with a non-significant decrease of hepatic IGF-II mRNA. In the chicken, a distinct regulation is therefore suggested for the two IGFs in liver. The most important and new observation concerns the effect of genotype on circulating IGFs and steady state hepatic mRNA levels. IGF-I exhibited significantly higher circulating concentrations in HG chickens across ages between 1 and 6 weeks in two generations. At the 38th generation, this difference was significant at each age and in each nutritional state at 6 weeks of age. Steady state IGF-I mRNA levels measured at 6 weeks of age were also significantly higher in the liver of HG chickens (+100% or more) in each nutritional state. Similar trends were also observed at 1 and 6 weeks of age in the first measurement (+60% and +30% respectively), which lead us to favour the hypothesis that differences in steady state IGF-I mRNA levels contribute to differences in circulating IGF-I levels at least around 6 weeks of age. In the HG chickens, circulating IGF-II and hepatic mRNA levels tended to be higher than in LG chickens, reproducing the feature observed with IGF-I, although at a lower extent and dependent upon age and nutritional state.

The present study also provided new information concerning circulating IGFBPs. All three IGFBPs (28, 34 and 40 kDa) increased with age, as previously observed in layer chickens (Radecki *et al.* 1997) and consistent with the idea that IGFs circulate as complexes with IGFBPs. The chicken IGFBP-40 corresponds to IGFBP-3 in mammalian species, while IGFBP-34 corresponds to IGFBP-2. The later was higher in HG chickens, consistent with the hypothesis that this could be the main IGF carrier in the chicken (see Duclos *et al.* 1999 and McMurtry *et al.* 1997). The chicken IGFBP-28 was presently shown to depend upon the nutritional state in both genotypes. Its radio-labelling increased with 16 h of fasting and surprisingly returned to fed levels by 48 h of fasting, while in a previous study (Beccavin *et al.* 1999) using older (9-week-old) and heavier (2.4 kg) chickens from the fat or lean genotypes, it was still higher at 48 h of fasting than in the fed state. IGFBP-28 was higher in HG than in LG chickens at 48 h of fasting. Re-feeding decreased its level in the former not the latter genotype. Similarly, IGFBP-34 levels dropped dramatically upon re-feeding only in HG chickens. This suggests that metabolic regulation depends upon age, body weight and/or genotype. From its size and its sensitivity to the nutritional state, it is very likely that chicken IGFBP-28 is the equivalent of IGFBP-1 in mammals, which is very sensitive to metabolic regulation and is mainly under dual control of glucose and insulin (see Thissen *et al.* 1994, for review). Presently, both plasma glucose and insulin in the two genotypes decreased at 16 h fasting which would account, at least in part, for the simultaneous and large increase in circulating IGFBP-28. At 48 h fasting, glucose, insulin and IGFBP-28 resumed

fed levels in the chicken, unlike in rats where glucose and insulin remained low and IGFBP-1 high (Frystyk *et al.* 1999). Consistent with the previous observations, IGFBP-28 decreased during re-feeding in the HG genotype while insulin significantly increased.

As a whole, HG and LG chickens clearly differ in circulating IGF-I concentrations and liver IGF-I mRNA, exhibiting high or low levels respectively. Similar, although less clear-cut, differences were observed for IGF-II concentrations and liver IGF-II mRNA levels. While a direct relationship between growth rate and IGF-I or IGF-II is clearly demonstrated in transgenic mice (total knock-out or over-expression experiments; see Efstratiadis 1998 for review), such observations are not so numerous in 'natural' models. Circulating IGF-I levels (but not IGF-II) also differed at 7 weeks of age (Scanes *et al.* 1989) in a comparable model of divergently selected broilers with high or low growth rate (Scanes *et al.* 1989). By contrast, no significant difference in circulating IGF-I levels could be observed from 1 to 10 weeks of age between three unrelated genotypes of chickens exhibiting different growth rates (in a layer line with low body weight and in two broiler lines with high or very high body weight) (Goddard *et al.* 1988) which is somewhat surprising. In mice divergently selected for high or low body weight (Hoefflich *et al.* 1998), higher circulating levels of IGF-I were observed in control or heavy animals compared with the smaller animals, together with higher levels of circulating insulin, as observed in the present study. In mice carrying the high growth (hg) gene (Medrano *et al.* 1991), higher IGF-I concentrations were also observed. Mice selected for high or low circulating IGF-I levels exhibited high or low growth rates as correlated responses (Baker *et al.* 1991). However, if body weight is the objective, direct selection on this character is more efficient than an indirect one on IGF-I levels (Baker *et al.* 1991). Altogether, the present data and most of the literature (Efstratiadis 1998) suggest a direct and causal relationship between circulating IGF-I levels and body growth rates. However, this hypothesis has been recently challenged in mice where specific and complete disruption of the liver IGF-I gene has been achieved in the post-natal period: circulating IGF-I levels were decreased by 75% while normal growth rate was maintained (Sjogren *et al.* 1999, Yakar *et al.* 1999). In these conditions, the liver appears to be the main source of circulating IGF-I whereas tissue growth would rely mostly, if not exclusively, on autocrine/paracrine mechanisms, without the need for a clear increase in the expression of IGF-I in peripheral tissues. In order to fully validate this hypothesis, potential compensatory mechanisms such as up-regulation of IGF-II peptide or IGF type-1 receptor synthesis and/or down-regulation of IGFBPs (although only IGFBP-3 has been investigated and found to be decreased) have to be ruled out. One can also speculate that in the post-natal period, 'normal' growth could be possible in the presence of minimal levels

of circulating IGF-I, whereas 'enhanced' growth would require higher than normal IGF-I levels, most likely provided by the liver in normal physiological conditions.

In conclusion, the high and low growth rates of divergently selected chickens were respectively associated with high and low levels of hepatic IGF-I mRNA and circulating IGF-I levels during the early post-hatching period. Correlated changes in circulating IGF-II, although of smaller magnitude, would also contribute to the divergence of growth rates between these two genotypes.

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