# Relationship of IGF-I mRNA Levels to Tissue Development in Chicken Embryos of Different Strains

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**ABSTRACT** : Insulin-like growth factor-I (IGF-I) mRNA levels in the eyes, heart, liver and breast muscle removed from dwarf egg-type, normal egg-type and normal meat-type chicken embryos at 7, 14 and 20 days of incubation were measured. There was no influence of chicken strain on IGF-I gene expression in the eyes and liver. The IGF-I gene expression in eyes increased significantly along with the incubation period. In the liver, IGF-I gene expression at 20 days of incubation was significantly higher than that at 14 days of incubation. In the muscle, the lowest value for IGF-I gene expression was observed in meat-type chicken embryos. Regression analysis revealed that IGF-I gene expression was significantly correlated to the weights of the eyes and liver, but not the muscle. We conclude that there is little influence of strain on tissue IGF-I gene expression in chicken embryos during incubation but that tissue development in chicken embryos is nevertheless at least partly regulated by the change in IGF-I gene expression. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1653-1658*)

**Key Words** : Chicken Embryo, Insulin-Like Growth Factor-I, mRNA, Dwarf, Broiler

## INTRODUCTION

Insulin-like growth factor-I (IGF-I) of chickens has been characterized and shown to consist from 70 amino acids (Ballard et al., 1990). Recently, a high correlation between plasma IGF-I concentration and body weight change in growing chickens has been reported, suggesting that IGF-I may play an important role in the control of growth in young chickens (Kita et al., 1996). It is well known that the growth rate of meat-type chickens after hatching is nearly twice as fast as the rate for egg-type chickens (NRC, 1984). On the contrary, the sex-linked dwarfing gene, dw (Hutt, 1959), is known to result in a major reduction in body weight even when compared to the effect of other dwarfing genes (Decuypere et al., 1991). The dwarfism caused by the dw gene is associated with a reduction in plasma IGF-I concentration from hatching through to sexual maturity (Huybrechts et al., 1985; Scanes et al., 1989). Recent work has suggested that during post-hatch growing periods the decrease in plasma IGF-I concentration in sex-linked dwarf chickens is a result of a splice mutation in the intracellular domain of the growth hormone receptor, which subsequently brings about a failure in the gene expression of hepatic IGF-I (Huang et al., 1993; Agarwal et al., 1994; Tanaka et al., 1995). Although IGF-I gene expression has been investigated in chicken embryonic tissues (Serrano et al., 1990; De Pablo et al., 1991; Kikuchi et al., 1991), there is virtually little comparison of IGF-I gene expression in various embryonic tissues between different strains of chickens. Moreover, the contribution of IGF-I gene expression to the development of tissues in chicken embryos has not been investigated. Therefore, in this study, we have examined the relationship of IGF-I gene expression to the development of various embryonic tissues in strains of chickens with different post-hatch growth rates.

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## MATERIALS AND METHODS

### Animals

Fertilized eggs of normal and dwarf egg-type chickens were obtained from normal and sex-linked dwarf single-comb White Leghorn layers maintained in our laboratories (Nagoya University, Nagoya) and the Aichi-ken Agricultural Research Center (Nagakute, Aichi), respectively. Fertilized eggs from meat-type chickens (White Rock×Cornish: Cobb strain) were purchased from a local hatchery (Fuso-en Co. Ltd., Togo, Aichi). All eggs were incubated at 37℃. At 7, 14 and 20 days of incubation, 5 embryos from each strain were taken from eggs selected at random and weighed. Eyes, heart, liver and breast muscle were removed after neck dislocation. All tissues were weighed after washing with physiological saline, frozen immediately in liquid nitrogen and stored at -80℃ until analysis.

### RNA extraction and ribonuclease protection assay

Total RNA was extracted from tissue samples

according to the acid guanidine thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). Chicken IGF-I cDNA was generously donated by Dr. P. Rotwein (Washington University School of Medicine, St. Louis, MO, USA) and subcloned into plasmid pSP73 (Bresatec, Adelaide, SA, Australia) for the generation of sense and antisense chicken IGF-I RNA. The $^{32}$P-labelled single-strand antisense chicken IGF-I RNA and unlabelled sense RNA were synthesized from the BamHI/EcoRI linearized plasmids using SP6 and T7 polymerases according to the protocol of an *in vitro* transcription kit (Ambion Inc., Austin, TX, USA), respectively. The ribonuclease protection assay was performed using an RNA II Ribonuclease Protection Assay Kit (Ambion Inc., Austin, TX, USA). The intensity of protected chicken IGF-I mRNA bands (329 bp) was measured using a bio-imaging analysis system (BAS 2000, Fuji Photo Film, Co. Ltd., Tokyo, Japan). Chicken $\beta$-actin cDNA, a generous gift from Dr. S. Dogra (University of Adelaide, Adelaide, SA, Australia), was cut by Pst I and Bgl II to obtain a fragment which was subcloned into plasmid pSP73. Chicken $\beta$-actin mRNA was measured according to the method used for IGF-I mRNA. The size of the protected $\beta$-actin mRNA band was 251 bp.

### Statistical analysis

Data was analysed by two-way analysis of variance to assess the significance of the effects of different chicken strains and incubation periods, and the protected LSD test was performed to compare between all pairs of means using the General Linear Model procedure of a commercial statistical package, SAS (SAS Institute, Cary, NC, USA).

## RESULTS

### Embryonic tissue weights

The body weights of dwarf egg-type, normal egg-type and normal meat-type chicken embryos were measured after various incubation periods (7, 14 and 20 days) but no interaction between the strain and the incubation period was detected. The mean embryo weight of meat-type chickens was significantly higher than that of normal egg-type chickens and tended to be higher, but not significantly, than that of dwarf egg-type chickens (table 1). Embryo weights increased significantly throughout the incubation period (table 1).

An interaction between the chicken strain and the incubation period was found on heart and liver weights. At 7 days of incubation, there was no significant influence of chicken strain on the weight of any tissues (figures 1A and 1B). At 14 days of incubation, the heart weight of meat-type chicken embryos was significantly higher than that of normal egg-type chicken embryos (figure 1A). During egg incubation, dwarf egg-type chicken embryos had almost identical heart weights to those of normal egg-type chicken embryos, whereas the heart weight in meat-type chicken embryos was higher than other strains

**Table 1.** Embryo weights of dwarf egg-type, normal egg-type and meat-type chickens following different incubation periods

| Lines | Embryo weight (g) |
|---|---|
| Dwarf egg-type | $17.44 \pm 4.98^{ab}$ |
| Normal egg-type | $16.66 \pm 4.63^{a}$ |
| Meat-type | $18.70 \pm 5.09^{b}$ |
| **Incubation period (days)** | **Embryo weight (g)** |
| 7 | $0.69 \pm 0.03^{a}$ |
| 14 | $9.21 \pm 0.40^{b}$ |
| 20 | $42.90 \pm 0.96^{c}$ |

The number of embryos in each treatment was fifteen. Values are mean ± SEM. There was no interaction between strain and incubation periods. Means not sharing with a common superscript letter are significantly different at $p<0.05$ (a, b, c).

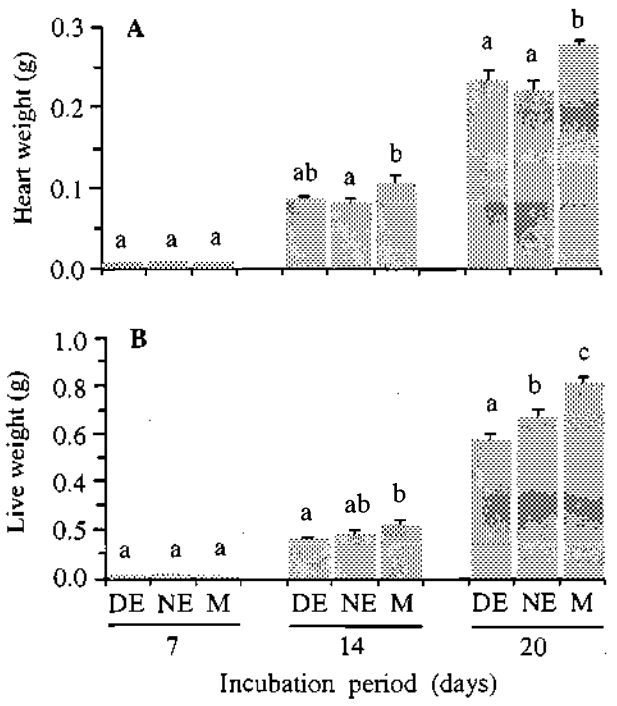


**Figure 1.** Heart (A) and liver (B) weights of dwarf egg-type (DE) and normal egg-type (NE) and meat-ype (M) chicken embryos following different incubaton periods. Vertical bars represent mean ± SEM. The number of tissues in each group was five. There was the interaction between strain and incubation periods. Means not sharing with a common letter within the same incubation days are significantly different at $p<0.05$ (a, b, c).

(figure 1A). The liver weight of meat-type chicken embryos was significantly higher than that of other strains at 14 and 20 days of incubation and the dwarfing gene had significantly decreased liver weight (figure 1B).

Eye and muscle weights of chicken embryos were also measured after different incubation periods. There was no significant influence of chicken strain on eye and muscle weights, and the weight of both tissues increased significantly throughout the incubation period (table 2).

**IGF- I gene expression in various tissues of chicken embryos**

As shown in figure 2, the bands of chicken IGF-I and $\beta$-actin mRNA in total RNA was successfully detected by using ribonuclease protection assay. The level of IGF- I mRNA in the eyes, liver and muscle was measured and corrected by that of $\beta$-actin mRNA, and the corrected value was used as an index of IGF- I gene expression in each tissue. There was no influence of chicken strain on IGF- I gene expression in eyes and liver. The IGF- I gene expression in eyes increased significantly along with the incubation period (figure 3A). In the liver, IGF- I gene expression at 20 days of incubation was significantly higher than at 14 days of incubation (figure 3B). Although there was no influence of incubation period on the value for muscle, IGF- I gene expression was lowered in meat-type chicken embryos (figure 3C).

## DISCUSSION

**IGF- I gene expression in tissues at different stages of embryogenesis**

In the present study, IGF- I mRNA was detected in embryonic tissues and quantified by using a ribonuclease protection assay. A protected IGF- I mRNA fragment of 329 bp was detected, which was similar to previous reports (Kikuchi et al., 1991; Kita et al., 1996). Since direct evidence for the production of IGF- I in response to growth hormone has been found by using a chicken hepatocyte culture system (O'Neill et al., 1990; Houston and O'Neill, 1991), it has been generally accepted that liver is the major site of IGF- I production during post-hatch of chickens. However, as shown in figure 3, extra-hepatic tissues also produced IGF- I mRNA throughout embryogenesis. Moreover, the developmental pattern of IGF- I mRNA expression differed widely among the tissues examined. No bands of IGF- I mRNA were detected in embryonic liver at 7 days of incubation, and thereafter the amount of hepatic IGF- I mRNA was quantified and increased from 14 to 20 days of incubation. Previously, a highly sensitive polymerase chain reaction has been used to try and to detect IGF- I mRNA in chicken embryos and using this strategy hepatic IGF- I mRNA was successfully amplified

**Table 2.** Eye and muscle weights of chicken embryos at different incubation period

| Incubation period (days) | 7 | 14 | 20 |
|---|---|---|---|
| Eye weight (g) | $0.12 \pm 0.008^{a}$ | $0.69 \pm 0.012^{b}$ | $0.91 \pm 0.025^{c}$ |
| Muscle weight (g) | - | $0.09 \pm 0.006^{a}$ | $0.20 \pm 0.007^{b}$ |

The number of tissues in each treatment was fifteen. Values are mean ± SEM. Both eyes were weighed. Breast muscle at both sides were weighed at 14 and 20 days of incubation. Muscle samples at 7 days of incubation could not be taken from embryos because of the small size of tissues. Means not sharing with a common letter within the same column are significantly different at $p<0.05$ (a, b, c).

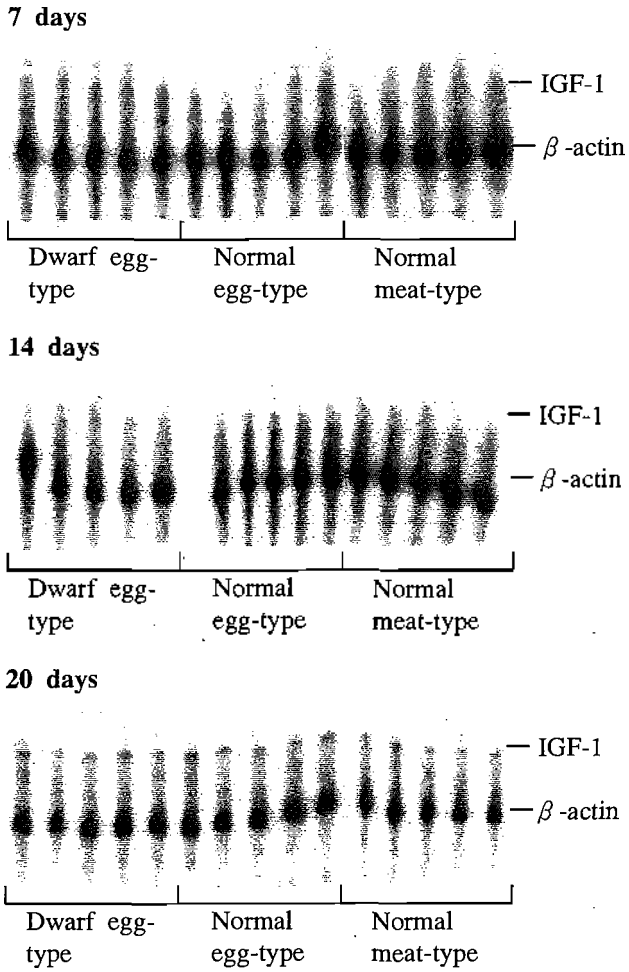


**Figure 2.** Chicken IGF-I and $\beta$-actin mRNA in total RNA from eyes of dwarf egg-type, normal egg-type and meat-type chicken embryos following different incubation periods (7, 14 and 20 days)

during late embryogenesis (18 to 19 days of incubation), but not mid-embryogenesis (12 to 16 days of incubation) (Serrano et al., 1990), which supports the data derived from the present study. In contrast to the liver, a protected IGF-I mRNA fragment was easily detected in the eyes. In eyes, a peak in IGF-I mRNA levels occurred at mid-embryogenesis, but by contrast, IGF-I mRNA was barely detectable in the heart throughout all incubation periods. These results are consistent with previous reports (Serrano et al., 1990; Kikuchi et al., 1991).

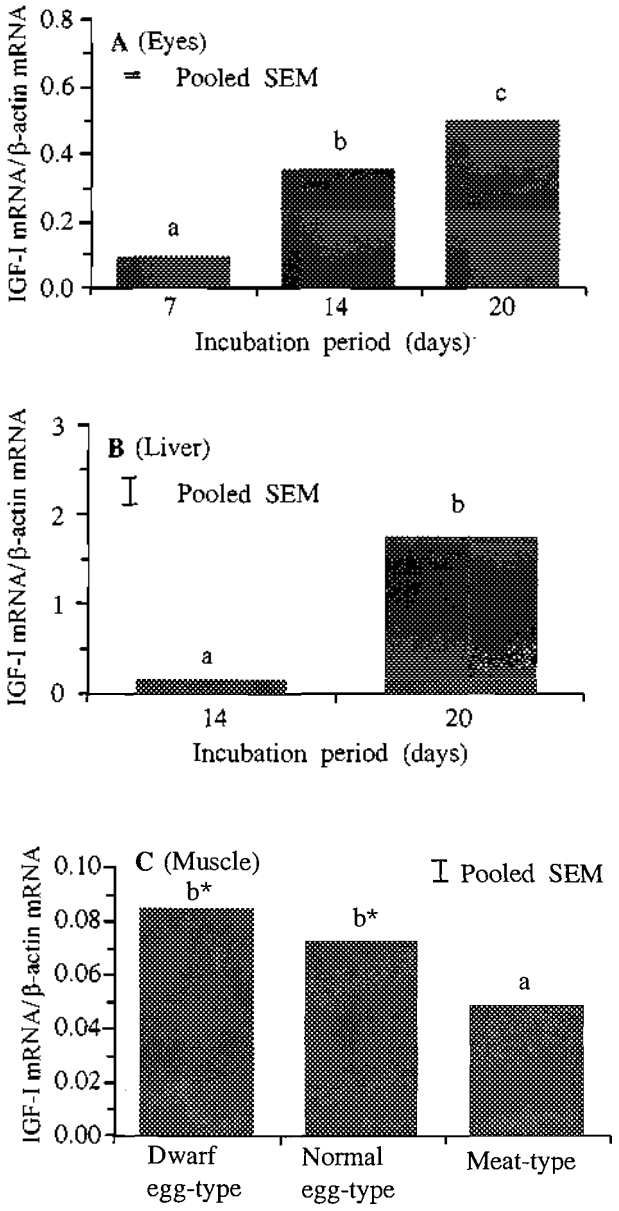


**Figure 3.** The IGF-I gene expression (IGF-I mRNA/$\beta$-actin mRNA ratio) in eyes (A), liver (B) and muscle (C) of chicken embryos. The number of tissues in each treatment was fifteen. * One missing value. There was no interactive effect between strain of chickens and incubation periods. The main effect of incubation periods was significant (eyes and liver). The main effect of strain of chickens was significant (muscle). Means not sharing with a common letter are significantly different at $p<0.05$ (a, b, c).

**The influence of strain on IGF-I gene expression in chicken embryos**

Recent studies have suggested that sex-linked dwarf chickens with the dw genotype have a splice mutation in the intracellular domain of the growth hormone receptor (Huang et al., 1993; Agarwal et al., 1994; Tanaka et al., 1995). Thus, the failure to stimulate IGF-I gene expression in the liver was caused by the mutated growth hormone receptor, results in a reduction in plasma IGF-I concentration after hatching (Huybrechts et al., 1985; Scanes et al., 1989; Tixier-Boichard et al., 1992). However, as shown in figure 3, sex-linked dwarf gene did not decrease IGF-I gene expression during embryogenesis. Similarly, it was reported that there was no difference in plasma IGF-I levels between normal and sex-linked dwarf chicken embryos (Huybrechts et al., 1989). These findings suggest that IGF-I gene expression in diverse tissues during chicken embryogenesis may be independent of the effects of growth hormone.

When meat-type chickens were selected for high and low body weight, plasma concentration of IGF-I in the high body weight line was significantly higher than that in the low body weight line (Scanes et al., 1989). However, the only effect of strain was a decrease in IGF-I gene expression in the muscle of meat-type chickens compared to normal and sex-linked dwarf egg-type chickens (figure 3C).

**The contribution of IGF-I gene expression to tissue development in chicken embryos**

As shown in table 1, the embryo weight of meat-type chickens was significantly higher than that of normal egg-type chickens. However, there was no significant difference in the embryo weight between normal and dwarf egg-type chickens, which is in good agreement with a previous report (Strong and Jaap, 1977). It has also been demonstrated that IGF-I has an important role in the growth, differentiation and metabolism of the whole-embryo of chickens (Girbau et al., 1987). However, there has been no reports of the relationship between tissue development and IGF-I levels during the embryonic stages of chickens. Therefore, we have examined the relationship between IGF-I gene expression and tissue weights. We found that the regression equation for IGF-I gene expression versus tissue weight had a significant and positive correlation in the eyes and liver (figures 4A and 4B). However, IGF-I gene expression did not correlate to the weight of muscles (figure 4C). In the

eyes and liver, the high correlation suggests that developmental changes in tissue weights of chicken embryos might be, at least partially, regulated by the change in tissue IGF- I gene expression. We conclude that there is little influence of strain on tissue IGF- I gene expression in chicken embryos and that the development of embryonic tissue are regulated, in part, by the change in IGF- I levels.

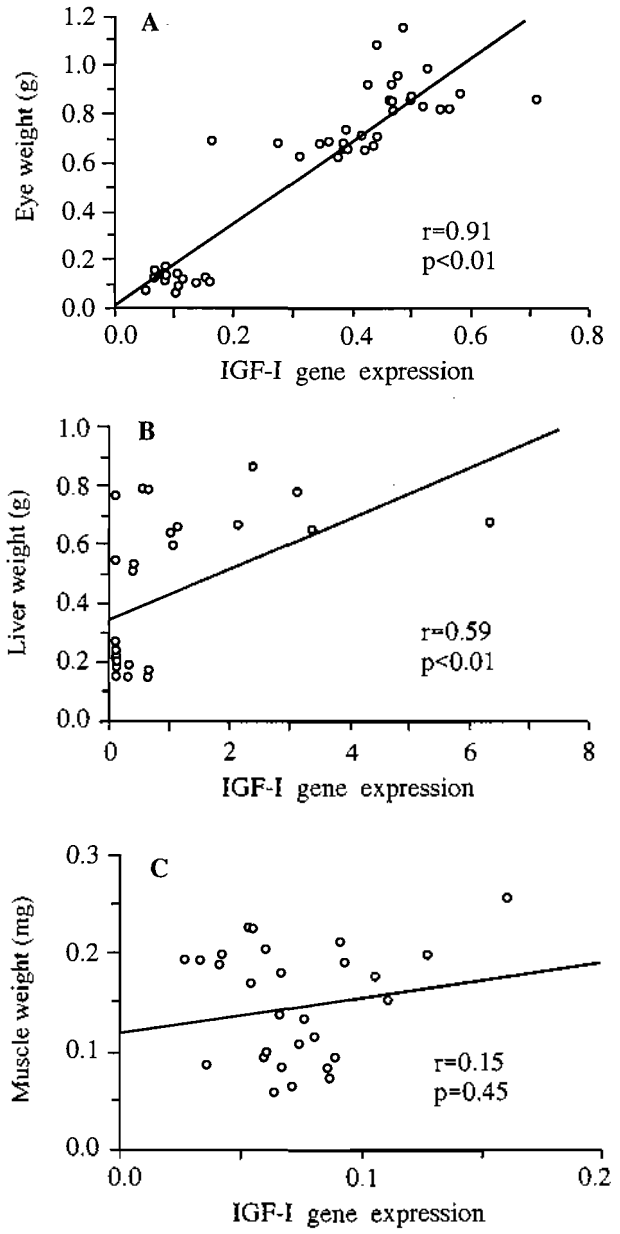


**Figure 4.** The correlation between tissue weight and IGF-I gene expression in chicken embryos. The quantitative ratio of IGF-I mRNA to $\beta$-actin mRNA was used as IGF-I gene expression in eyes (A), liver (B) and muscle (C). Tissue weight and IGF-I gene expression were measured at 7, 14 and 20 days of incubation. Embryos were derived from dwarf egg-type, normal egg-type chickens and meat-type chickens.

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