

Association of Insulin-Like Growth Factor-I (IGF-I) Gene Polymorphism with Serum IGF-I Concentration and Body Weight in Korean Native Ogol Chicken

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ABSTRACT : IGF-I is involved in the regulation of growth and differentiation in mammals, but its role as a modulator of growth and metabolism in poultry is poorly understood. And, no studies have so far been reported for the comparison between serum IGF-I concentration and body growth in the egg type or the dual purposes (meat and egg type) chicken including the Korean Native Ogol Chicken (KNOC). Therefore, in order to improve the body growth and meat production of the KNOC, this study was conducted for the identification of the polymorphism of IGF-I gene and for its possible association with both body weight and IGF-I concentration. The RFLP patterns for IGF-I gene were identified by the *Pst*I restriction enzyme. The frequencies of +/+, +/-, and -/- genotype were 16.9%, 51.7%, and 31.4%, respectively. Any statistical significance was not observed in all variations except for sex variation ($p < 0.01$) by covariate quadratic model. The significant effect of the IGF-I genotype on body weight by sex indicates that there are different physiological characteristics in gender. Although the body weights of male KNOCs in most ages were not significant, there was a tendency of KNOCs with +/+ IGF-I genotype to be heavier than those with any other genotypes. But all IGF-I genotypes in female did not influence on body weight. The ANOVA revealed no significant effects of IGF-I genotypes on serum IGF-I concentration but sex effect was highly significant on the IGF-I concentration at 20 and 40 weeks ($p < 0.01$). Although the +/+ genotype, in gender, tended to express a higher IGF-I concentration than the other genotypes at all ages in males, a statistical difference among the genotypes was not found except for 60 weeks ($p < 0.05$). Furthermore, since body weight and IGF-I genotypes are associated, it is possible to improve KNOC to a meat type breed if a continuous selection can be made for the body weight and/or IGF-I traits. (*Asian-Aust. J. Anim. Sci.* 2001, Vol 14, No. 7: 915-921)

Key Words : IGF-I, RFLP, Body Weight, Korean Native Ogol Chicken

INTRODUCTION

Korean Native Ogol Chicken (KNOC) is one of the most famous fowl in Korean indigenous livestock. In the view of genetic resources conservation, the genetic analysis of the KNOC is valuable. And the improvement of its genetic characteristics can be a means to further develop the poultry industry in Korea. Therefore, scientists have analyzed genetic characteristics to improve the economic traits of the KNOC. Han et al. (1988) provided the research results on meat production ability and on genetic correlations between economic traits in the KNOC. However, the genetic variability of the KNOC has not been analyzed in detail at the gene (DNA) level, although there were some studies with the KNOC using molecular biology techniques (Lee et al., 1995; Seo et al., 1995; Hwang, 1996).

The meat production ability is closely associated with muscle growth. Recent researches on polypeptides growth factors have identified several growth factors, such as IGFs, epidermal growth factor (EGF), transforming growth factors (TGFs), and platelet-derived growth factor (PDGF), as modulators of muscle (Florini et al., 1996;

Duclos, 1998). There is considerable evidence that IGF-I is involved in the regulation of growth and differentiation in mammals, but its role in determining the extremely rapid growth of young meat-type chickens is unknown. However, Baker et al. (1991) and Siddiqui et al. (1992) reported that plasma IGF-I concentration is a heritable character by repeated divergent selection for high or low circulating IGF-I concentrations in rats. In particular, they reported a positive correlation between IGF-I concentration and body weight. Thus, these findings suggest the possibility of selection for body weight using plasma IGF-I concentration.

Chicken IGF-I is structurally similar to mammalian IGF-I, but there are some fundamental differences in IGF-I physiology between mammals and poultry (Daughaday et al., 1985; Buonomo et al., 1987). Although absolute IGF-I concentrations in poultry appear to be lower than that in mammals (Daughaday et al., 1985), the plasma concentration of IGF-I increases with age in chickens (Huybrechts et al., 1985). Moreover, its role as a modulator of growth and metabolism in poultry is poorly understood (McMurtry, 1994).

Recent research by Kang et al. (2000) into the association between insulin-like growth factor-I (IGF-I) and egg productivity of the KNOC has shown that IGF-I is loosely related to egg productivity. The association of IGF-I concentrations with economic traits has been studied mainly in broilers (Goddard et al., 1988; Scanes et al., 1989). No studies have so far been reported for the comparison between

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serum IGF-I concentration and body growth in the egg type or the dual purposes (meat and egg type) chicken including the KNOC. However, Nagaraja et al. (2000) studied the association between feed intake, body weight, laying rate, and egg weight with IGF-I polymorphism and reported that it was possible to improve the economic traits by IGF-I genotypes in an unselected population.

So, in order to improve the body growth and meat production of the KNOC, this study was conducted for the identification of the polymorphism of IGF-I gene and for its possible association with both body weight and IGF-I concentration by weeks after sexual maturity.

MATERIALS AND METHODS

Animals

Unselected, randomly mated 89 KNOCs (19 males and 70 females) that were 15-weeks old were purchased from YeonSan Farm, the only place in Korea allowed to raise the pure breed of the KNOC. The KNOCs were vaccinated against Marek's disease, avian encephalomyelitis, Newcastle disease, and avian infectious bronchitis following standard procedures. The KNOCs were housed and reared in individual cages under a photoperiod of 15 hrs light : 9 hrs dark at 17 weeks. The step-up lighting system was conducted up to 17 hrs light : 7 hrs dark with a 15 min light increase every week (Ohh, 1988). The KNOCs were cared for in the Korea University Animal Breeding Station and were fed with a commercial diet (16.0% crude protein, 3.0% crude fat, 7.0% crude fiber, 15.0% crude ash, 3.0% calcium, 0.45% phosphate, 0.58% methionine + cysteine, 2.75 Mcal/kg metabolizable energy) ad libitum from 19 weeks to 60 weeks.

Blood and serum sampling

Blood samples were collected from a wing vein by 10-week intervals from 20 weeks. And blood collection was conducted at AM 11:00~PM 3:00. Sera were made by allowing collected blood samples to stand at room temperature for 1 to 2 hrs, to be centrifuged at 1000×g for 20 min. And sera were aliquoted, stored at -70°C, and assayed at the same time to avoid inter-assay variation (Daughaday et al., 1980).

IGF-I iodination

One microgram of recombinant human IGF-I (GroPep Pty Ltd.) was iodinated to a specific activity of 270~300 µCi/µg protein using 1 mCi Na [¹²⁵I] (Amersham) by the chloramine T method (Lee and Henricks, 1990). Iodinated IGF-I was purified on a Sephadex G-50 column and stored at -20°C until used.

IGF-I radioimmunoassay (RIA)

Serum IGFBPs were removed using the acid-ethanol method (Daughaday et al., 1980). Briefly, 0.2 ml of each sample was acidified with 0.8 ml of acid-ethanol (87.5% ethanol, 12.5% HCl) and stabilized for 30 min at room temperature. After samples were centrifuged at 1,000 × g for 30 min, the supernates were neutralized with 0.2 ml of 0.855 M Tris-base. IGFBPs-removed supernatant was added to 0.1 ml of RIA buffer (30 mM sodium phosphate, 0.02% protamine sulfate, 10 mM EDTA, 0.05% Tween-20, 0.02% sodium azide ; pH 7.5), incubated with rabbit anti-human IGF-I polyclonal antiserum (GroPep Pty Ltd.), and 20,000 cpm [¹²⁵I] IGF-I in RIA buffer for 16 h at 4°C. Then, 0.1 ml of goat anti-rabbit IgG antibody (GroPep Pty Ltd.) was added and the mixture was incubated for 1 hr followed by additional incubation with 0.1 ml of normal rabbit serum at 4°C. After the addition of 1 ml RIA buffer, the tubes were centrifuged for 30 min at 1,000 × g at 4°C. The supernatant was aspirated and the pellet was measured for its radioactivity in a gamma-counter. The IGF-I amount was determined by logit - log plots and the intra-assay coefficient of variation was 7.8%.

DNA extraction

KNOC genomic DNA was extracted from the clotted blood (Seo et al., 1999). In brief, 250 µl of lysis solution (360 µg/ml proteinase K, 150 mM sodium chloride, 50 mM EDTA, 2% SDS) was mixed to lyse the clotted blood and the mixture was incubated at 55°C for 3 h. After 5.5 M NaCl and 600 µl of phenol : chloroform (25 : 24) were added, the mixture was centrifuged for 10 min at 5,000 × g. The supernatant was mixed with absolute ethanol and the mixture was centrifuged again at the same condition. The pellet was dried, resuspended with TE solution (10 mM Tris-Cl, 1 mM EDTA), and stored at -20°C.

IGF-I RFLP

IGF-I genotypes were analyzed with PCR-RFLP using primers as reported (Nagaraja et al., 2000). Primers were as follows: Forward 5'-GAC TAT ACA GAA AGA ACC CAC-3', Reverse 5'-TAT CAC TCA AGT GGC TCA AGT-3'. The PCR reaction was performed with AccuPower™ Premix-Top (BIONEER Co., Korea) including 50 ng of extracted DNA under the following conditions: 1 cycle at 94°C for 4 min; 30 cycles of 94°C for 1 min, 61°C for 2 min, and 72°C for 1.5 min; 1 cycle at 72°C for 8 min ; hold at 4°C in a GeneAmp PCR System 2400 (Perkin Elmer Co.). PCR products were digested with *Pst*I and separated using 10% polyacrylamide gel electrophoresis for RFLP analysis.

Statistical analysis

IGF-I gene and genotype frequency : Simple gene counting method was used to determine an estimate of IGF-I

gene and genotype frequency (Pirchner, 1983), and the formula was as follows:

$$PA = (2AA+AB) / 2N,$$

$$PB = 1 - PA$$

where, PA = + gene frequency;
 PB = - gene frequency;
 AA = number of ++ genotype;
 AB = number of +/- genotype; and,
 N = total number of chickens.

Statistical model : To evaluate the effects of sex and genetic locus on body weight in the KNOC, the genotypes of 19 males and of 70 females were scored for genetic locus (IGF-I). The statistical model used for the effect of sex and genetic locus of IGF-I on the body weight was the covariate model using the SAS package (1996).

$$Y_{ijk} = \mu + s_i + g_j + sg_{ij} + b_1 IGF_k + b_2 IGF_k^2 + e_{ijk} \text{ ----- (Model 1)}$$

where; Y_{ijk} = body weight record on the kth chicken
 μ = overall mean;
 s_i = ith sex;
 g_j = jth IGF-I genotype;
 sg_{ij} = interaction effect between IGF-I genotypes and sex;
 IGF_k = serum IGF-I effect;
 IGF_k^2 = quadratic effect of serum IGF-I;
 b_1 = regression coefficient of serum IGF-I effect;
 b_2 = regression coefficient of quadratic serum IGF-I effect; and,
 e_{ijk} = error term.

And, to evaluate the effects of each sex and genetic locus on serum IGF-I concentration in the KNOC, the genotypes of 19 males and of 70 females were scored for the IGF-I locus. The statistical model (SAS, 1996) used for the analysis of the effect of IGF-I genotype on IGF-I concentration was given as;

$$Y_{ijk} = \mu + s_i + g_j + sg_{ij} + b BW_k + e_{ijk} \text{ ----- (Model 2)}$$

where; Y_{ijk} = serum IGF-I concentration record on the kth chicken;
 μ = overall mean;
 s_i = ith sex;
 g_j = jth IGF-I genotype;
 sg_{ij} = interaction between IGF-I genotypes and sex;
 BW_k = body weight;
 b = regression coefficient for BW; and,
 e_{ijk} = error term.

RESULTS

PCR-RFLP

The PCR-amplified product of the IGF-I locus was identified at 621 bp in 10% polyacrylamide gel (Data not shown). Figure 1 shows the RFLP patterns for PCR products by the *Pst*I restriction enzyme. The *Pst*I (-) allele revealed a single band at 621 bp, whereas the *Pst*I (+) allele revealed two bands at 364 bp and 257 bp. The resultant band patterns for each allele were similar to the reports by Nagaraja et al. (2000).

Frequency of IGF-I gene and genotypes

The result of the gene and genotype frequency for the IGF-I genetic loci in 89 KNOCs (70 females and 19 males) was given in table 1. The frequencies of ++, +/-, and -/- genotype were 16.9%, 51.7%, and 31.4%, respectively. And the overall gene frequencies for (+) and (-) alleles were 0.427 and 0.573, respectively, in KNOCs tested. But in the female group, the gene frequencies for (+) and (-) alleles were 0.421 and 0.579, respectively. In the male group, the gene frequencies for (+) and (-) alleles were 0.447 and 0.553. The observed distribution of genotypes was not different from the expected distribution under the assumption of the Hardy-Weinberg equilibrium for the IGF-I alleles.

Association between IGF-I genotypes and body weight

Table 2 shows the analysis of variance for body weight at

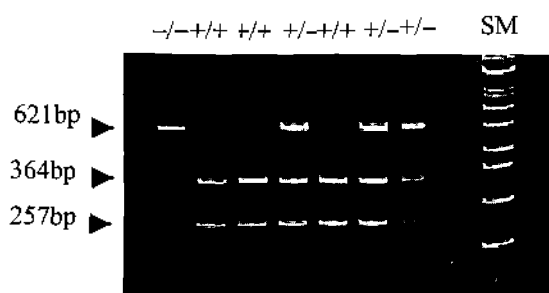


Figure 1. RFLP band patterns for insulin-like growth factor-I by *Pst* I restriction enzyme. SM: size marker (1kb ladder)

Table 1. Distribution of IGF-I genotypes and gene frequency determined by PCR-RFLP

Sex	No. of birds	No. of Genotypes (%)			Gene frequency	
		+/+	+/-	-/-	+	-
Male	19	3 (15.8)	11 (57.9)	5 (26.3)	0.447	0.553
Female	70	12 (17.1)	35 (50.0)	23 (32.9)	0.421	0.579
Total	89	15 (16.9)	46 (51.7)	28 (31.4)	0.427	0.573

Table 2. ANOVA table for body weight at different ages

Weeks	S. V. ¹⁾	d.f	Type III SS	Mean SS	F-value	P-value
20 weeks	Sex	1	1,836,105	1,836,105	97.61	0.0001**
	Genotype	2	50,119	25,059	1.33	0.2706
	Sex × Genotype	2	76,808	38,404	2.04	0.1376
	IGF20	1	27,145	27,145	1.44	0.2338
	IGF20 × IGF20	1	54,036	54,036	2.87	0.0946
30 weeks	Sex	1	1,862,532	1,862,532	46.77	0.0001**
	Genotype	2	154,987	77,493	1.95	0.1501
	Sex × Genotype	2	173,083	86,541	2.17	0.1210
	IGF30	1	2,352	2,352	0.06	0.8086
	IGF30 × IGF30	1	3,310	3,310	0.08	0.7739
40 weeks	Sex	1	1,411,675	1,411,675	29.07	0.0001**
	Genotype	2	213,792	106,896	2.20	0.1181
	Sex × Genotype	2	187,230	93,615	1.93	0.1530
	IGF40	1	11,825	11,825	0.24	0.6232
	IGF40 × IGF40	1	14,624	14,624	0.30	0.5849
50 weeks	Sex	1	1,334,518	1,334,518	22.78	0.0001**
	Genotype	2	81,525	40,762	0.70	0.5020
	Sex × Genotype	2	262,129	131,064	2.24	0.1140
	IGF50	1	1,712	1,712	0.03	0.8647
	IGF50 × IGF50	1	5,728	5,728	0.10	0.7554
60 weeks	Sex	1	875,990	875,990	13.83	0.0004**
	Genotype	2	66,530	33,265	0.53	0.5936
	Sex × Genotype	2	204,630	102,315	1.62	0.2057
	IGF60	1	33,223	33,223	0.52	0.4712
	IGF60 × IGF60	1	47,958	47,958	0.76	0.3870

** indicates statistically significant differences ($p < 0.01$); ¹⁾ S.V. : Source of variation.

Table 3. Least-squares means of body weight for IGF-I genotypes by sex

Sex	Genotype	No. of bird	Body weight (g)				
			20weeks	30weeks	40weeks	50weeks	60weeks
Male	+/+	3	1,696 ± 116 ¹⁾	2,058 ± 138 ^a	2,070 ± 164	2,139 ± 144	2,036 ± 127
	+/-	11	1,536 ± 57	1,755 ± 75 ^{ab}	1,895 ± 80	1,985 ± 77	1,895 ± 69
	-/-	5	1,455 ± 88	1,684 ± 102 ^a	1,775 ± 127	1,840 ± 129	1,778 ± 113
Female	+/+	12	1,104 ± 39	1,368 ± 60	1,476 ± 67	1,548 ± 76	1,504 ± 84
	+/-	35	1,182 ± 22	1,436 ± 34	1,546 ± 40	1,640 ± 44	1,626 ± 48
	-/-	23	1,118 ± 25	1,392 ± 40	1,469 ± 47	1,658 ± 51	1,638 ± 55

¹⁾ Standard error.

Superscripts with different letters in the same column significantly differ ($p < 0.05$).

different ages by covariate quadratic model (Model 1). Any statistical significance was not observed in all variations except for sex variation ($p < 0.01$). The significant effect of the IGF-I genotype on body weight by sex indicates that there are different physiological characteristics in gender. Table 3 shows the least squares means of body weight for IGF-I genotype by sexes at different ages. Differences were not found in all ages except for the body weight of a male at 30 weeks ($p < 0.05$). Although the body weights of male KNOCs in most ages were not significant, there was a tendency of KNOCs with +/+ IGF-I genotype to be heavier than those with any other genotypes (+/-, -/-). The body weights of female chickens with +/- genotype were heavier at 20, 30, and 40 weeks, and with -/- genotype were heavier

at 50, 60 weeks than at other ages. But all IGF-I genotypes in female did not influence on body weight.

Association between IGF-I genotypes and serum IGF-I concentration

Table 4 shows the analysis of variance for serum IGF-I concentration at different ages by Model 2. The ANOVA revealed no significant effects of IGF-I genotypes on serum IGF-I concentration but sex effect was highly significant on the IGF-I concentration at 20 and 40 weeks ($p < 0.01$).

The least squares means of serum IGF-I concentration for the IGF-I genotypes were shown in table 5. Although the +/- genotype, in gender, tended to express a higher IGF-I concentration than the other genotypes at all ages in males, a statistical difference among the genotypes was not found

Table 4. ANOVA table for serum IGF-I concentration at different ages

Weeks	S. V. ¹⁾	d.f	Type III SS	Mean SS	F-value	P-value
20 weeks	Sex	1	1,631	1,631	16.99	0.0001**
	Genotype	2	189	94	0.98	0.3789
	Sex×Genotype	2	45	22	0.23	0.7912
	BW20	1	249	249	2.59	0.1118
30 weeks	Sex	1	3	3	0.05	0.8230
	Genotype	2	110	55	0.93	0.4000
	Sex×Genotype	2	50	25	0.42	0.6583
	BW30	1	7	7	0.11	0.7380
40 weeks	Sex	1	674	674	13.65	0.0004**
	Genotype	2	121	60	1.23	0.2985
	Sex×Genotype	2	136	68	1.38	0.2585
	BW40	1	6	6	0.11	0.7358
50 weeks	Sex	1	11	11	0.06	0.8033
	Genotype	2	19	10	0.06	0.9462
	Sex×Genotype	2	0.06	0.03	0.00	0.9998
	BW50	1	40	40	0.23	0.6297
60 weeks	Sex	1	209	209	1.97	0.1643
	Genotype	2	376	188	1.78	0.1762
	Sex×Genotype	2	307	153	1.45	0.2407
	BW60	1	44	44	0.42	0.5173

¹⁾ S. V. : Source of variation.

** indicates statistically significant differences (p<0.01).

Table 5. Least-squares means of serum IGF-I concentrations analyzed for IGF-I genotypes by sex

Sex	Genotype	No. of bird	IGF-I concentration (ng/ml)				
			20 weeks	30 weeks	40 weeks	50 weeks	60 weeks
Male	+/+	3	52.7±5.2 ¹⁾	38.3±4.5	31.3±3.4	26.6±4.3	34.1±3.7 ^a
	+/-	11	46.9±2.7	29.7±2.3	24.2±1.7	25.2±2.1	24.0±1.9 ^{ab}
	-/-	5	44.6±4.0	30.4±3.4	24.3±2.9	25.0±3.7	32.4±3.3 ^b
Female	+/+	12	35.9±3.4	35.4±2.4	34.9±2.3 ^a	25.1±4.5	27.5±3.5
	+/-	35	35.2±2.0	33.4±1.4	34.2±1.4 ^{ab}	25.0±2.6	23.7±2.0
	-/-	23	32.5±2.2	32.5±1.6	38.6±1.6 ^b	26.3±2.9	22.2±2.3

¹⁾ Standard error

Superscripts with different letters in the same column significantly differ (p<0.05).

except for 60 weeks (p<0.05). Similarly, serum IGF-I concentrations of female KNOCs for the IGF-I genotypes were significantly different only at 40 weeks (p<0.05).

DISCUSSION

The constituent part of the growth hormone (GH) axis affects a wide range of biological processes such as growth, differentiation, reproduction, immune responsiveness, and aging. GH released from the anterior pituitary gland may act either directly on muscle or other target tissues or indirectly by releasing IGF-I from the liver. Along with specific cell surface receptors and binding proteins, IGF-I provides a complex regulatory network. Thus, IGF-I is thought to be a positive modulator in body growth and muscle development in many species.

The chicken IGF-I gene has been shown to be in the short arm of chromosome 1 near the centromere and to be conserved in several vertebrate species (Klein et al., 1996). Also, the RFLP analysis of the chicken IGF-I gene

revealed a single *Pst*I polymorphism in the 5' region (Nagaraja et al., 2000). In this study, the *Pst*I - digested PCR product for the IGF-I gene revealed polymorphic fragments of 257 bp, 364 bp, and 621 bp (figure 1), which is consistent with the previous reports by Nagaraja et al. (2000). The comparison for two allele sequences indicated the loss of the *Pst*I restriction enzyme site (CTGCA↓G) by point mutation.

The result of the gene and genotype frequency for the IGF-I genetic locus in this study with the KNOCs is somewhat different from the report with unselected White Leghorn (Nagaraja et al., 2000). IGF-I genotype was not associated with a body weight but egg and eggshell weight in White Leghorns, while *Pst*I (+/+) genotype in male KNOC tended to express a higher body weight than the other genotypes.

Such differences may reflect differences in hereditary characters among chicken breeds because the genetic distance is long between the White Leghorn and the KNOC. It is likely to say that the allele frequency of IGF-I in the White Leghorn breed is biased since the breed has been

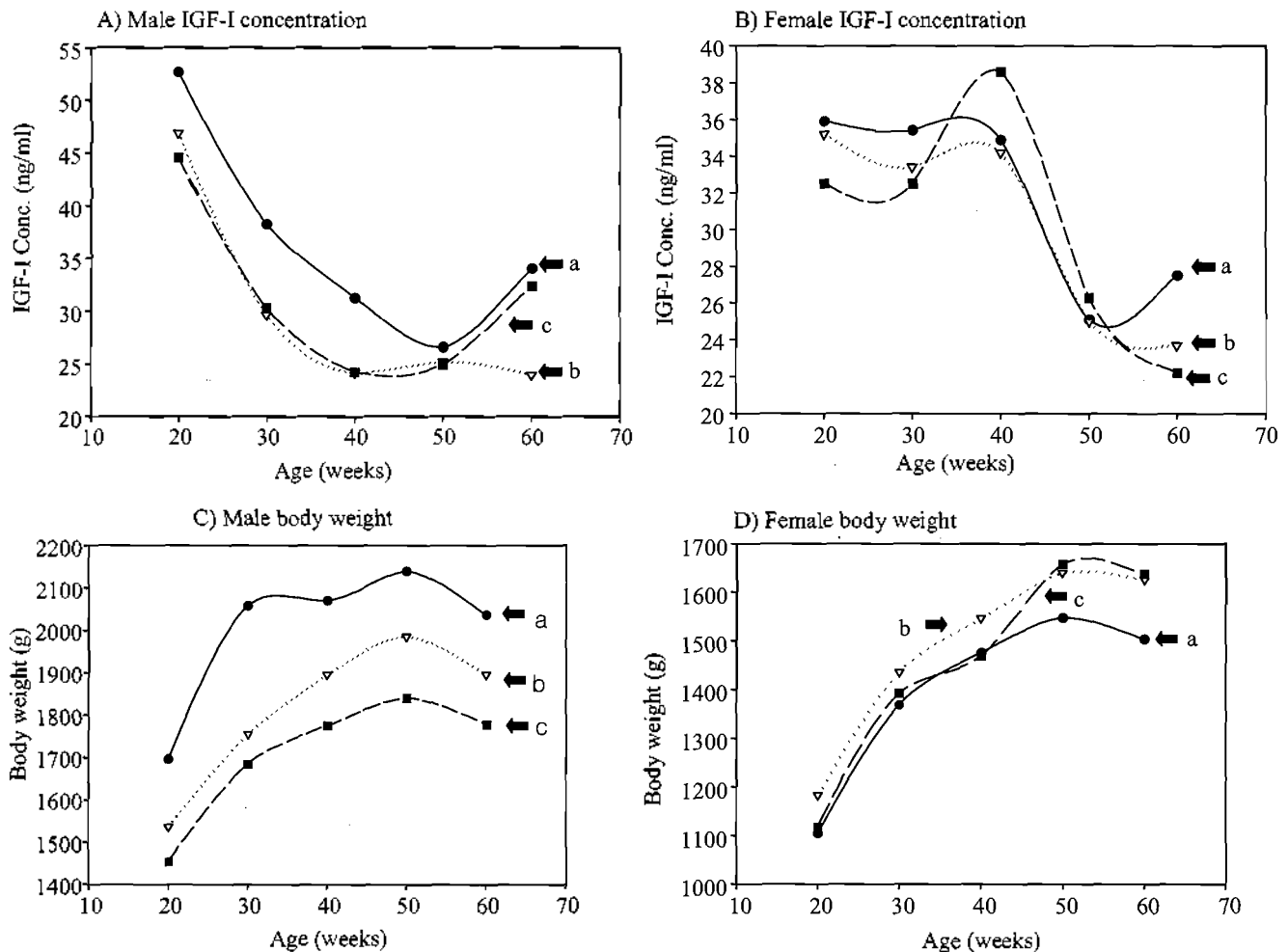


Figure 2. Change of IGF-I concentration and body weight gain by IGF-I genotypes after sexual maturation. Arrow a: +/+ genotype; arrow b: +/- genotype; arrow c: -/- genotype.

selected to improve the egg productivity using schematic breeding programs. However, since KNOCs have been conserved for dual purposes of meat and eggs, the allele frequency is not biased.

The IGF-I has been reported to have significant effects on the overall body and muscle growth in chicken (Duclos et al., 1999). And the study of repeated divergent selection for high or low circulating IGF-I concentration concluded that the IGF-I concentration is a heritable character in rat (Siddiqui et al., 1992). In this study, the IGF-I expression was significantly different depending on the sex but not by the genotypes. Although there were the limitations due to small sample size, the finding that male IGF-I expression in +/+ genotype is higher than in other genotypes in all ages implies that +/+ genotype is more closely associated with the IGF-I expression than the other genotypes in the male KNOC (figure 2A). However, any distinct tendency of IGF-I levels by the genotypes was not observed in the female KNOC (figure 2B). Thus, analyses of family and individual are required for a more precise

estimation of the genetic parameters in the KNOC. There was a report that IGF-I levels were significantly higher for the normal strain (White Leghorn) as compared to the fast growing strain (Rhode Island Red) (Goddard et al., 1988).

In contrast, any statistical associations of serum IGF-I levels and body weight gain with +/+ IGF-I genotype at all of ages except for 60 week old of the male KNOCs were not detected in this study (tables 3 and 5), although +/+ IGF-I genotype had positive effects on body weight gain (figure 2A, C) similar to White Leghorn layer (Nagaraja et al., 2000). These conflict results are seem to be contributed by differences in the degree of selection for any particular economic traits of breeds.

On the contrary, the female KNOC did not show a tendency between body weight and serum IGF-I concentration by IGF-I genotypes (figure 2B, D). Therefore, all these results in this study suggest that serum IGF-I plays dual roles (McMurtry et al., 1997; Kang et al., 2000) depending on the sex: serum IGF-I is important for body growth in the male KNOC whereas the IGF-I is important for

egg production in the female KNOC.

Accordingly, it is necessary to develop a characterized feed for the preservation of KNOC characteristics that is a dual (egg and meat) purposes breed and for the minimization of physiological difference in sex variation. Furthermore, since body weight and IGF-I genotypes are associated, it is possible to improve KNOC from a dual purposes breed to a meat type breed if a continuous selection can be made for the body weight and/or IGF-I traits. In addition, the fact that IGF-I acts primarily as a paracrine/autocrine growth factor could supplement studies on the tissue content of IGF-I in normal and fast growing KNOC strains to address this possibility.

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