Developmental Changes of Plasma Insulin, Glucagon, Insulin-like Growth Factors, Thyroid Hormones, and Glucose Concentrations in Chick Embryos and Hatched Chicks

J. W. Lu,* J. P. McMurtry,† and C. N. Coon*[1]

*Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701; and †USDA, ARS, Growth Biology Laboratory, Beltsville, MD 20705

ABSTRACT Developmental hormonal changes in Cobb 500 chick embryos and hatched chicks were determined by measuring plasma insulin, glucagon, insulin-like growth factor (IGF)-I, IGF-II, triiodothyronine, thyroxine, and glucose concentrations at different ages of embryogenesis and posthatch development. Plasma samples were obtained daily from 10 d of embryogenesis (10E) through 13 d posthatch and also at 17 and 21 d posthatch. A significant increase in plasma insulin was observed with increasing age from 10E to hatch. Plasma glucagon levels remained low until 17E, and then significantly increased approximately 3-fold at hatch, which corresponded with increasing plasma glucose levels during late embryo development. The plasma insulin to glucagon molar ratio of incubation from 14E to 17E ranged from 2 to 4, and was significantly higher than at any other time during incubation. These results indicate that insulin may be an important promoter of chick embryonic growth by the anabolic drive to promote protein deposition. Insulin and glucagon increased after hatch, which may be due to increased feed consumption and increased utilization of carbohydrates as the key energy source, compared with nutrients obtained through lipolysis and proteolysis in the embryos. Plasma triiodothyronine increased 4-fold from 18E to 20E, and thyroxine increased 3-fold from 16E to 19E. Insulin-like growth factor-I and IGF-II peaked at 14E. Insulin-like growth factor-I steadily increased above embryonic levels during the 3 wk of the posthatch period, whereas IGF-II levels steadily declined. These results suggest that IGF-II may be a more important functionary for chick embryonic development than IGF-I, and that IGF-I may be more important than IGF-II after hatch. The profile of metabolic hormones in the present study may help support an understanding of significant changes that occur in embryonic development and posthatch growth in chicks.

Key words: chick embryo, insulin, glucagon, insulin-like growth factor, thyroid hormone

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INTRODUCTION

Previous research has shown that insulin is the key hormone regulating the concentrations of amino acids and related compounds in plasma, amniotic fluid, and allantoic fluid of 13-d-old chicken embryos (Hohlweg et al., 1999). Insulin has been shown to accelerate chick embryonic growth and morphological development (de Pablo et al., 1991). The thyroid hormones triiodothyronine ($\mathbf{T_3}$) and thyroxine ($\mathbf{T_4}$) are involved in numerous physiological processes in mammals and birds (Cogburn et al., 1989; McNabb, 2000). In addition, the thyroid hormones regulate heat production during the incubation of chick eggs (McNabb, 2000). The roles of IGF-I and IGF-II in poultry (de Pablo et al., 1991; McMurtry, 1998) are similar to those in mammals, including the pleiotropic effects on growth and development and on intermediary metabolism, although some unique differences between these 2 species exist (McMurtry et al., 1998). Both IGF-I and IGF-II stimulate hepatic glycogen, RNA, and protein synthesis in chick embryo hepatocytes, even though IGF-I is extrahepatic in origin (McMurtry, 1998). In addition, both IGF-I and IGF-II have been found in amniotic and allantoic fluid in chick embryos, and IGF-I may have a role in regulating amino compounds in the fluid of chick embryos (Karcher et al., 2005).

Developmental changes of the following hormones have been reported: growth hormone in late chick embryos and hatched chicks (Goddard et al., 1988; McGuinness and Cogburn, 1990); T_3 in late chick embryos and hatched chicks (Thommes and Hylka, 1977; Goddard et al., 1988; McGuinness and Cogburn, 1990); and IGF-I and IGF-II in turkey and chick embryos (Scanes et al., 1997; McMurtry et al., 1998), in turkey poults and chicks (Radecki et al., 1997; McMurtry et al., 1998), and in the amniotic and allantoic fluid of avian embryos (chick, duck, and turkey; Karcher et al., 2005). It has been suggested

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[1]Corresponding author: ccoon@uark.edu

Table 1. The effect of sex on plasma hormones and glucose in chick embryos and hatched chicks[1]

Item	Male	Female
Insulin (pg/mL)	674.80 ± 22.34[a]	726.27 ± 27.91[a]
Glucagon (pg/mL)	290.89 ± 12.74[a]	296.27 ± 13.75[a]
Glucose (mg/dL)	218.18 ± 3.22[a]	227.09 ± 5.50[a]
T_4 (ng/mL)	4.39 ± 0.18[a]	4.89 ± 0.17[b]
T_3 (pg/mL)	669.22 ± 28.00[a]	632.49 ± 26.95[a]
Insulin-like growth factor-I (ng/mL)	13.41 ± 0.41[a]	13.20 ± 0.43[a]
Insulin-like growth factor-II (ng/mL)	46.47 ± 1.58[a]	44.38 ± 1.38[a]
Insulin:glucagon molar ratio	2.26 ± 0.15[a]	2.69 ± 0.19[a]

[a–b]Means within a row lacking a common superscript are different ($P < 0.05$).

[1]Each value is mean ± SEM (n = 210). Ten male and 10 female samples were taken daily from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. Therefore, the number of samples for each sex is 210. T_4, thyroxine; T_3, triiodothyronine.

that growth hormone is unlikely to stimulate embryo growth because circulating concentrations of growth hormone are very low in the embryo (Hazelwood, 2000). Insulin immunoreactivity has been found in chicken eggs (de Pablo et al., 1991), and pancreas insulin levels have been reported in chick embryos (Hazelwood, 2000). However, plasma insulin, glucagon, and the insulin:glucagon molar ratio (**intogl**) in developing chicks have not been reported during consecutive days of embryonic development. The importance of glucagon and IGF-II during chick embryonic development is not well established. Studies that have simultaneously addressed multiple metabolic hormone levels in broiler chick embryos and hatched chicks are limited. The objective of the present experiment was to provide a developmental profile of plasma insulin, glucagon, intogl, glucose, T_3, T_4, IGF-I, and IGF-II levels in chick embryos and hatched chicks.

MATERIALS AND METHODS

Animal Care

Hatching eggs from a flock of 39-wk-old Cobb 500 females being fed a standard Breeder I diet supplied by Cobb-Vantress (Siloam Springs, AR) were collected and incubated in a model 252 incubator and hatcher (Jamesway Co., Fort Atkinson, WI) at the University of Arkansas hatchery on the poultry research farm. The hatching eggs were incubated and hatched with a temperature of 37.8°C and 60% RH. Hatched chicks were reared for 3 wk and fed a standard corn-soybean chick starter diet (22% CP, 3,000 kcal/kg, 0.90% TSAA, 1.20% Lys) that met or exceeded minimum NRC (1994) standards. Feed and water were provided ad libitum during the 3 wk posthatch. A 24-h constant light schedule was maintained. The temperature of the environmental room for hatched chicks was set at 35°C on d 1, 32°C from d 2 to 7, 29°C from d 8 to 14, and 26°C from d 15 to 21.

Sample Collection

Blood samples were obtained daily by cardiac puncture from 10 d of embryogenesis (**10E**) through 13 d after hatch (**13H**). Hatched chicks were also sampled by cardiac puncture at 17H and 21H. The plasma sample was obtained from EDTA-treated whole blood. Embryos and young chicks up to 7 d posthatch were pooled (4 to 10 for embryos; 2 to 3 for chicks) to acquire 2,000 μL of plasma per sample. Ten plasma samples were collected from 10E to 15E for each day. Beginning with 16E, all embryos and hatched chicks were sexed and 20 plasma samples were obtained (10 males and 10 females) for each day.

Hormones and Glucose Assays

Specific RIA were used to determine plasma hormone concentrations. All samples were analyzed within 1 assay to avoid interassay variations. Double-antibody RIA were used to determine plasma concentrations of IGF-I, with an intraassay CV of 2.8% (McMurtry et al., 1994); chicken IGF-II, with an intraassay CV of 3.7% (McMurtry et al., 1998); and insulin, with an intraassay CV of 2.2% (McMurtry et al., 1983). Triiodothyronine and T_4 were determined as previously described (McMurtry et al., 1988) and had CV of 2.5 and 2.8%, respectively. Plasma glucagon was determined using commercial kits (Linco Research, Inc., St. Charles, MO), with an intraassay CV of 1.9%. For glucagon analysis, an aliquot of plasma was stored in the presence of 1,000 kIU of aprotinin. Colorimetric enzymatic assay kits (Sigma Chemical Co., St. Louis, MO) were used to determine plasma levels of glucose.

Statistical Analysis

All the data were subjected to ANOVA analysis using SAS procedures (SAS Institute, Cary, NC). Differences between means were compared using the Duncan multiple range test. There were no significant differences in hormone levels between males and females, except for T_4. The plasma hormone levels of males and females were pooled to increase statistical sensitivity. The correlation coefficients between BW and hormones in chick embryos and hatched chicks were determined by the CORR procedure of SAS.

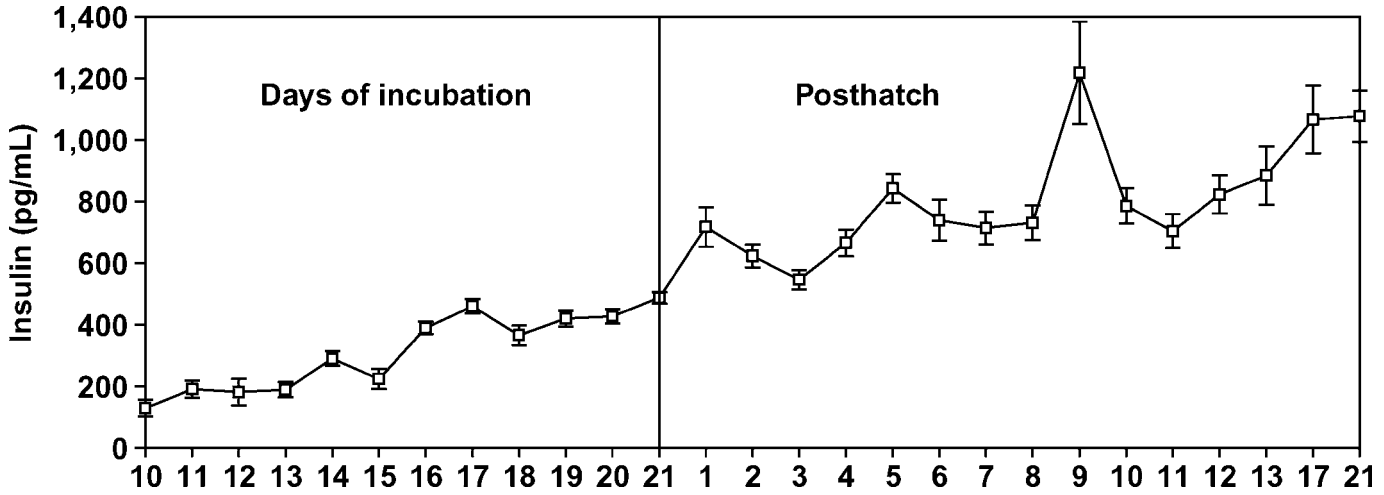

Figure 1. Developmental changes in plasma insulin of chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 d to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in plasma insulin.

RESULTS

Sex

There were no significant effects of sex on plasma insulin, glucagon, intogl, T_3, IGF-I, IGF-II, and glucose levels in chick embryos and hatched chicks (Table 1). Plasma levels of T_4 in hatched chicks, however, were significantly affected by the difference in sex (Table 1 and Figure 6). Significantly higher plasma levels of T_4 were observed in female chicks at 6H, 8H, and 17H than in male chicks (Figure 6).

Insulin

Plasma insulin levels increased significantly with increasing age from 10E (130 pg/mL) to 9H (1217 pg/mL; Figure 1). Four peaks were observed (460 pg/mL at 17E; 717 pg/mL at 1H; 842 pg/mL at 5H; and 1,217 pg/mL at 9H), with a plateau during pipping and hatch. The first day posthatch after 24 h of feed consumption significantly increased the insulin levels by approximately 50% compared with newly hatched chicks. After the peak at 9H, the plasma insulin level decreased to 703 pg/mL at 11H, and then increased and reached 1,000 pg/mL at 17H.

Glucagon

A low plasma glucagon level (59 pg/mL) was detected in embryos at 10 d of incubation as compared with 130 pg/mL for insulin (Figure 2). A small peak (169 pg/mL) in glucagon during midincubation was noted in 12-d-old embryos. Glucagon levels increased from 15E and reached a significantly greater level at 18 d of incubation (205 pg/mL). During pipping and hatch, plasma gluca-

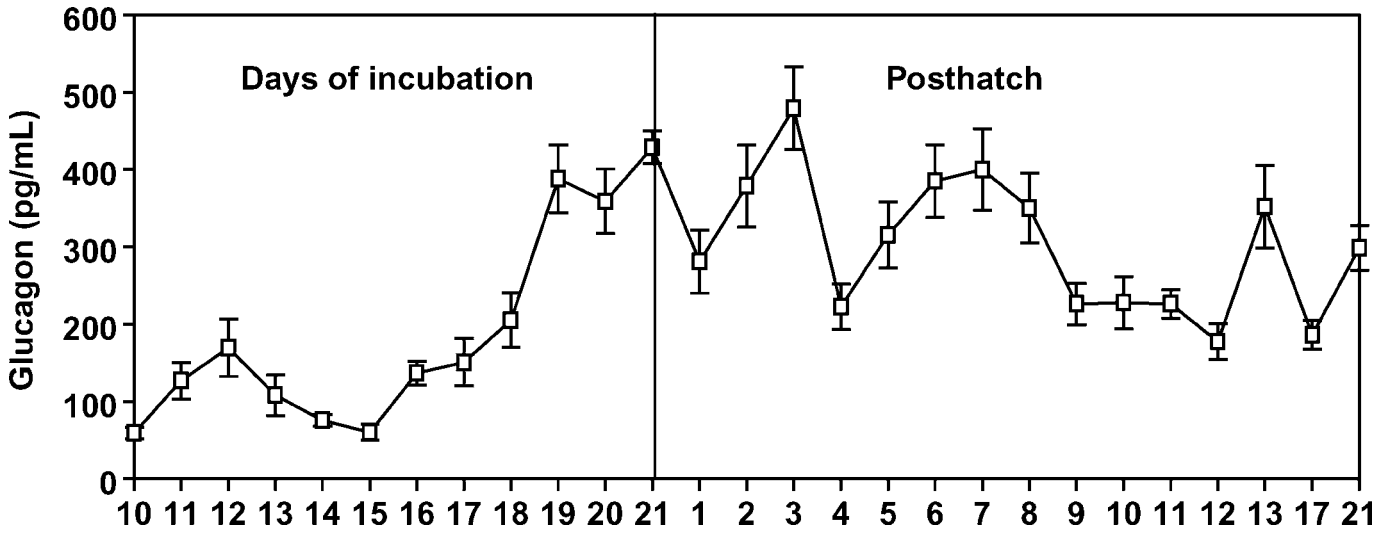

Figure 2. Developmental changes in plasma glucagon of chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 d to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in plasma glucagon.

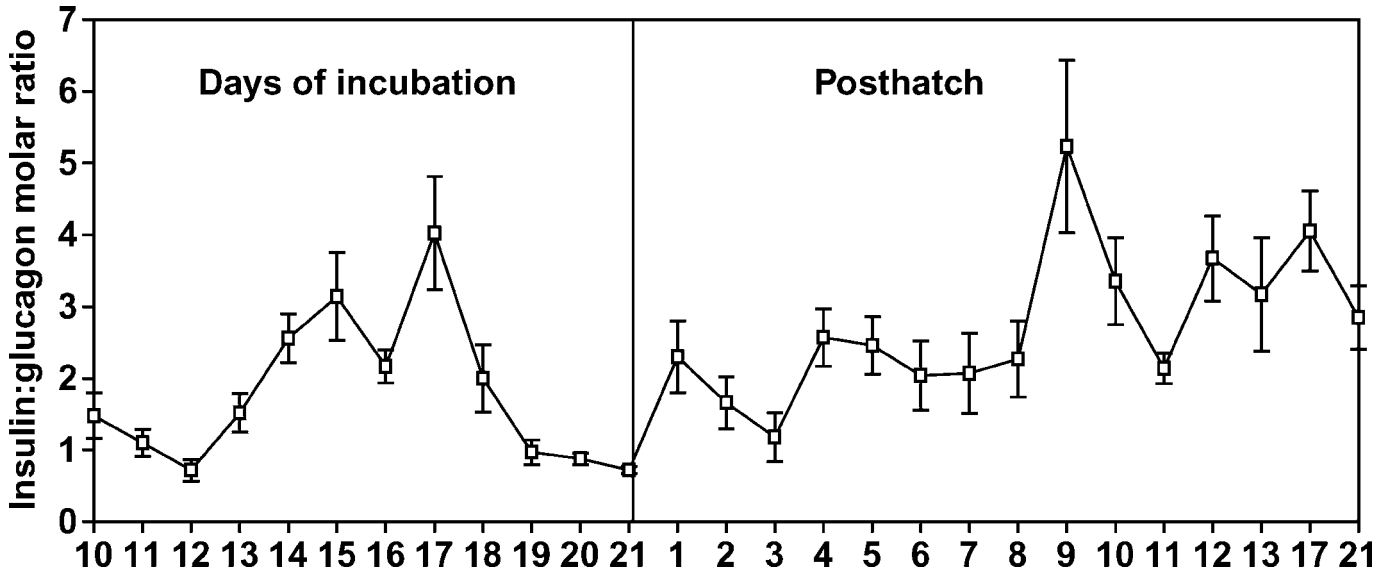

Figure 3. Developmental changes of the molar ratios of insulin to glucagon in chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 d to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in the molar ratios of insulin to glucagon.

gon levels continued to increase significantly (387 pg/mL at 19E; 429 pg/mL at hatch). Plasma levels of glucagon in chicks in the first day posthatch after 24 h of feed consumption were 40% lower compared with those of newly hatched chicks. Fluctuating plasma glucagon levels in chicks were observed during the 3 wk of feeding after hatch. Plasma glucagon levels in the first week posthatch were greater than those in the second and third weeks posthatch.

Insulin:Glucagon Molar Ratio

There was a significant increase in intogl from 12E (0.7, lowest among all observations) to 15E (3.1; Figure 3). The intogl from 14E to 18E ranged from 2.0 to 4.0, with a maximum of 4.0 at 17E. A significantly lower intogl was observed during pipping and hatch compared with intogl at 15E. The intogl in all hatched chicks was above 2.0, with a maximum of 5.2 at 9H with the exception of 2H and 3H (1.6 and 1.2, respectively). The intogl during the first week posthatch was lower than that in the second and third weeks posthatch.

Glucose

Plasma glucose levels increased significantly with age from 10E (116 mg/dL) to 3H (233 mg/dL; Figure 4), with a plateau during pipping and hatch. A significantly lower

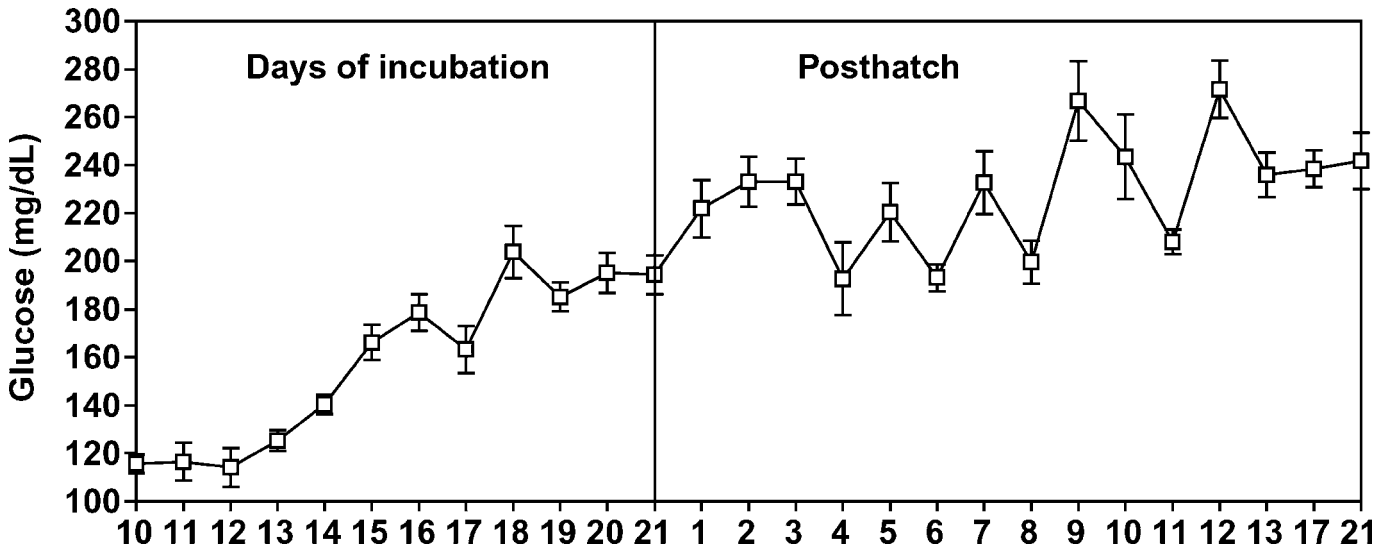

Figure 4. Developmental changes in plasma glucose of chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 d to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in plasma glucose.

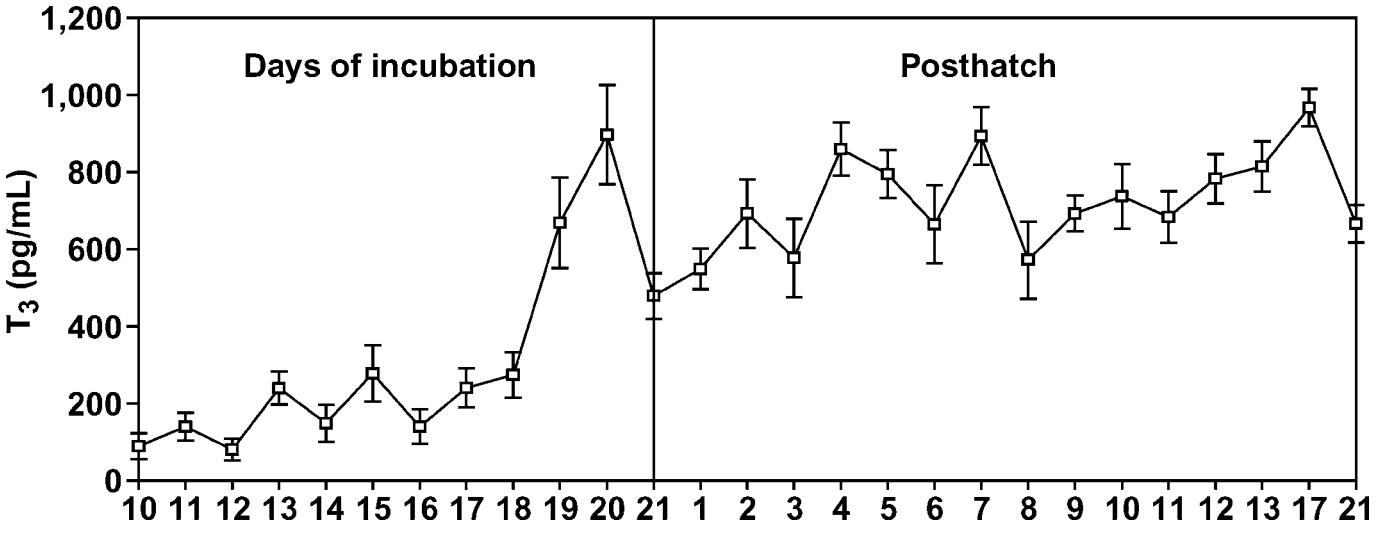

Figure 5. Developmental changes in plasma triiodothyronine (T_3) of chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 d to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in plasma T_3.

plasma glucose level was found at 17E compared with that at 16E or 18E. The first 24 h of feed consumption significantly increased glucose levels by approximately 20% as compared with glucose levels of newly hatched chicks. After 3H, chicks had fluctuating plasma glucose levels at the second week after hatch, which remained at approximately 240 mg/dL at 17H and 21 H.

T_3 and T_4

There were no significant changes in plasma T_3 levels during the middle period of incubation (Figure 5). Plasma T_3 significantly increased during pipping and hatch, compared with plasma T_3 in earlier embryos, and reached a peak at 20E (897 pg/mL). After hatch, chicks had significantly higher T_3 levels than did embryos before 18 d of incubation. Developmental changes in T_4 in sexed chick embryos and hatched chicks are presented in Figure 6. There was a significant effect of sex on plasma T_4. The lowest plasma T_4 level (0.2 ng/mL) was detected in 10-d-old embryos. Embryos had significantly higher T_4 levels at 15E and 16E than at 10E. Plasma T_4 continued to increase significantly at 17E and 18E compared with 15E and 16E, and reached a peak at 19E (6.2 ng/mL). After hatch, chicks exhibited a low T_4 plasma level for the first 4 d, and then elevated levels at 11H (7.9 ng/mL) and at 21H (7.7 ng/mL).

IGF-I and IGF-II

Plasma IGF-I levels increased significantly from 10E (4.4 ng/mL) to 14E (11.3 ng/mL) and then slowly decreased until hatch (5.0 ng/mL; Figure 7). A significant increase in the plasma IGF-I level was observed after hatch for each day up to 21H. Like IGF-I levels, plasma IGF-II levels increased significantly from 10E (34 ng/mL) to 14E (80 ng/mL). A significantly lower plasma IGF-II level was found at 15E (58 ng/mL) compared with that at 14E or 16E (72 ng/mL; Figure 8). Plasma IGF-II for the 17E to 21E period remained between 60 and 75 ng/mL, and levels then decreased posthatch to 25 ng/mL at 21H. Plasma levels of IGF-II were 5- to 12-fold higher than IGF-I in embryos.

Correlations Between BW and Plasma Hormones During Embryogenesis or the Posthatch Period

The correlation coefficients between plasma hormone levels and chick embryo BW during embryogenesis are shown in Table 2. The results indicated that embryonic BW was positively related to insulin (r = 0.6311, $P < 0.001$), glucagon (r = 0.6334, $P < 0.001$), T_3 (r = 0.5287, $P < 0.001$), and T_4 (r = 0.5583, $P < 0.001$) levels. Plasma insulin in chick embryos was positively related to glucagon (r = 0.4228, $P < 0.001$) and T_4 (r = 0.4642, $P < 0.001$) levels. Plasma glucagon in chick embryos was negatively related to intogl (r = –0.5273, $P < 0.001$).

The correlation coefficients between plasma hormone levels and chick BW during the posthatch period are presented in Table 2. Chick BW was positively related to insulin (r = 0.3458, $P < 0.001$) and T_4 (r = 0.4521, $P < 0.001$) levels. Hatched chick plasma insulin was positively related to intogl (r = 0.8014, $P < 0.001$) and T_4 (r = 0.4642, $P < 0.001$). Hatched chick plasma glucagon was negatively related to intogl (r = 0.6265, $P < 0.001$). There was a positive correlation between plasma T_4 and IGF-I in hatched chicks (r = 0.4549, $P < 0.001$).

DISCUSSION

Sex

No previous reports in which the plasma levels of insulin, glucagon, and IGF-II were separated for male and

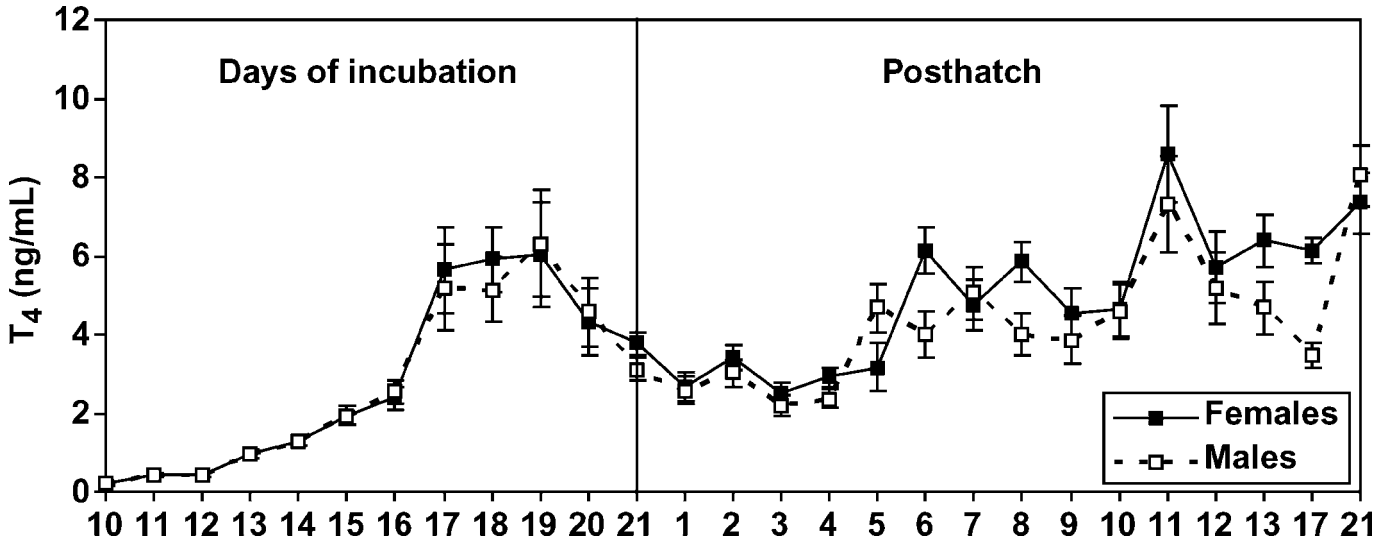

Figure 6. Developmental changes in plasma thyroxine (T_4) of sexed chick embryos and hatched chicks. Values are means ± SEM; n = 10 from 10 d to 15 d of incubation (not sexed); n = 10 from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch (sexed). There was a significant difference in T_4 between male and female chicks at 5, 6, 8, and 17 d posthatch.

female chicks were found to corroborate the nonsignificant differences reported herein (Table 1). Leenstra et al. (1991) reported no sex differences in plasma glucose levels for 2-, 4-, and 6-wk-old broilers, which would imply that a sex difference in plasma insulin and glucagon would not be expected. Human adult studies conducted by Vahl et al. (1997) reported no differences in the levels of serum insulin between men and women in a younger group (average age: 29 yr) and an older group (average age: 50 yr), which is also consistent with the data reported herein. Leenstra et al. (1991) reported no significant difference between the sexes for plasma IGF-I levels in 2- and 4-wk-old broilers or for T_3 levels in hatched broiler chicks. However, Leenstra et al. (1991) reported that 6-wk-old female broilers had higher plasma levels of IGF-I than did males, suggesting that the hormone may not be different in the 2 sexes until a specific level of maturity is reached. Leenstra et al. (1991) also reported higher T_4 levels in female broilers at 2, 4, and 6 wk of age, which agrees with the findings reported herein (Table 1 and Figure 6). However, Segal et al. (1982) reported that serum T_4 concentrations were higher in male rats than in female rats, but this may have been due to species difference. The mechanism involved that causes a sex difference in thyroid hormone concentrations remains unclear and needs further study.

Insulin

A previous study addressed the possible role of insulin as a growth factor in chick embryos (de Pablo et al., 1991), but a developmental profile of plasma insulin levels

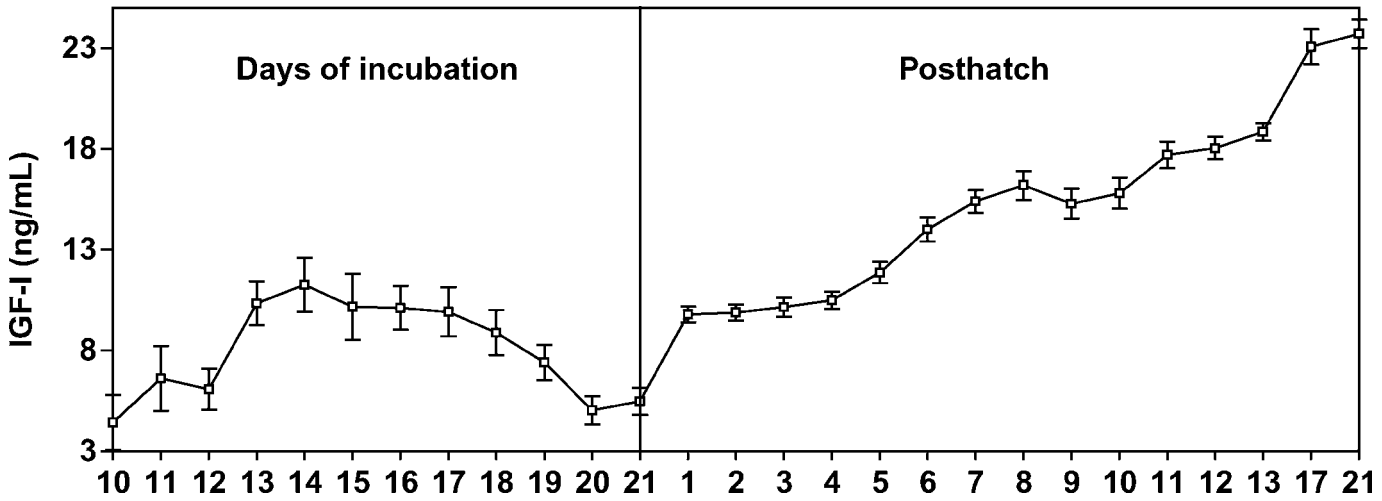

Figure 7. Developmental changes in plasma insulin-like growth factor-I (IGF-I) of chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 d to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in plasma IGF-I.

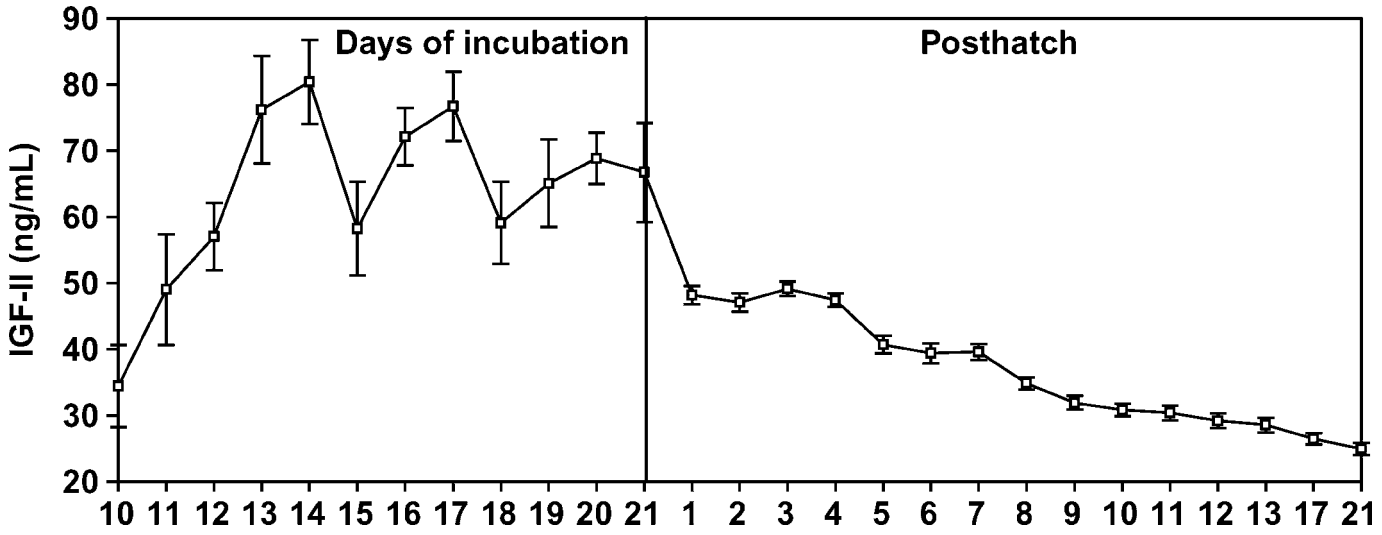

Figure 8. Developmental changes in plasma insulin-like growth factor-II (IGF-II) of chick embryos and hatched chicks. Values are means ± SEM; n = 10 (not sexed) from 10 to 15 d of incubation; n = 20 (10 males and 10 females) from 16 d of incubation to 13 d posthatch and at 17 and 21 d posthatch. There was no sex difference in plasma IGF-II.

during the major part of chick embryogenesis has not been reported. The plasma insulin levels increased significantly to 488 pg/mL at hatch, supporting the previous reports that pancreatic insulin mRNA increased at the end of embryogenesis (de Pablo et al., 1991). Our finding of increased plasma insulin levels with chick embryo age (Figure 1) is in accordance with other studies in which chick embryonic pancreatic insulin levels increased from 9 ng/mg at 5E to 100 ng/mg at 12E to 15E, and to 250 to 300 ng/mg at hatch (Hazelwood, 2000). Hohlweg et al. (1999) demonstrated that insulin levels in 13-d-old chick embryos appear to be much more sensitive to amino acid levels than to carbohydrate levels. The low sensitivity of insulin to carbohydrate in chick embryos is compatible with the report by McNabb (2000) suggesting that the importance of chick insulin for regulating carbohydrate metabolism is reduced, compared with the regulation of carbohydrate metabolism in mammals. Low plasma insulin levels at 15E in the present study may be due to a reduced protein or amino acid supply from albumin, and the first major peak of plasma insulin at 17E could be caused by the embryo drinking the amniotic fluid. The plateau in plasma insulin levels at hatch in the current study may be caused by a shift in the embryo from using fuel for growth to providing energy needed for pipping and hatch. Hohlweg et al. (1999) showed that insulin can control and regulate the concentration of amino acids in embryo plasma, amniotic fluid, and allantoic fluid during embryogenesis. Davis and Reeds (1998) reported that the anabolic drive by insulin in mammals can directly promote protein deposition during fetal and early postnatal life and can suppress proteolysis in the adult. A significant

Table 2. Correlation coefficients between BW and plasma metabolic hormones in chick embryos and hatched chicks[1]

Item	Insulin	Glucagon	Intogl	T_4	T_3	IGF-I	IGF-II
Chick embryos							
BW	0.6311	0.6334	−0.1666	0.5583	0.5287	−0.2094	0.1384
	<0.0001	<0.0001	0.0257	<0.0001	<0.0001	0.0077	0.0709
Insulin	1	0.4228	0.1724	0.4642	0.2900	−0.0198	0.2725
		<0.0001	0.0210	<0.0001	<0.0001	0.8024	0.0003
Glucagon	0.4228	1	−0.5273	0.1630	0.2043	−0.2670	0.0423
	<0.0001		<0.0001	0.0292	0.0061	0.8024	0.5824
Hatched chicks							
BW	0.3458	−0.1272	0.1600	0.4521	0.1147	0.7285	−0.6430
	<0.0001	0.0275	0.0055	<0.0001	0.0471	<0.0001	<0.0001
Insulin	1	−0.3614	0.8014	0.0358	0.1829	0.2403	−0.2669
		<0.0001	<0.0001	0.5368	0.0015	<0.0001	<0.0001
Glucagon	−0.3614	1	−0.6265	0.0677	−0.3912	−0.1558	0.2151
	<0.0001		<0.0001	0.2417	<0.0001	0.0069	0.0002
T_4	0.0358	0.0677	−0.1115	1	−0.1052	0.4549	−0.4530
	0.5368	0.2417	0.0545		0.0688	<0.0001	<0.0001

[1]For each pair, the top number is the correlation coefficient and the bottom number is the *P*-value. Intogl = insulin:glucagon molar ratio; T_4 = thyroxine; T_3 = triiodothyronine; IGF-I = insulin-like growth factor-I; IGF-II = insulin-like growth factor-II.

strong positive correlation between chick embryo BW and plasma insulin levels (r = 0.6311, $P < 0.001$; Table 2) may also indicate that insulin is an important promoter of embryonic growth by the anabolic drive to promote protein deposition, especially during the period of rapid embryonic growth.

The plasma insulin data in growing chicks reported herein (Figure 1) is in good agreement with Obi and Coon (1989), who showed that plasma insulin increased proportionately with age. The finding of higher insulin in posthatch chicks compared with chick embryos in the present study would suggest that the chick pancreas might have a more important role in partitioning substrates into tissue accretion or catabolism compared with the embryonic pancreas. The positive correlation between the BW and plasma insulin levels hatched chick (r = 0.3458, $P < 0.001$) observed in the current study (Table 2) confirms the critical role of insulin in regulating postnatal metabolism and growth.

Glucagon

Very few reports have shown glucagon concentration data in chick embryos and hatched chicks, although glucagon appears to be the dominant pancreatic hormone in birds compared with mammals. Glucagon has been shown to maintain "chronic hyperglycemic" blood glucose levels in birds that are 2 to 3 times greater than in most mammals (Hazelwood, 2000). The glucagon data in chick embryos reported herein (Figure 2) indicates that glucagon plays a critical role in providing the glucose requirement of chick embryos during embryogenesis. Picardo and Dickson (1982) reported a dramatic activation of glycogen synthesis during the second half of chick embryogenesis, which is consistent with the lowest level of glucagon (Figure 2) and higher intogl at 15E (Figure 3) in this study. Picardo and Dickson (1982) suggested that the sudden depletion they observed in glycogen 1 or 2 d before hatching could be associated with the energy requirement for successful pipping and hatching, in support of the present observation of a significant increase in glucagon levels during pipping and hatch. The increased demand by chick embryos for glucose during pipping and hatching has been suggested because the use of lipids as an energy source would require too much oxygen (Christensen et al., 2001). Picardo and Dickson (1982) further demonstrated that insulin did not inhibit glucagon-stimulated glycogenolysis in isolated hepatocytes from 18-d chick embryos. Glycogenolysis has been shown to support a successful hatch, with adequate energy being supplied from glycogen (Picardo and Dickson, 1982).

Previous studies have shown that glucagon has an important role in the nutritional transition between the high-fat, low-carbohydrate supply of the chick embryo to the low-fat, high-carbohydrate diet of the hatched chick (Langslow et al., 1979). Langslow et al. (1979) reported a 10-fold increase in the lipolytic sensitivity of adipocytes to glucagon in 8-d-old chickens compared with 18-d-old chick embryos. Subcutaneous adipose tissue accumulates during chick embryogenesis until the first day after hatch and declines thereafter (Langslow et al., 1979). In the current study, young chicks had a higher plasma level of glucagon compared with chick embryos (Figure 2), which is consistent with the report of Langslow et al. (1979).

A significant positive correlation between plasma glucagon levels and chick embryonic BW (r = 0.6334, $P < 0.001$) was found in the current study, suggesting that glucagon may be considered an embryonic growth-stimulating factor. Elevated glucagon at 18 d of incubation (Figure 2) relates very well to data showing glycogenolysis and gluconeogenesis (McNabb, 2000) when the chick embryo mass was deposited quickly. However, decreased glucagon was observed between the 13th and 15th day of incubation (Figure 2), which correlates with the beginning of rapid organ growth in the chick embryo (Romanoff, 1967). Elirick et al. (1958) also reported that injecting highly purified crystalline glucagon had no effect on chick embryo weight, although crude glucagon preparations did cause a significant increase in chick embryonic BW. Therefore, this seems to indicate that glucagon functions as an embryonic growth-stimulating factor in a complex way or by interacting with other hormones (e.g., insulin). Plasma glucagon was negatively correlated ($P < 0.05$) with intogl during embryogenesis (r = –0.62734, $P < 0.001$), but plasma insulin was not (Table 2). However, in hatched chicks, plasma insulin showed a higher correlation with intogl compared with plasma glucagon. These findings in the current study may suggest that glucagon is the main factor affecting intogl during chick embryonic development and that insulin has a major effect on intogl during hatched chick growth. Glucagon is known as a "hormone of fasting" and insulin as a "hormone of feasting" (Hazelwood, 2000). Thus, the current findings indicate that chick embryos could be in a "fasting" status and that hatched chicks may stay in a "feasting" status.

Insulin:Glucagon Molar Ratio

The significantly greater intogl from 14E to 17E (Figure 3) corresponds with a previous report by Cogburn (1991) showing that somatostatin (**SRIF**) remains at low levels in the embryonic pancreas from 7 to 13 d of incubation. Hazelwood (2000) has reported that SRIF is supposed to directly adjust to intogl to meet changing metabolic demands at any given moment, because SRIF from D cells inhibits the secretion from pancreas A, B, and F cells. Combined with data from plasma insulin and glucagon, the increase in intogl in midembryogenesis indicates that insulin may be an important promoter of chick embryonic growth by an anabolic drive to promote protein and fat deposition. The significantly lower intogl found during pipping and hatch in the present study seems to support the high demand for energy in chick embryos for hatching. Although feeding status can affect pancreatic hormones, the increased intogl (2.0 to 5.2; Figure 3) for young chicks from hatch to 3 wk of age still strongly indicates an adaptive pancreatic ability to support nutrient deposition. A previous study using chicks in the postabsorptive

state showed intogl to be only 1.2 to 1.8 (Hazelwood, 2000). The elevated levels of insulin and glucagon and the higher intogl that occurred in hatched chicks in the present study may be due to increased feed intake and subsequent utilization of carbohydrates as a key energy source, compared with nutrients obtained through lipolysis and proteolysis in the embryo.

Glucose

The increase in plasma glucose levels with age during embryogenesis (Figure 4) coincides with an increase in plasma insulin levels. The increased plasma glucose during late embryonic development may result from the increased power of glucagon (Figure 2), causing glycogenolysis. The findings of this study agree with the previous suggestion by Davis and Reeds (1998) that glucose is an important regulator of protein anabolism in the mammalian fetus via its ability to suppress amino acid oxidation. Hazelwood (2000) suggested that a certain synergism exists between glucose and amino acids when β-cell responses are considered. A significantly lower plasma glucose level at 17E (Figure 4), with the corresponding maximum level of plasma insulin during embryogenesis, may indicate that the glucose at 17E was utilized for body glycogen accumulation. This glycogen may accumulate in preparation for extra carbohydrate requirements that will be needed during pipping and hatch (Christensen et al., 2001). A plateau of plasma glucose levels during pipping and hatch may indicate that glucose is being utilized as an energy source during this critical period in order for the embryo to survive. Compared with chick embryos, 1- to 21-d-old chicks had higher plasma glucose levels (Figure 4), which reflects a change in energy metabolism from using lipids and amino acids as an energy source during embryo development to glucose utilization in the posthatch state.

T_3

The developmental changes in plasma T_3 concentrations during embryogenesis (Figure 5) agree with a previous report by Thommes and Hylka (1977) showing that plasma T_3 was detectable at 9.5 d of incubation and was relatively unchanged until late embryonic development. In the present study, we also observed a sharp rise in T_3 activities at the developmental stage when the embryo switches to lung respiration (McNabb, 2000; Reyns et al., 2003). This is a critical time for the chick embryo to survive because the embryos need oxygen and energy for hatching. Plasma T_3 levels in chicks posthatch increased with age (Figure 5) although fluctuations occurred. Goddard et al. (1988) also reported a trend of increasing T_3 levels in broilers and White Leghorns during the first 3 wk after hatch. These fluctuating levels of T_3 in young chicks may be due to changes in temperature and feed availability. McNabb (2000) reported that temperature and feed availability have the greatest influences on thyroid function. Prior research has shown that feed restriction in chicks decreases circulating T_3 levels and that feeding increases T_3 concentrations (McMurtry et al., 1988; McNabb, 2000).

T_4

The increased plasma T_4 concentrations in midincubation (Figure 6) parallel the findings of Bellabarba et al. (1988), which showed that an increase in plasma T_4 was directly related to elevated thyroid hormone receptors in the liver and brain during midstage chick embryogenesis. The significantly elevated plasma T_4 levels before hatch (Figure 6) agreed with previous reports by McNabb (2000) and Reyns et al. (2003) suggesting that thyroxine is important for stimulating a variety of developmental and metabolic processes necessary for successful hatching (Decuypere et al., 1991). The increased T_4 levels with age after hatch (Figure 6) were consistent with previous results by Goddard et al. (1988), which showed that T_4 increased consistently with age in broilers and White Leghorns. Thyroid hormones, both T_4 and T_3, showed significant positive correlations with chick embryonic BW in the present study (Table 2), suggesting that thyroid hormones appear to be critically important in maintaining normal growth and development during chick embryogenesis. King and May (1984) reported that the greatest effect of goitrogens on chick embryonic growth occurs during late embryogenesis when normal thyroid hormone levels are increasing.

IGF-I

The observed rise in plasma IGF-I in chick embryos at midincubation in the current study (Figure 7) confirms the findings of Robcis et al. (1991), de Pablo et al. (1991), and McMurtry et al. (1998) showing an IGF-I peak at midincubation for chick embryos and turkey embryos. In addition, Robcis et al. (1991) reported that growth-retarded ex ovo-cultured chick embryos did not show an IGF-I peak at midembryogenesis, implicating IGF-I in the control of normal chick embryonic development. The cause of the IGF-I peak during midincubation is not clear, although the rise in IGF-I secretion midembryogenesis in the current study may be explained in part by a significant increase in plasma insulin levels (Figure 1). The pattern of a large increase in plasma insulin levels in hatched chicks (Figure 1) following 24 h of feed consumption was similar to the pattern of increased plasma IGF-I levels in the same chicks (Figure 7). Our finding confirmed the previous report by Guernec et al. (2003) that feed intake can directly or indirectly enhance plasma levels of IGF-I in day-old chicks. The steady increase in plasma levels of IGF-I from hatch to 21-d-old broiler chicks in the current study confirms the findings of McGuinness and Cogburn (1990) and McMurtry et al. (1998). This increase is related to the increased expression of IGF-I mRNA in chicks (McMurtry et al., 1998). A significant positive correlation between hatched chick BW and plasma IGF-I levels ($r = 0.7258$, $P < 0.001$; Table 2) agrees with previous reports that IGF-I levels are correlated with chick BW gain (God-

dard et al., 1988; Leenstra et al., 1991), especially with regard to protein deposition and the relative growth rate (McGuinness and Cogburn, 1990).

IGF-II

Few studies have focused on the role of IGF-II in chicks, especially during embryogenesis. The 5- to 10-fold higher concentration of IGF-II in plasma (Figure 8), compared with IGF-I, (Figure 7) was consistent with the finding of Richards et al. (2005) that IGF-II mRNA expression was greater in the developing turkey embryo, supporting the suggestion by McMurtry (1998) that IGF-II is an important functionary for chick embryonic development. McMurtry et al. (1998) also reported that plasma IGF-II in 50-d-old broiler chicks was significantly lower than plasma levels in day-old chicks, confirming the current finding of a significant decline in plasma IGF-II levels in chicks after hatch to 3 wk of age (Figure 8). Radecki et al. (1997) reported no variations in IGF-II levels posthatch in White Leghorn chicks, but this may have been due to a strain difference. Previous research with mammals has shown that IGF-II is almost exclusively expressed in embryonic and neonatal tissues and that after birth the level of detectable IGF-II protein also falls significantly (Stewart and Rotwein, 1996).

The developmental profile of plasma levels of insulin, glucagon, T_3, T_4, IGF-I, and IGF-II in embryos and hatched broiler chicks indicates that a close relationship exists between circulating levels of metabolic hormones and known developmental events. These data suggest that insulin and IGF-II during embryogenesis and IGF-I and T_4 from hatch to 3 wk of age may be the most important contributors to chick growth (body mass deposition). Glucagon may work as an adjuster between growth and function during embryogenesis, and thyroid hormones appear to be critically important to development during chick embryogenesis, especially during hatching. The relationship between nutritional status and the endocrine system during the early development period requires further study.

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