Role of Corticosterone in Lipid Metabolism in Broiler Chick White Adipose Tissue

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Excessive accumulation of body fat in broiler chickens has become a serious problem in the poultry industry. However, the molecular mechanism of triglyceride accumulation in chicken white adipose tissue (WAT) has not been elucidated. In the present study, we investigated the physiological importance of the catabolic hormone corticosterone, the major glucocorticoid in chickens, in the regulation of chicken WAT lipid metabolism. We first examined the effects of fasting on the mRNA levels of lipid metabolism-related genes associated with WAT, plasma corticosterone, and non-esterified fatty acid (NEFA). We then examined the effects of corticosterone on the expression of these genes *in vivo* and *in vitro*. In 10-day-old chicks, 3 h of fasting significantly decreased mRNA levels of lipoprotein lipase (*LPL*) in WAT and significantly elevated plasma concentrations of NEFA. Six hours of fasting significantly increased mRNA levels of adipose triglyceride lipase (*ATGL*) in WAT and significantly elevated plasma concentrations of corticosterone. On the other hand, fasting significantly reduced mRNA levels of *LPL* in WAT and elevated plasma concentrations of NEFA in 29-day-old chicks without affecting mRNA levels of *ATGL* in WAT or plasma corticosterone concentrations. Oral administration of corticosterone significantly reduced mRNA levels of *LPL* and significantly increased the mRNA levels of *ATGL* in WAT in 29-day-old chicks without affecting plasma NEFA concentrations. The addition of corticosterone to primary chicken adipocytes significantly increased mRNA levels of *ATGL*, whereas mRNA levels of *LPL* tended to decrease. NEFA concentrations in the culture medium were not influenced by corticosterone levels. These results suggest that plasma corticosterone partly regulates the gene expression of lipid metabolism-related genes in chicken WAT and this regulation is different from the acute elevation of plasma NEFA due to short-term fasting.

Key words: abdominal fat, broiler, FFA, lipid, TG

Introduction

Broiler chickens have been intensively selected for productive traits over the last half century. As a result, increased body fat in broiler chickens has led to serious problems in the poultry industry, such as increases in metabolic diseases in chickens (Julian 2005; Nijdam *et al*., 2006) and fat energy in chicken meat (Wang *et al*., 2010). Thus, the regulatory mechanisms of lipid metabolism and body fat accumulation in chickens have been the focus of research for poultry nutritionists in recent decades.

Triglyceride (TG) lipases, including lipoprotein lipase (LPL),

hormone-sensitive lipase (HSL), and adipose triglyceride lipase (ATGL), play critical roles in TG metabolism in mammals (Lee *et al*., 2014; Nakamura *et al*., 2014). LPL hydrolyzes blood lipoprotein TG into non-esterified fatty acids (NEFAs) and promotes the cellular uptake of NEFAs (Lee *et al*., 2014; Nakamura *et al*., 2014). Since *de novo* lipogenesis in avian species is centered in the liver (Bergen and Mersmann 2005), the growth of white adipose tissue (WAT) depends mainly on circulating TG transported from the liver in chickens (Alvarenga *et al*., 2011). In fact, a positive correlation has been found between LPL activity and fat deposits in chickens (Griffin *et al*., 1987). Chronic administration of anti-LPL antibodies is effective in reducing the fat content of broiler chickens (Sato *et al*., 1999). These findings suggest that LPL plays a critical role in TG accumulation in the WAT of chickens. However, the physiological roles of the lipolytic enzymes ATGL and HSL in chickens have not yet been clarified. For example, no homolog of the HSL gene has been identified in the chicken genome, although an HSL-like enzyme has been purified from chicken WAT (Anthonsen *et al*., 1997). However, we found that 4 h of fasting significantly

increased mRNA levels of *ATGL* and reduced mRNA levels of *LPL* in chicken WAT (Saneyasu *et al.*, 2013b; Honda *et al.*, 2017). We also showed that plasma NEFA concentrations were significantly elevated after 4 h of fasting (Honda *et al.*, 2017). Therefore, transcriptional regulation of *LPL* and *ATGL* may play important roles in the regulation of body fat accumulation in chickens.

In mammals, the physiological roles of insulin in adipocytes, such as stimulation of lipogenesis and suppression of lipolysis, have been well established (Santoro *et al.*, 2021). However, the role of insulin in chicken WAT is unclear or questionable (Scanes, 2009; Dupont *et al.*, 2012). Ji *et al.* (2012) reported that significant changes in the gene expression of *LPL* and *ATGL* in WAT were not included in the list of differentially expressed genes in insulin-neutralized vs. fed chickens. Although glucagon is known to be the major lipolytic hormone in chickens (Scanes, 2009), it does not affect mRNA levels of *LPL* and *ATGL* in adipocytes *in vitro* (Honda *et al.*, 2017). Serr *et al.* (2011b) reported that injection of dexamethasone, a synthetic glucocorticoid, upregulates *ATGL* gene expression in chicken WAT. There is evidence that more than 2 h of fasting significantly elevates plasma corticosterone concentration in chicks (Kadhim *et al.*, 2019). It is therefore possible that the transcriptional changes in *LPL* and *ATGL* are induced by corticosterone in chicks, which in turn results in metabolic changes in chicken WAT.

The aim of this study was to evaluate the role of corticosterone, the major glucocorticoid in chickens, in the regulation of gene expression of *LPL* and *ATGL* in chicken WAT. In addition to *LPL* and *ATGL*, comparative gene identification-58 (*CGI-58*), an activator protein of ATGL in mammals (Zimmermann *et al.*, 2009), was also analyzed.

Materials and Methods

Animals and Diet

This study was approved by the Institutional Animal Care and Use Committee (permission number: 25-08-01 and 27-10-07) and was carried out according to the Kobe University Animal Experimentation Regulations. Day-old male chicks (*Gallus gallus domesticus*, Ross 308) were purchased from a local supplier (Ishii Co., Ltd. Tokushima, Japan) and maintained in an electrically heated brooder. The temperature was maintained at $31 \pm 2℃$ during the first 7 days, and then reduced gradually with age to $25 \pm 2℃$ at 21 days. Chicks were given free access to water and a commercial chick starter diet (Nippon Formula Feed Mfg. Co., Ltd., Kanagawa, Japan) with continuous lighting.

Experiment 1: Effects of Fasting on Lipid Metabolism in 10- and 29-day-old Chicks
Sampling and Analysis of Plasma Corticosterone and NEFA

Abdominal fat accumulation in chicks significantly increased after hatching. For example, abdominal adipose tissue weight, as a percentage of body weight, was dramatically elevated from 4 days to 14 days of age (Bai *et al.*, 2015). We previously showed that percentages of abdominal adipose tissue weight at 14, 21, and 28 days of age was significantly higher than that at 7 days of age in broiler chicks, and there were no significant differences in the percentages between 14, 21, and 28 days of age (Saneyasu *et al.*, 2013a). Therefore, in order to evaluate the effects of fasting on transcriptional changes in lipid metabolism-related genes at different stages of WAT development, we used two differently aged chicks in Experiment 1. To represent the period of increase, we used 10-day-olds and to represent stabilization period, we used 29-day-olds.

A total of 18 male broiler chicks were weighed and allocated to three cages (1,725×425×320 mm, six birds in each group) based on body weight under *ad libitum* feeding conditions. Chicks in the feeding group were euthanized by decapitation by a trained person. Chicks in the fasting groups were similarly euthanized after 3 and 6 h of fasting. Blood was collected after euthanasia from carotid arteries. Plasma was separated immediately by centrifugation at 1,910×g for 10 min at 4℃, and NEFA and corticosterone levels were measured using commercial kits (LabAssay™ NEFA, Wako Pure Chemical Industries, Ltd., Osaka, Japan; Corticosterone ELISA Kit, AssayPro LLC, MO, USA). Abdominal WAT was excised and frozen immediately by using liquid nitrogen for real-time PCR analyses.

Real-time PCR Analysis

Total RNA was extracted from WAT using Sepazol-RNA I (Nacalai Tesque, Inc., Kyoto, Japan). First-strand cDNA was synthesized from total RNA using a ReverTra Ace® qPCR RT Master Mix with a gDNA remover (Toyobo Co. Ltd., Osaka, Japan). Complementary DNAs of *LPL* (NM_205282), *ATGL* (NM_001113291), *CGI-58* (CGI-58, NR_103454), and ribosomal protein S17 (*RPS17*, NM_204217) were amplified using the following primers: *LPL* sense, 5′-GAC AGC TTG GCA CAG TGC AA-3′, *LPL* antisense, 5′-CAC CCA TGG ATC ACC ACA AA-3′; *ATGL* sense, 5′-GCT GAT CCA GGC CTG TGT CT-3′, *ATGL* antisense, 5′-TGG AGG TAT CTG CCC ACA GTA GA-3′; *CGI-58* sense, 5′-TGG ACA CAA TCT GGG TGG ATT-3′, *CGI-58* antisense, 5′-GGC TTA GAC CTT GAT GGG TAT TTT AA-3′; *RPS17* sense, 5′-GCG GGT GAT CAT CGA GAA GT-3′, and *RPS17* antisense, 5′-GCG CTT GTT GGT GTG GAA GT-3′. *RPS17* was used as an internal standard. All primers were purchased from Hokkaido System Science Co., Ltd. (Hokkaido, Japan). The mRNA levels were quantified in duplicate using the Applied Biosystems 7300 Real-Time PCR system and THUNDERBIRD™ SYBR® qPCR Mix (Toyobo Co. LTD) according to the supplier's recommendations.

Experiment 2: Effects of Oral Corticosterone Administration on the Expression of Lipid Metabolism-related Genes in 29-day-old Chicks

Twelve 29-day-old male broiler chicks were weighed and allocated to two cages (1,725×425×320 mm, six birds in each group) based on body weight. After 4 h of fasting, either 0 (control) or 2 mg/kg body weight corticosterone (Sigma-Aldrich Japan K.K., Tokyo, Japan) in 0.5% carboxymethyl cellulose solution was orally administered. After 2 h of administration, the chicks were euthanized by decapitation, and

abdominal WAT was excised. The mRNA levels of lipid metabolism-related genes in WAT and plasma NEFA concentrations were analyzed as described in Experiment 1.

Experiment 3: Effect of Corticosterone on the Expression of Lipid Metabolism-related Genes in Primary White Adipocytes Isolated from Chicks

Six 10-day-old chicks were euthanized by decapitation and abdominal WAT was excised. Adipocytes were isolated as described previously (Oscar *et al*., 1991), and then incubated with Dulbecco's Modified Eagle Medium (DMEM, 1.0 g/L glucose with L-glutamine and sodium pyruvate; 08456-65, Nacalai Tesque, Inc.) containing 25 mM HEPES, 80 µg/m*l* kanamycin, and 3% bovine serum albumin, supplemented with either 0 (control) or 1,040 µg/mL corticosterone (Sigma-Aldrich Japan K.K., Tokyo, Japan) for 16 h. After removing the culture medium, the cells were washed twice with PBS and used for real-time PCR analysis. The mRNA levels of lipid metabolism-related genes in adipocytes and the NEFA concentration in the medium were analyzed as described in Experiment 1.

Data Analysis

Data from Experiment 1 were analyzed using the Tukey-Kramer test using a commercial package (StatView version 5, SAS Institute, Cary, NC, USA, 1998). Data from Experiments 2 and 3 were analyzed by Student's *t*-test using Excel 2013 (Microsoft Corporation, WA, USA).

Results

As shown in Table 1, three hours of fasting significantly elevated plasma NEFA concentrations in both 10- and 29-day-old chicks, suggesting that lipolysis was stimulated. However, elevated plasma NEFA levels were abolished in 10-day-old chicks after 6 h of fasting. Plasma corticosterone concentrations were significantly elevated after 6 h of fasting in 10-day-old chicks, but not in 29-day-old chicks.

Fig. 1a shows the effects of fasting on mRNA levels of lipid metabolism-related genes in 10-day-old chicken WAT. Three and six hours of fasting significantly reduced mRNA levels of *LPL*, whereas 6 h of fasting significantly increased mRNA levels of *ATGL* and *CGI-58*. All these significant changes were abolished by 6 h of refeeding. On the other hand, fasting and re-feeding did not influence mRNA levels of *ATGL* or *CGI-58* in 29-day-old chicks, although mRNA levels of *LPL* showed similar changes as those in 10-day-old chicks (Fig. 1b). These results suggest that fasting-increased plasma corticosterone upregulates the transcription of *ATGL* and *CGI-58* in the WAT of 10-day-old chicks.

We next examined the effects of oral corticosterone administration on the transcription of LPL, ATGL, and CGI-58 genes in the WAT of 29-day-old chicks. For corticosterone doses, we carried out a screening experiment to examine the effect of oral corticosterone administration (dose: 0, 2, and 4 mg/kg body weight) on plasma corticosterone concentrations in 4 h-fasted chicks. We found that 0, 2, and 4 mg of oral corticosterone administration significantly elevated plasma corticosterone levels to 8.22 ± 0.97 ng/m*l*, 40.8 ± 7.0 ng/m*l*, and 126.2 ± 12.5 ng/m*l*, respectively, 2 h after administration. Based on these data, a 2 mg dose was used in Experiment 2. As shown in Fig. 2, oral administration of corticosterone significantly increased mRNA levels of *ATGL* and significantly reduced mRNA levels of *LPL*, whereas mRNA levels of *CGI-58* were not affected. Plasma concentrations of NEFA were not affected by corticosterone (control, 534.1 ± 75.6 µEq/L; corticosterone, 488.3 ± 35.2 µEq/L). These results suggest that the plasma corticosterone elevation is involved in the upregulation of *ATGL* and downregulation of *LPL* gene expression in chick WAT.

To examine the direct effect of corticosterone on white adipocytes, we examined the effects of corticosterone on the expression of lipid metabolism-related genes *in vitro*. As shown in Fig. 3, mRNA levels of *ATGL* in primary white adipocytes were significantly increased by corticosterone. The mRNA levels of *LPL* in primary white adipocytes tended to be reduced by corticosterone ($P=0.071$). In contrast, mRNA levels of *CGI-58* were not affected by corticosterone. Medium concentration of NEFA was not affected by corti-

Table 1. **Effects of fasting on the weights of body and abdominal adipose tissue, and plasma concentraton of non-esterified fatty acid and corticosterone in different age of chicks**

		Fasting periods		
		0 h	3 h	6 h
Body weight (g)	10-day-old	209.5 ± 5.1	203.7 ± 3.8	198.7 ± 3.6
	29-day-old	1519 ± 24	1402 ± 34	1485 ± 37
Abdominal adipose tissue weight (g)	10-day-old	1.93 ± 0.14	1.89 ± 0.16	1.84 ± 0.11
	29-day-old	18.2 ± 2.1	16.7 ± 1.7	17.7 ± 1.6
Abdominal adipose tissue weight (%)	10-day-old	0.92 ± 0.05	0.93 ± 0.08	0.93 ± 0.05
	29-day-old	1.20 ± 0.14	1.20 ± 0.14	1.19 ± 0.09
Non-esterified fatty acid (µEq/L)	10-day-old	274.5 ± 36.7^a	542.3 ± 74.2^b	320.0 ± 61.9^a
	29-day-old	126.9 ± 11.7^a	529.0 ± 50.9^b	543.0 ± 108.9^b
Corticosterone (ng/mL)	10-day-old	1.56 ± 0.22^a	5.15 ± 0.88^{ab}	10.89 ± 2.77^b
	29-day-old	4.33 ± 1.76	5.56 ± 3.23	2.43 ± 0.31

Data are the means±S.E.M. of six birds in each group. Data were analyzed by Tukey-Kramer test. Groups with different letters are significantly different in each age ($P<0.05$).
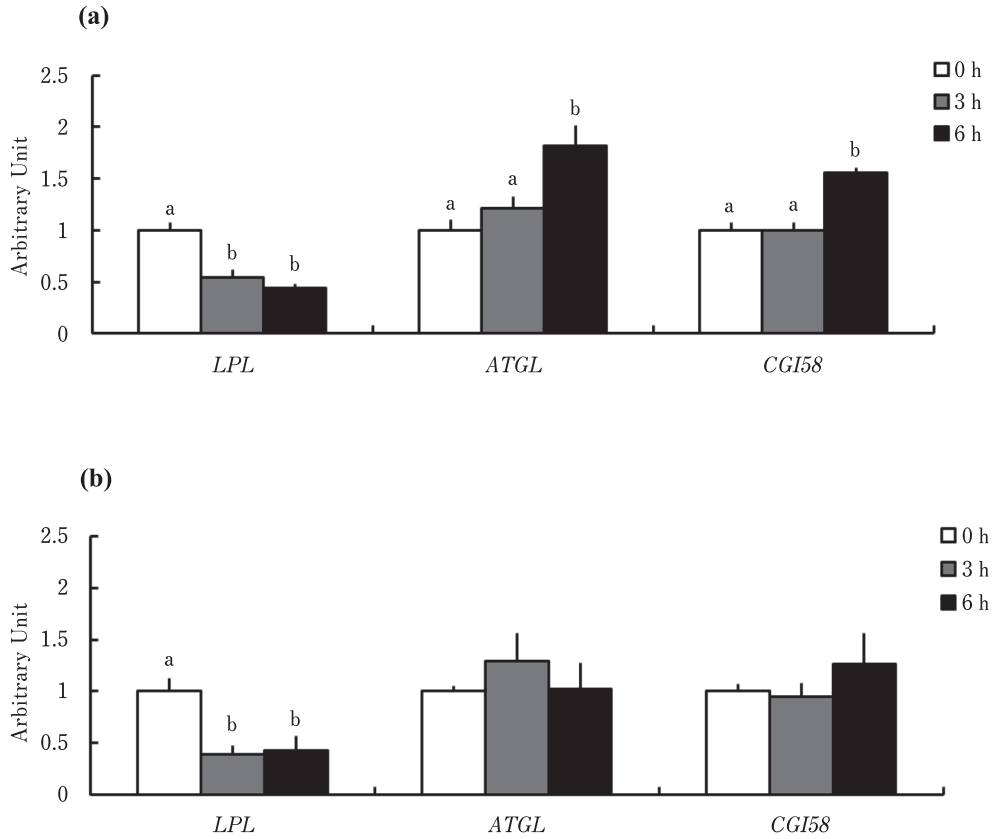
(a)

(b)

Fig. 1. **Effects of fasting on mRNA levels of lipid metabolism-related genes in white adipose tissue in (a) 10-day-old and (b) 29-day-old chicks.** Data are represented as means±SEM of six chicks in each group. Data were analyzed by Tukey-Kramer *t*-test (*, $P < 0.05$).
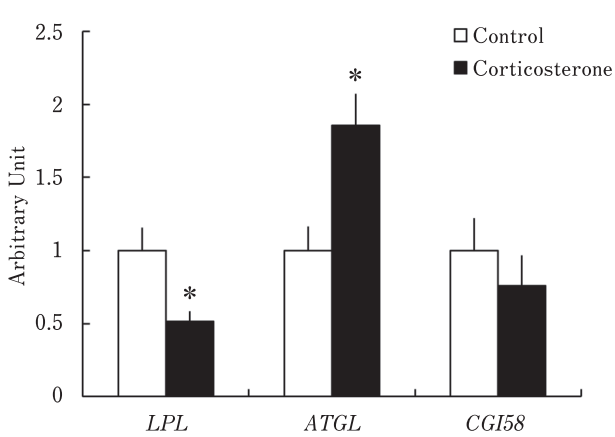

Fig. 2. **Effects of oral administration of corticosterone on mRNA levels of lipid metabolism-related genes in white adipose tissue in chicks.** Data are represented as means± SEM of six chicks in each group. Data were analyzed by Student's *t*-test (*, $P < 0.05$).
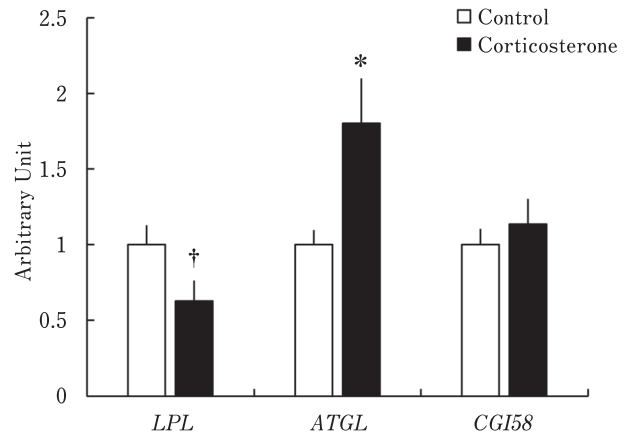

Fig. 3. **Effects of corticosterone on mRNA levels of lipid metabolism-related genes in adipocytes *in vitro*.** Data are represented as means±SEM of five tubes in each group. Data were analyzed by Student's *t*-test (†, $0.05 \leqq P < 0.1$; *, $P < 0.05$, respectively).

costerone (Control, 536.6±69.9 µEq/L; Corticosterone, 735.8 ±140.5 µEq/L). These results suggest that corticosterone influences the expression of *ATGL* and *LPL* genes in chicken white adipocytes.

Discussion

Serr *et al*. (2011b) showed that ATGL mRNA and protein are stimulated by dexamethasone, contributing to elevated plasma NEFA levels in chicks. We also showed that corticosterone significantly increased the mRNA levels of *ATGL in vivo* and *in vitro*. Six hours of fasting significantly increased mRNA levels of *ATGL* in WAT and elevated plasma corticosterone concentrations in 10-day-old chicks. However, in the present study, 3 h of fasting elevated plasma NEFA concentrations without affecting the plasma corticosterone concentrations or *ATGL* mRNA levels in the WAT of 10-day-old chicks. These results suggest that plasma corticosterone upregulates ATGL gene expression in chicken WAT, but this regulatory mechanism does not play an important role in the acute elevation of plasma NEFA caused by short-term fasting in 10-day-old chicks.

In the present study, 2 mg of corticosterone was administered to chicks, because this dose significantly elevated plasma corticosterone (40.8±7.0 ng/m*l*) after 2 h of administration in the preliminary experiment. This plasma corticosterone concentration was higher than that prevailing under physiological conditions (Table 1). However, Geris *et al*. (1999) reported that intravascular administration of 40 µg of corticosterone in broiler chicks resulted in a fivefold increase in plasma corticosterone (17.2±2.6 ng/m*l* vs. 98.8±9.3 ng/m*l*) after 15 min, but this increase was no longer present after 1 h. Therefore, the effect of exogenously administered corticosterone seems to be immediately reduced in chicks. In addition, Hassanzadeh *et al*. (2004) reported that plasma corticosterone levels were significantly decreased with age in broiler chicks (day 7, 42.5±8.9 ng/m*l*; day 42, 12.1±2.8 ng/m*l*). It is therefore possible that the results of Experiment 2 show physiological effects of corticosterone in chicks.

In this study, oral administration of corticosterone significantly reduced *LPL* mRNA levels in the WAT of 29-day-old chicks. In addition, mRNA levels of *LPL* in primary chicken adipocytes tended to be reduced by corticosterone. However, 3 h of fasting significantly reduced mRNA levels of *LPL* in WAT without affecting plasma corticosterone concentrations in both age groups of chicks. It is therefore likely that corticosterone downregulates LPL gene expression in chicken WAT, but the role of corticosterone is minor, at least under these experimental conditions.

In Experiment 3, 1,040 ng/mL of corticosterone was used, but this concentration was higher than that of plasma corticosterone in chicks (Table 1), suggesting that the results may indicate pharmacological effects. However, it is difficult to incubate primary cells under physiological conditions *in vitro*. In general, experimental results from cell cultures are only one piece of evidence. For example, additional data from knockdown or knockout experiments targeting the glucocorticoid receptor gene would possibly reinforce the

findings from our experiments. Further studies are needed to evaluate the physiological importance of corticosterone in the transcriptional regulation of ATGL.

In mammals, ATGL activity is regulated by the activator protein CGI-58 (Lass *et al*., 2006; Schweiger *et al*., 2008). For example, CGI-58 significantly increased TG hydrolase activity in WAT in wild-type and *HSL*-null mice 1.7- and 2.1-fold, respectively (Schweiger *et al*., 2006). There is evidence that silencing either *ATGL* or *CGI-58* significantly reduced lipolysis in a human white adipocyte model (Bezaire *et al*., 2009). Serr *et al*. (2011a) reported that *CGI-58* mRNA levels in WAT were increased by long-term (24 h) fasting in 21-day-old broiler chicks. We showed that the increase in *ATGL* mRNA levels after fasting was accompanied by an increase in *CGI-58* mRNA levels in 10-day-old chicks. These findings suggest that ATGL and CGI-58 coordinate to regulate WAT lipolysis in both mammals and birds. However, corticosterone directly stimulates *ATGL* expression in chicken WAT without affecting *CGI-58* expression. Serr *et al*. (2011b) also showed that dexamethasone injection significantly increased the mRNA and protein levels of ATGL without affecting *CGI-58* mRNA levels in subcutaneous adipose tissue in 25-day-old broiler chicks. All these findings suggest that both ATGL and CGI-58 play important roles in fasting-induced lipolysis, but that the regulatory mechanism underlying fasting-induced transcriptional changes in WAT is different between *ATGL* and *CGI-58* genes in chicks.

In chickens, short-term fasting elevates plasma concentrations of glucagon (Christensen *et al*., 2009), corticosterone (Kadhim *et al*., 2019), NEFA (Dupont *et al*., 2008; Honda *et al*., 2017), and mRNA levels of *ATGL* in WAT (Saneyasu *et al*., 2013b; Honda *et al*., 2017). We showed that corticosterone directly upregulates *ATGL* gene expression in chicken WAT without affecting NEFA release *in vivo* or *in vitro*. In contrast, we previously showed that glucagon stimulated NEFA release in a culture medium without affecting the mRNA level of *ATGL* in chicken primary adipocytes (Honda *et al*., 2017). It is well known that glucagon injection elevates plasma NEFA levels in chicks. All these findings suggest that glucagon plays a more important role compared to corticosterone in the acute elevation of plasma NEFA levels resulting from short-term fasting in chicks.

In the present study, 3 h of fasting significantly elevated plasma NEFA concentrations, but this elevation was not observed after 6 h of fasting in 10-day-old chicks. We previously showed that 4 h of fasting significantly increased mRNA levels of carnitine palmitoyltransferase 1a, a rate-limiting enzyme of fatty acid oxidation, and *PPARα*, a transcription factor of fatty acid oxidation-related genes, in the livers of 13-day-old chicks (Saneyasu *et al*., 2013b). Plasma NEFA released from WAT induces fatty acid oxidation in the liver and skeletal muscles via PPARα and PPARδ in mammals (Nakamura *et al*., 2014). Therefore, it seems likely that the significant elevation of circulating NEFA was abolished by exhausting plasma NEFA in the liver and other tissues after 6 h of fasting in 10-day-old chicks.

In 29-day-old chicks, significant elevation of plasma NEFA

concentrations was observed in both the 3 and 6 h fasting groups, although a significant elevation of plasma NEFA concentrations in 10-day-old chicks was not observed in the 6 h fasting group. One possible explanation is that the ability to supply NEFAs from adipocytes in 29-day-old chicks was higher than that in 10-day-old chicks. Bai *et al*. (2015) showed that most adipocytes (82.88%) had a mean diameter of 5–15 μm in 4-day-old chicks, whereas larger adipocytes (mean diameter $>$ 15 μm) comprised 51.89% of the total number of adipocytes in 14-day-old chicks. A similar phenomenon was observed in a 10- and 29-day-old chicks in our laboratory (unpublished data). In addition, after 6 h of fasting, the percentage of abdominal adipose tissue in 29-day-old chicks was significantly higher than that in 10-day-old chicks (Student's *t*-test; $P<0.05$). It is therefore likely that sufficient NEFA could be supplied from WAT to the circulation after 6 h of fasting in 29-day-old chicks.

In the present study, 6 h of fasting increased mRNA levels of *ATGL* in WAT in 10-day-old chicks. Similar results have been reported in 10- (Honda *et al*., 2017) and 13-day-old chicks (Saneyasu *et al*., 2013b). However, Wang *et al*. (2017) reported that 3 h of fasting significantly reduced mRNA levels of *ATGL* in clavicular fat in 4-day-old broiler chicks fed a high-carbohydrate diet. One possible explanation is that the decrease in mRNA levels of *ATGL* may result from the suppression of adipocyte differentiation. The mRNA levels of adipocyte-specific genes are increased after adipocyte differentiation (Kim *et al*., 2020). Bai *et al*. (2015) reported adipose tissue histology that indicated a low level of differentiation in 4-day-old compared to 14-day-old chicks. Lee *et al*. (2009) reported that mRNA levels of *ATGL* in the adipocyte fraction were approximately 30-fold higher than those in a preadipocyte fraction prepared to WAT. All these findings suggest that changes in mRNA levels of WAT in 10-day-old chicks could be influenced not only by lipid metabolism, but also by adipocyte differentiation. Further studies are required to examine the effects of fasting on adipocyte differentiation in chicken WAT during the neonatal period.

In the present study, plasma corticosterone concentrations were elevated by fasting in 10-day-old, but not in 29-day-old chicks. The reasons for the different responses between the age groups are not clear. However, Delezie *et al*. (2007) reported that 3 and 13 h of fasting did not influence plasma corticosterone concentrations in 6-week-old broiler chicks. Plasma corticosterone levels significantly decreased with age in broiler chicks (Decuypere *et al*., 1989; Hassanzadeh *et al*., 2004). A single injection of ACTH significantly elevated plasma corticosterone concentration in 4- and 10-day-old chicks, but not in 17-day-old chicks, indicating an apparent failure of the ACTH response (Kalliecharan 1981). Therefore, it is possible that the hypothalamic-pituitary-adrenal axis is inactivated in an age-dependent manner in broiler chicks.

In conclusion, mRNA levels of *ATGL* in chicken WAT were affected by 6 h of fasting and corticosterone *in vivo* and *in vitro*. However, plasma NEFA concentrations could be elevated by 3 h of fasting without elevation of plasma corticosterone concentrations. These results suggest that corticosterone upregulates gene expression of *ATGL* in chicken WAT, but that this regulation is not involved in the acute stimulation of lipolysis by fasting.

Conflict of Interest

The authors declare no conflict of interest.

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