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Increased *de novo* lipogenesis in liver contributes to the augmented fat deposition in dexamethasone exposed broiler chickens (*Gallus gallus domesticus*)

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ABSTRACT

Effect of dexamethasone (DEX, a synthetic glucocorticoid) on lipid metabolism in broiler chickens (*Gallus gallus domesticus*) was investigated. Male Arbor Acres chickens (1 wk old, $n=30$) were injected with DEX or saline for 1 wk, and a pair-fed group was included. DEX administration resulted in enhanced lipid deposition in adipose tissues. Plasma insulin increased about 3.3 fold in DEX injected chickens as against the control and hepatic triglyceride was higher as compared with the pair-fed chickens. In DEX injected chickens, the hepatic activities of malic enzyme (ME) and fatty acid synthetase (FAS) were significantly increased, while the mRNA levels of acetyl CoA carboxylase (ACC), ME, and FAS were significantly up-regulated, compared with the control. Although the mRNA levels of lipoprotein lipase (LPL), peroxisome proliferator-activated receptor-γ (PPARγ) and adipose triglyceride lipase (ATGL) genes in adipose tissue were not affected by DEX injection, ME activity and mRNA levels in abdominal fat pad of chickens treated with DEX are higher than those of control chickens. The results indicated that the increased hepatic *de novo* lipogenesis and in turn, the increased circulating lipid flux contributes to the augmented fat deposition in adipose tissues and liver in DEX-challenged chickens. The results suggest that glucocorticoids together with the induced hyperinsulinemia should be responsible for the up-regulated hepatic lipogenesis.

1. Introduction

In intensive rearing systems, chickens are challenged by various stress factors such as high environmental temperature, ammonia, crowding, immune challenge and so on. As the final effectors of the hypothalamus-pituitary-adrenal (HPA) axis, corticosterone (CORT) evokes a series of essential physiological responses related to the mobilization of energy stores and energy redistribution towards inhibiting non-essential functions such as growth, in favor of the maintenance of homeostasis and survival (Matteri et al., 2000).

In rats, high circulating levels of glucocorticoids (GCs) stimulate fat accumulation in the mesenteric region (Rebuffé-Scrive et al., 1992), as a result of redistribution of energy stores toward an intra-abdominal deposition (Strack et al., 1995a,b; Bell et al., 2000). In our previous work, CORT could stimulate the relative feed consumption, while resulting in decreased body weight gain and reduced feed and caloric efficiency (Dong et al., 2007; Yuan et al., 2008). This was accompanied with a retarded development of skeletal muscles in CORT-challenged broiler chickens on the one hand, and enhanced fat deposition on the other hand, suggesting promoted energy stores as toward fat deposition (Dong et al., 2007; Jiang et al., 2008; Yuan et al., 2008).

In mammals, the up-regulated circulating CORT together with the altered insulin sensitivity is suggested to be involved in the enhanced fat deposition (Geraert et al., 1996). The development of obesity was attenuated by adrenalectomy and reversed by administration of GCs (Freedman et al., 1986). In stressed chickens, the augmented CORT and induced hyperinsulinism were suggested to be responsible for the enhanced fat deposition as well (Jiang et al., 2008; Yuan et al., 2008).

Triglyceride (TG) storage in adipose tissue depends on the availability of plasma triglyceride-rich lipoproteins originating from either diet or liver. Lipoprotein lipase (LPL) catalyzes the hydrolysis of plasma lipoproteins, which is a rate-limiting step in the lipid transport into peripheral tissues (Sato et al., 1999). In mammals, increased LPL activity is strongly associated with fat deposition and obesity, which is regulated by both insulin and GCs (Fried et al., 1993). The major site of lipogenesis in birds, however, is the liver rather than the adipose tissue in mammals (Leveille, 1969). Also, the LPL activity in adipose tissue of CORT-challenged broiler chickens seemed not to be the only critical factor contributing to the augmented fat deposition (Jiang et al., 2008; Yuan et al., 2008). Therefore, we hypothesize that the up-regulated GCs may enhance the fat deposition in adipose tissues in stressed chickens mainly via the augmented hepatic *de novo* lipoõgenesis, and, in turn, the increased blood lipid flux.

The objective of the study was to investigate the effect of GCs on the lipid metabolism in broiler chickens. Dexamethasone (DEX), a synthetic glucocorticoid exhibiting a high affinity for glucocorticoid

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receptors and a delayed plasma clearance that enhances tissue exposure (Foucaud et al., 1998) was employed to induce the hyperglucocorticoid status in the present study. Lipid metabolism in both liver and adipose tissues was investigated.

2. Materials and methods

2.1. Animals and diets

Ninety male broiler chicks (Arbor Acres, *Gallus gallus domesticus*) were obtained from a local hatchery (Taiyu Broiler Comp., Taian, Shandong, P.R. China) at 1 d of age and reared in an environmentally controlled room. The brooding temperature was maintained at 35 °C for the first 2 d, and then decreased gradually to 26 °C (45% RH) until 14 d of age. The light regime was 24 h illumination. All chicks received a starter diet with 21.5% crude protein and 12.33 MJ/kg of metabolizable energy (Zhao et al., 2009). The experiment was conducted in accordance with laws and regulations that control experiments and procedures with live animals in China, as laid down by the China Animal Research Authority.

All chicks were randomly divided into three groups and each group had three pens of 10 birds. At 7 d of age, the three groups of chickens were randomly subjected to one of the following treatments for 1 wk. Daily subcutaneous injection (8:00 h) of dexamethasone in a dose of 2.0 mg/kg body mass (BW) (DEX treatment, 1 mg/mL), sham-treated (injection of saline) pair-fed treatment that maintains the same feed consumption of DEX chickens, or the control treatment (injection of saline). Feed intake and BW gain were recorded daily and feed efficiency (feed:gain) was calculated.

At 14 d of age, 12 chickens in each treatment (4 chickens from one of the three pens) were randomly selected and a blood samples were obtained with a heparinized syringe within 30 s from the heart and collected in iced tubes. Plasma was obtained after centrifugation at 400 g for 10 min at 4 °C and was stored at −20 °C for further analysis. After bleeding, the chickens were subjected to cervical dislocation and then exsanguinations (Close et al., 1997). Tissue samples were obtained from liver, abdominal and cervical adipose tissues. The tissue samples were washed with ice-cold sterilized saline, cooled down in liquid nitrogen and stored at −80 °C for further analysis. Thereafter, total liver, abdominal fat, cervical fat and thigh fat were harvested, weighed and expressed as the percentage of BW (%).

2.2. Plasma and liver metabolites analysis

The concentration of glucose (GLU, No. F006), urate (No. C012), non-esterified fatty acids (NEFA, No. A042), TG (No. F001) and LPL (No. A067) was measured spectrophotometrically with colorimetric enzymatic methods by using commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, P.R. China). The concentrations of very low density lipoprotein (VLDL) were determined as previously described (Griffin and Whitehead, 1982).

2.3. Plasma insulin determination

Plasma insulin was measured by radioimmunoassay with a guinea pig anti-porcine insulin serum (3V Biochem. Engineering Comp., Weifang, P.R. China) using chicken insulin as the standard (Simon et al., 1974). A large cross-reaction has been observed between chicken insulin and this anti-serum (porcine) (Simon et al., 1974). The insulin in this study is referred to as immunoreactive insulin. The sensitivity of the assay was 6.9 pmol/L and all samples were included in the same assay to avoid interassay variability. The intra-assay coefficient of variation was 6.9%.

2.4. Enzyme activity assays

The activities of fatty acid synthase (FAS, EC 2.3.1.85) and malic enzyme (ME; EC 1.1.1.40) in liver and adipose tissues were measured. The liver and adipose tissue samples were homogenized in ice-cold 0.25 mol/L sucrose, 1 mol/L dithiothreitol and 1 mol/L EDTA at pH 7.4. The cytosolic fractions were obtained by centrifugation at 100,000 g for 1 h at 4 °C and used for the enzyme assays. The activity of FAS was measured according to the method of Halestrap and Denton (1973). ME activity was determined by a modified method from Hsu and Lardy (1969).

2.5. RNA isolation and analysis

The expression of genes in liver and adipose tissue was quantified using quantitative real-time PCR with SYBR Green I labeling.

Total RNA from liver and adipose tissue was isolated using the guanidinium isothiocyanate method with Trizol Reagent (Invitrogen, San Diego, CA, USA). The quality of the RNA was tested by electrophoresis on an agarose-gel and the quantity of the RNA was determined with biophotometer (Eppendorf, Germany).

RT reactions (10 μL) consisted of 500 ng total RNA, 5 mmol/L $MgCl_2$, 1 μL RT buffer, 1 mmol/L dNTP, 2.5 U AMV, 0.7 nmol/L oligo d(T) and 10 U Ribonuclease inhibitor. Real-time PCR analysis was conducted using the Applied Biosystems 7500 Real-time PCR System (Applied Biosystems, Foster, CA, USA). Each RT-reaction served as a template in a 20 μL PCR reaction containing 0.2 μmol/L of each primer and SYBR green master mix (Takara). Primer-set sequences are described in Table 1. Real-time PCR reactions were performed at 95 °C for 10 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 34 s. SYBR green fluorescence was detected at the end of each cycle to monitor the amount of PCR product. When calculating the efficiency of qPCR primers, a standard curve was made in 5 fold dilutions, and its slope was used to calculate efficiency.

The relative amount of mRNA for a gene was calculated according to the method of Livak and Schmittgen (2001). The mRNA levels of these genes were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels (ΔC_T). The ΔC_T was calibrated against an average of the control chickens. The linear amount of target molecules relative to the calibrator was calculated by $2^{-\Delta\Delta CT}$. Therefore, all gene transcription results are reported as the *n*-fold difference relative to the calibrator. Specificity of the amplification product was verified by electrophoresis on a 0.8% agarose-gel and by DNA sequencing.

Table 1
Gene-specific primer of the lipid metabolism related enzyme.

Gene	GenBank accession no.	Primers sequences (5′→3′)	Orientation	Product size (bp)
ACC	NM_205505	aatggcagctttggaggtgt	Forward	136
		tctgtttgggtgggaggtg	Reverse	
FAS	J03860	ctatcgacacagcctgctcct	Forward	107
		cagaatgttgacccctcctacc	Reverse	
ME	NM204303	tgccagcattacggtttagc	Forward	175
		ccattccataacagccaaggtc	Reverse	
LPL	NM205282	cagtgcaacttcaaccataccа	Forward	150
		aaccagccagtccacaacaa	Reverse	
PPARγ	AF163811	ccagcgacatcgaccagtt	Forward	145
		ggtgatttgtctgtcgtctttcc	Reverse	
GAPDH	NM_204305	ctacacacggacacttcaag	Forward	244
		acaaacatgggggcatcag	Reverse	
ATGL	EU852334	tccttcaccttcagcgtcca	Forward	113
		agtgttgtcctccatctggtc	Reverse	

Abbreviations used: ACC, acetyl CoA carboxylase; ATGL, adipose triglyceride lipase; FAS, fatty acid synthase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LPL, lipoprotein lipase; ME, malic enzyme; PPARγ, peroxisome proliferator-activated receptor-γ.