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Cholesterol deregulation induced by chronic corticosterone (CORT) stress in pectoralis major of broiler chickens

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ABSTRACT

Chronic endogenous glucocorticoid (GC) excess in mammals is associated with metabolic dysfunction and dyslipidemia that are characterized by increased plasma triglyceride and total cholesterol (Tch) levels. However, the effects of chronic GC administration on cholesterol metabolism, particularly in muscle tissues of broiler chickens, are unknown. In this study, broiler chickens were treated chronically with vehicle (CON) or corticosterone (CORT) for 2 weeks. Chronic CORT treatment significantly increased Tch levels in pectoralis major muscle (PMC) ($p < 0.001$) as well as in leg muscle ($p < 0.01$), and CORT enhanced triglyceride levels in the PMC ($p < 0.001$). Real-time PCR results showed that HMGCR ($p < 0.05$) mRNA expression was up-regulated by CORT in PMC, and 11β-HSD1 gene transcription ($p = 0.08$) was not significantly downregulated, whereas glucocorticoid receptor (GR) mRNA expression, 11β-HSD2, CYP7A1, CYP27A1, ApoB and LDLR were unchanged by CORT ($p > 0.05$). Western blot results showed that the levels of total GR ($p = 0.08$) tended to be increased and nuclear GR protein ($p < 0.05$) was increased in PMC by CORT administration. Parallel to an increase in gene expression, HMGCR protein expression in PMC was significantly increased ($p < 0.05$) by CORT. Moreover, LDLR ($p < 0.05$), ApoA1 ($p = 0.06$) and 11β-HSD2 ($p = 0.07$) protein expression in PMC tended to be increased by CORT compared to control. These results indicate that chronic CORT administration causes cholesterol accumulation in PMC tissues of broiler chickens by increasing cholesterol synthesis and uptake.

1. Introduction

Hyperlipidemia and metabolic abnormalities caused by endogenous glucocorticoid (GC) excess have been well documented in mammals and birds. Much information is available regarding long-term dexamethasone (DEX) induction of common metabolic dysfunction and dyslipidemia characterized by increased fasting plasma triglyceride and total and low-density lipoprotein cholesterol (LDLC) concentrations, and decreased high-density lipoprotein cholesterol (HDLC) concentration. In mammals, increased circulating GCs together with the altered insulin sensitivity are suggested to be responsible for enhanced visceral fat deposition and hyperlipidemia (Geraert et al., 1996). In previous studies, GC administration increased hepatic lipogenesis and triglyceride accumulation in adipose tissues in rats (Bowes et al., 1996). In chicken, exogenous GC administration increased hepatic lipogenesis as well as intramyocellular lipid uptake and accumulation in broiler chickens (Lin et al., 2004; Wang et al., 2010; Wang et al., 2012). Our previous study demonstrated that chronic corticosterone (CORT) administration did not change plasma lipid profile, except for a moderate increase of HDLC levels in broiler chickens (Wang et al., 2013). However, until now, data regarding cholesterol metabolic status in skeletal muscle has not been available.

Cholesterol levels in tissues reflect a balance among dietary uptake, endogenous de novo synthesis, efflux, and utilization to bile acids (Faust and Kovacs, 2014). A previous study focused on HMGCR transcription level in the liver, breast or thigh muscles in Beijing-you chickens whose 3′-untranslated region (UTR) of the HMGCR gene was mutated (Cui et al., 2010). With respect to cholesterol uptake, the LDL receptor (LDLR) and ApoA1 are well-known for their important roles in regulating plasma and intracellular cholesterol homeostasis (Soto-Acosta et al., 2013); they are primarily modulated by intracellular cholesterol levels (Liu et al., 2012). Two key factors, cholesterol-7-alpha hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27A1), are prominently involved in the biosynthesis of bile acid from cholesterol and participate in the degradation of cholesterol in the liver (Bjorkhem et al., 2002). However, information regarding HMGCR,

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; APOA1, apoliprotein A1; ApoB, apoliprotein B; CORT, corticosterone; CYP7A1, cholesterol-7-alpha hydroxylase; CYP27A1, sterol 27-hydroxylase; DEX, dexamethasone; GC, glucocorticoid; GR, glucocorticoid receptor; HDLC, high-density lipoprotein cholesterol; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDLC, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LDLs, low-density lipoproteins; PMC, pectoralis major muscle; Tch, total cholesterol; TG, total triglycerides.

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LDLR and sterol hydroxylase in the regulation of cholesterol metabolism in skeletal muscle following CORT-treatment is scarce. CORT conveys its signals primarily through the glucocorticoid receptor (GR), which is the target of endogenous CORT and certain synthetic steroids (Goodwin et al., 2013). As a nuclear hormone receptor, GR is widely conserved and presented in most organs; it is involved in both healthy and disease conditions (Stolte et al., 2006). The intracellular levels of active GC are regulated by several GC-metabolizing enzymes. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) activates, whereas 11β-HSD2 inactivates GCs (Holmes and Seckl, 2006; Tomlinson et al., 2004).

In commercial production, chickens are subjected to a number of stressors prior to slaughter, including feed deprivation, crating density and transportation, which results in a negative impact on meat quality (Delezie et al., 2007). The serum concentrations of total and LDL-cholesterol were increased but serum HDL-cholesterol decreased in heat stress broilers (Habibian et al., 2013). As an essential component of cell structure and the precursor of steroid hormone, the amount of cholesterol in chicken muscle will affect avian well-being, and may ultimately influence human health through dietary intake. It has been reported that, in humans, dietary intake of cholesterol and saturated fatty acids is strongly associated with coronary heart disease and arteriosclerosis (Simopoulos, 2006). Moreover, the muscle plays a critical role in maintaining systemic energy homeostasis and accounts for about 80% of insulin-directed glucose disposal (Nguyen et al., 2014). Investigating the effect of GC on cholesterol metabolism in skeletal muscle in chickens would help elucidate the mechanism of intramyocellular cholesterol accumulation. Therefore, the objective of the present study was to investigate the effect of chronic CORT administration via drinking water on cholesterol metabolism in pectoralis major muscle (PMC), and to clarify the underlying mechanism through the measurement of gene and protein expressions involved in the metabolic process.

2. Materials and methods

2.1. Ethics statement

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Agricultural University and the sampling procedures complied with the "Guidelines on Ethical Treatment of Experimental Animals" (2006) No. 398 set by the Ministry of Science and Technology, China and "the Regulation regarding the Management and Treatment of Experimental Animals" (2008) No. 45 set by the Jiangsu Provincial People's Government.

2.2. Animals and experimental design

Experimental design and animal management have been well documented in the previous publication (Wang et al., 2013). In brief, broiler breeder eggs (Ross 308) were incubated under standard conditions. Newly hatched chickens received a starter diet (12.5 ME/kg; 21% CP) from 1 d to 20 d and a finish diet (12.8 ME/kg; 19.5% CP) from 21 d to 42 d and water was provided ad libitum throughout the experiment. The light was continuous during the first week and decreased gradually to 18L:6D. On d27, the chickens were divided into control and CORT-treated groups. Chickens in CORT group were supplied with water supplemented with 5 mg/L CORT (C2505, SIGMA, USA), in contrast, those in control groups were supplied with water supplemented with equivalent volume of the solvent (absolute ethanol) for the following 15 d. On d42, all chickens were sacrificed by decapitation, and PMC and leg muscle samples were rapidly frozen in liquid nitrogen and then stored at −70 °C.

2.3. RNA isolation, cDNA synthesis and real-time PCR

Total RNA was extracted from PMC samples with TRIzol Reagent (15596026, Invitrogen). Quantity of the RNA was measured by NanoDrop ND-1000 Spectrophotometer (Thermo, USA). The ratios of absorption (260/280 nm) of all samples were between 1.8 and 2.0. Aliquots of RNA samples were subjected to electrophoresis through a 1.4% agarose–formaldehyde gel to verify their integrity. Two micrograms of total RNA was treated with RNase-Free DNase (M6101, Promega, USA) and reverse-transcribed according to the manufacturer's instructions. 2 μL of diluted cDNA (1:40, vol/vol) was used for real-time PCR which was detected in Mx3000P (Stratagene, USA). β-Actin, which is not affected by the experimental factors (CORT) (Duan et al., 2013), was chosen as the reference gene. All the primers chosen to study the expression of genes related to CORT and cholesterol metabolism, as listed in Table 1, were synthesized by Generay (Shanghai, China). The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR results and gene mRNA levels were expressed as the fold change relative to the mean value of control group (Livak and Schmittgen, 2001).

2.4. Nuclear and total protein extracts and western blotting

Nuclear protein extracts were prepared from the muscle as previously described (Rudiger et al., 2002; Sun et al., 2013). Total protein extracts were prepared as previously described (Duan et al., 2013). Protein concentrations were determined using a BCA Protein Assay kit (Pierce, Rockford, IL, USA). Forty micrograms of protein extract from each sample was then loaded onto 7.5% SDS-PAGE gels and the separated proteins were transferred onto the nitrocellulose membranes (BioTrace, Pall Co, USA). After transfer, membranes were blocked for 2 h at room temperature in blocking buffer and then membranes were incubated with the following primary antibodies: GR (1:200; sc1004, Santa Cruz), 11β-HSD1 (1:200; sc20175, Santa Cruz), 11β-HSD2 (1:200; sc20176, Santa Cruz), HMGCR (1:200; sc33827, Santa Cruz), CYP7A1 (1:1000; ab65596, Abcam), CYP27A1 (1:500; BS2192, Bioworld, USA), APOA1 (1:500; BS06158, Bioworld, USA), LDLR (1:500; 10785-1-AP, Proteintech, USA), Lamin A/C (1:500; BS1446, Bioworld, USA), and GAPDH (1:10000; KC-5G4, Changchen, China), in dilution buffer overnight at 4 °C. After several washes in Tris-Buffered-Saline with Tween (TBST), membranes were incubated with goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies (1:10,000; Bioworld, USA) in dilution buffer for 2 h at room temperature. After several washes, bands were visualized by enhanced chemiluminescence (ECL) using the LumiGlo substrate (Super Signal West Pico Trial Kit, Pierce, USA), and the signals were recorded by an imaging System (Bio-Rad, USA), and analyzed with Quantity One software (Bio-Rad, USA).

2.5. Cholesterol and triglyceride contents in the muscle

A modified procedure was used for extraction of total lipids from tissue samples, as described (Cong et al., 2012). Briefly, 50 mg of frozen muscle sample was homogenized in 1 mL ice-cold buffer RIPA, then 200 μL of homogenates was homogenized with 800 μL mixture of chloroform/methanol (2:1, vol/vol) and centrifuged at 3000 *g* for 10 min. The bottom (chloroform) layer was removed, air-dried and reconstituted in 30 μL mixture of tert-butyl alcohol and methanol (13:2, vol/vol). The Tch content was determined by cholesterol assay kit (006301, Beijingbeihua, China), the triglyceride content was determined by triglyceride assay kit (006304, Beijingbeihua, China).

2.6. Statistical analysis

The results are presented as means ± SEM. The general linear model was conducted to evaluate the effects of CORT. All analyses were performed using SPSS 18.0 software. p values less than 0.05 were considered statistically significant.