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Effect of Dietary Conjugated Linoleic Acid (CLA) on Abdominal Fat Deposition in Yellow-feather Broiler Chickens and Its Possible Mechanism*

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ABSTRACT : A total of 60 one-day-old Yellow-feather broiler chickens were allotted into treatment and control groups. The treatment group was fed with the diet supplemented with 3% conjugated linoleic acid (CLA) for 48 d, while control group was fed with the diet supplemented with 3% rapeseed oil. Chickens were slaughtered in each group at the age of 49 d, and the blood and the abdominal adipose tissue were sampled. Serum cLeptin and serum cAdiponectin were measured by ELISA. The total RNA was extracted from adipose tissue to measure the abundance of the chicken growth hormone receptor (cGHR), insulin-like growth factor 1 (cIGF-1), insulin-like growth factor I receptor (cIGF-IR), peroxisome proliferator-activated receptor gamma (cPPARγ), cAdiponectin and cAdipoIR mRNA by RT-PCR using β-actin as an internal standard. Results showed that the CLA decreased the abdominal fat index by 20.93% ($p<0.05$). The level of serum cLeptin but not serum cAdiponectin was significantly increased by CLA treatment ($p<0.05$). CLA down-regulated the relative abundance of cGH-R mRNA and cPPARγ mRNA in abdominal adipose tissue by 24.74% ($p<0.05$) and 66.52% ($p<0.01$) respectively. However, no differences were found between CLA treatment group and control group ($p>0.05$) in the relative abundance of cIGF-1, cIGF-IR, cAdiponectin, and cAdipoIR mRNA in abdominal adipose tissue. The data suggested that CLA inhibited abdominal fat deposition in broiler chicken may be determined by decreasing the GHR available for GH, and by inhibiting the differentiation of preadipocytes via down-regulation of PPARγ, but independent of IGF and (or) GH-IGF pathway or adiponectin action.
(**Key Words :** Conjugated Linoleic Acid (CLA), Yellow-feather Broiler Chicken, Adipose Tissue, Somatropic Axis, Adiponectin)

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term for positional and geometric isomers of linoleic acid. CLA has been reported to reduce body fat gain in mice, rats, hamsters, pigs, dogs, humans (Pariza et al., 2001; Wiegand et al., 2001) and chickens (Szymczyk et al., 2001; Du and Ahn, 2002), and decrease lipid metabolism in the liver of broilers (Jang et al., 2004). However, the underlying neuroendocrine and molecular mechanisms are poorly understood, especially in chickens.

Somatotropic axis related genes play an important role in modulation of adipocyte differentiation, proliferation and fat deposition in animals (Boney et al., 1998; Hausman et al., 2001). The understanding of the physiological systems regulating CLA on somatotropic axis is fundamental to cognize the effects of CLA. Peroxisome proliferator activated receptorsγ (PPARγ) are ligand-activated nuclear hormone receptors that heterodimerize with the retinoid X receptor and act to control the expression of genes involved in cellular metabolism and differentiation (Desvergne and Whahli, 1999). Leptin is synthesized by the Ob gene in white adipose tissue (WAT) and plays a major part in the control of body fat storage through the regulation of food intake and total body energy consumption (Niswender and Schwartz, 2003). Adiponectin is also produced and secreted exclusively from WAT and plays a role in glucose and lipid homeostasis (Scherer et al., 1995). Adiponectin has great importance in adipocyte functions (Ding et al., 2004), and as an important candidate gene affecting fat deposition and carcass traits in pigs (Dai et al., 2006). Serum levels of adiponectin are reduced in humans and rodents with insulin resistance and obesity (Havel, 2002).

The response of somatotropic axis related genes mRNA expressions to CLA administration in broiler have not been

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Table 1. Components of basic diet (%)

Item	0-3 wk (Starter diet)	4-7 wk (Grower-finisher diet)
Ingredients		
Corn	56.0	62.0
Soybean meal	34.0	30.0
Fish meal	4.0	2.5
Soybean oil	2.24	1.97
Monocalcium phosphate	1.0	0.7
Met	0.17	0.07
Lys	0.05	0.05
Limestone	1.19	1.36
Salt	0.35	0.35
Premix	1.0	1.0
Nutrient levels		
Crude protein	21.0	19.0
Crude fiber	4.5	5.5
Ash	7.0	7.0
Ca	0.8-1.3	0.7-1.2
P	0.65	0.60

reported, and much less is known about the relationships between adiponectin and its receptor expressed in adipose tissues of broiler and their coordinated response to CLA administration. Therefore, the present study is aimed to elucidate the mechanisms underlying the action of CLA on fat deposition through examination of the hormones which are related with fat metabolism and mainly secreted by adipose tissue, and on the target gene of growth hormone (GH) including growth hormone receptor (cGHR), insulin-like growth factor-1 (cIGF-1) and its I receptor (cIGF-IR) in abdominal fat, and cPPARγ, cAdiponectin and its I receptor (cAdipoIR) in abdominal fat of broiler chickens.

MATERIALS AND METHODS

CLA source and dietary treatments

The Conjugated linoleic acid (CLA) obtained from Auhai Bio-tech Co., Ltd, Qingdao (P. R. China) contains 80.10% CLA (cis-9, tran-11-18: 2: 39.20, cis-10, tran-12-18: 2: 38.90, and other CLA isomers: 2.0, 16:0: 3.90, 18:0: 2.57, 18:1: 11.55, 18:2: 1.88, %). The basal diet was given in accordance with the standard ration specification for the starter diet and the grower-finisher diet of Yellow-feather broiler chickens (Table 1). The crumbled diets were provided in the first few weeks and then the pellet diets were followed from 22 to 49 d. After a 1-wk adaptation period with basal starter diet, the broiler chickens were put into two groups: control group which is given the diet consisting of basic diet+3% rapeseed oil and experiment group which is provided with the diet consisting of basic diet+3% CLA. Both groups were fed with a standard starter diet from 8 to 21 d, and a grower-finisher diet from 22 to 49 d. The rapeseed oil and CLA source have been added "on top" of basal diets.

Animals and experimental design

A total of 60 (thirty male and thirty female) one-day-old Yellow-feather broiler chickens (the Chinese local strain) were assigned to two dietary treatments with different inclusion level of CLA (0%, and 3% CLA). Day-old broilers were maintained with a ratio of 20 h light: 4 h darkness (the ratio was down-regulated gradually as broilers grew) in a controlled environment room in two identical heated-battery brooders until 21 days of age. At 22 days of age, broilers were individually housed to wire cages (0.4×0.4×0.4 m) in controlled environment rooms and maintained under the same 10 h light: 14 h darkness cycle until the end of the experiment. Broiler chickens were allowed an access to spontaneous feed and water. Individually housed broiler chickens were weighed at 7 d intervals and feed disappearance was determined everyday. Broilers were slaughtered at the age of 49 d, blood samples were collected from the vena jugularis immediately when chickens were slaughtered, and serum was prepared and stored at -20°C. Abdominal fat was weighed and taken immediately (within 5-10 min of death) to be frozen in liquid nitrogen and then stored at -70°C.

ELISA for serum cLeptin and cAdiponectin

Serum cLeptin and cAdiponectin were measured by double-antibody ELISA using commercial kits (Innogent Bio-Sci & Tech Co. Ltd, Shenzhen, P. R. China).

RNA extraction

Total RNA was extracted from the tissue samples with Trizol and the RNA concentration was then quantified by measuring the absorbance at 260 nm and the quality of RNA was examined through a 1.4% agarose-formaldehyde gel.

Reverse transcription (RT) and polymerase chain reaction (PCR)

2 μg total RNA was reversely transcribed by incubation at 42°C for 1 h in a 20 μl mixture consisting of 10 U avian myeloblastosis virus reverse transcriptase, 20 U RNase inhibitor, 5 μmol random primers, 50 mmol/L tris-HCl (pH 8.3), 10 mmol/L $MgCl_2$, 50 mmol/L KCl, 10 mmol/L DDT, 0.5 mmol/L spermidine and 0.5 mmol/L each dNTP. The reaction was terminated by heating at 95°C for 5 min and quickly cooling on ice.

2 μl adipose tissue sample RT reaction mix was used for PCR in a final volume of 20 μl containing 0.1 U Taq DNA polymerase, 2 μl 10×PCR Buffer, 0.2 mmol/L dNTP, 2.0 mmol/L $MgCl_2$, 0.5 μmol each of PCR primers for cGHR, cIGF-1, cIGF-IR, cPPARγ, cAdiponectin, cAdipoIR and cβ-actin were designed using Primer Premier 5.0 and were synthesized by Haojia Biotech. Ltd. (Table 2). PCR was performed for 5 min at 94°C, followed by 33 cycles for 30 s

Table 2. Primer sequences of target genes and conditions for PCR

Target genes	GenBank No.	Primer sequences	PCR products/bp
cβ-actin	L08165	F : 5'- ACGTCGCACTGGATTTCGAG -3'	282 (721-1,002)
		R : 5'- TGTCAGCAATGCCAGGGTAC -3'	
cGHR	AB075215	F : 5'-TTACTTCAACACATCCTACACC-3'	346 (283-628)
		R : 5'-TCATAATCTCTTCCCATCTTCA-3'	
cIGF-1	NM_001004384	F : 5'- CTGGTTGATGCTCTTCAGTTCG -3'	182 (172-353)
		R : 5'- GCAGATTTAGGTGGCTTTATTGG -3'	
cIGF-IR	NM_205032	F : 5'- GTACTTCAGTGCTTCGGATGTG -3'	397 (2,940-3,336)
		R : 5'- CTTCTTCAGAGTTGGAGGTGCT -3'	
cPPARγ	NM_001001460	F : 5'- GACCTTAATTGTCGCATCCAT -3'	237 (439-675)
		R : 5'- CGGGAAGGACTTTATGTATGA -3'	
cAdiponectin	AY523637	F : 5'- CCAACTGGATGGGAGGAG -3'	385 (104-488)
		R : 5'- GTCAGGTGGTAGGCAAAGA -3'	
cAdipoIR	DQ072275	F : 5' GGAAGAGGAGGAAGTTGT-3'	161 (174-334)
		R : 5' GCAGGAGGTAATCGTTGT-3'	

at 94°C, 30 s at 48°C (cGHR), 52°C (cIGF-1), 53°C (cIGF-IR, cPPARγ), 56°C (cAdiponectin) and 49°C (cAdipoIR) and 1 min at 72°C and for 7 min at 72°C in the last cycle.

Different controls were set to monitor the possible contaminations of genomic DNA and environment DNA both at the stage of RT and RCR. The pooled samples made by mixing equal quantity of total RNA from all samples were used for optimizing the PCR condition and normalizing the intra-assay variations. All samples were included in the same run of RT-PCR and repeated at least for 3 times.

Quantitation of PCR products and statistical analysis

An aliquot (10-20 μl) of PCR products was analyzed by electrophoresis on 2% agarose gels. The gel was stained with ethidiun bromide and photographed with digital camera. The net intensities of individual bands were measured by using Digital Science 1D software. The ratios of net intensity of target genes to cβ-actin bands were adopted to represent the relative level of target gene expression. The average level of three repeats was employed for statistical analysis. All data were tested by one-way ANOVA with SPSS for Windows 11.0 (SPSS Soft, Inc.). Mean values and SE are reported. The experiment was conducted with strict observance of the guidelines set by the regional Animal Ethics Committee.

RESULTS

Body weight gain and abdominal fat deposition

Treatment of 3% CLA significantly reduced abdominal fat deposition of broiler chickens at the age of 49 d ($p<0.05$). However, average daily body weight gains (ADG) and feed intake were not affected by the dietary CLA (Table 3).The effects of CLA on the results showed no gender difference.

Table 4. Effect of CLA on serum level of cleptin and cadiponectin in Yellow-feather broiler chicken

	cLeptin (ng/ml)	cAdiponectin (ng/ml)
Control	0.31±0.03	164.47±18.67
CLA treatment	0.40±0.03*	154.99±9.92

Mean±SE. * $p<0.05$.

Serum level of cleptin and cAdiponectin

CLA treatment increased serum concentration of cLeptin by 29.03% ($p<0.05$) compared with control group, while no significant difference of cAdiponectin concentration in serum between CLA-treated group and control group were observed (Table 4).

cGHR, cIGF-1, cIGF-IR, cPPARγ, cAdiponectin, and cAdipoIR mRNA expression in abdominal adipose tissue

The relative abundance of cGHR mRNA expression in abdominal adipose tissue was reduced by 24.74% ($p<0.05$) with CLA treatment (Figure 1A). No marked changes of relative abundance of cIGF-1 mRNA and cIGF-IR mRNA ($p>0.05$) between CLA-treated group and control group were observed (Figure 1B, 1C). CLA down regulated cGHR mRNA expression. The relative abundance of cPPARγ mRNA expression in abdominal adipose tissue was reduced by 66.51% ($p<0.01$) (Figure 1D). However, no marked

Table 3. Effect of CLA on growth performance and fat depot in *Yellow-feather* broiler chicken

	ADG (g/d)	Total Feed intake for 49 d (kg)	Abdominal fat weight (g)	Abdominal fat index (%)	Mortality level (%)
Control	32.70±1.17	3.69±1.17	58.41±2.25	5.12±0.24	0
CLA treatment	31.56±1.16	3.78±1.17	45.79±2.72*	4.05±0.17*	0

Mean±SE. * $p<0.05$.

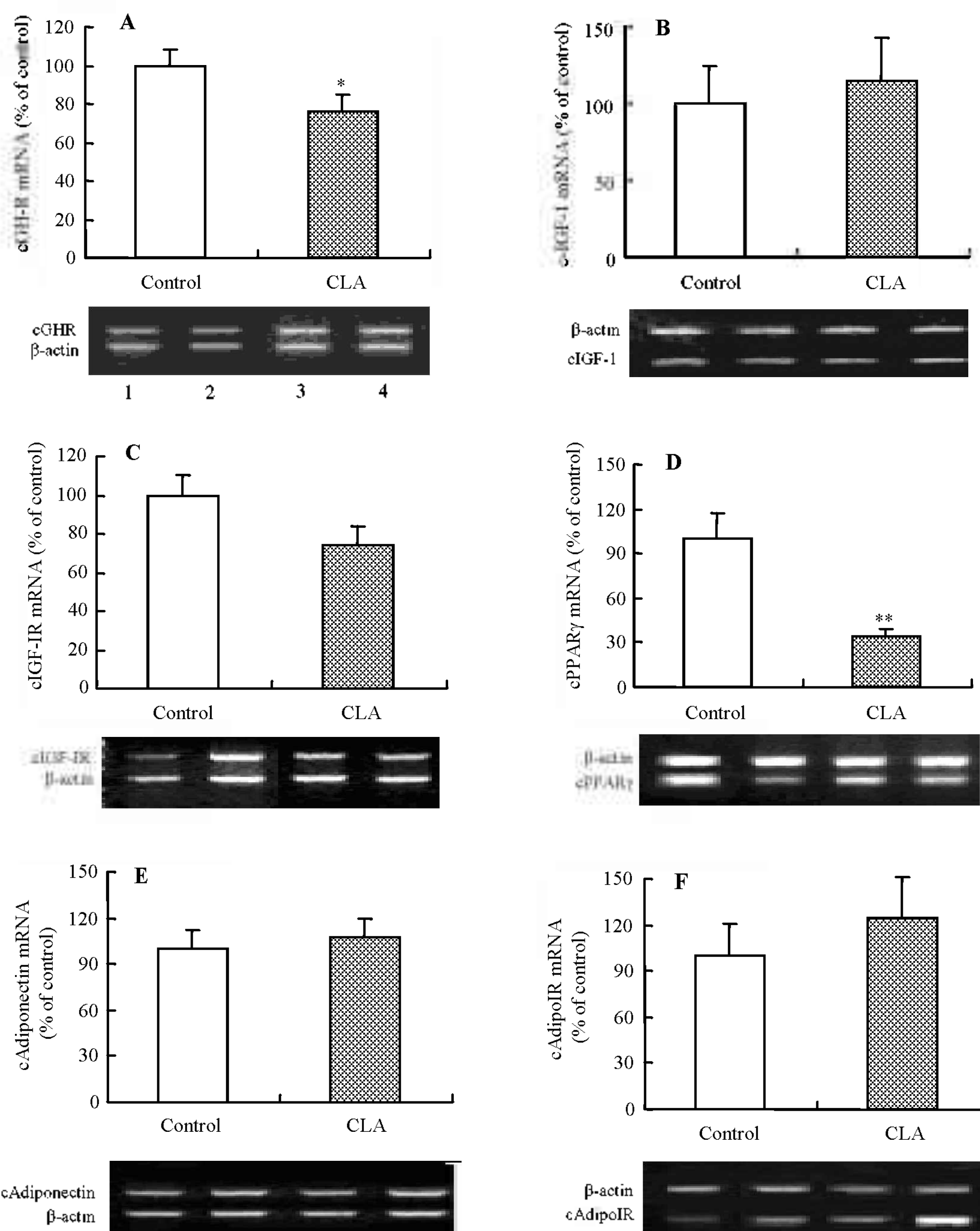

Figure 1. Effect of CLA on expression of cGHR, cIGF-1, cIGF-IR, cPPARγ, cAdiponectin, and cAdipoIR mRNA of abdominal adipose tissue. In electrophpresis photo, lanes 1-2: broilor fed rapeseed oil, lanes 3-4: broilor fed CLA. * $p<0.05$, ** $p<0.01$.

changes of relative abundance of cAdiponectin mRNA and cAdipoIR mRNA ($p>0.05$) between CLA-treated group and control group were observed (Figure 1E, 1F). CLA treatment down regulated cPPARγ mRNA expression.

DISCUSSION

Like mammals, the neuroendocrine system integrates internal such as genotype with external factors such as nutrition to regulate bird growth. However, the underlying mechanisms are not always the same. The levels of cGHR mRNA in the liver and abdominal fat relevant with growth and fat deposition of different strain broiler chickens respectively. In contrast, the level of cGH protein is not relevant with growth and (or) fat deposition, and there is no difference in cIGF-1 level in different strain (Mao et al., 1998). The hypothalamic somatostatin (cSS) and pituitary cGH mRNA expression as well as the plasma cGH levels were higher in the slower growth layer chickens than the fast-growing broiler while the opposite was true for hepatic

cGHR mRNA (Zhao et al., 2004). These results show that the growth of chickens and fat deposition mainly depends on the target mechanism of GH.

Although IGF-1 is considered to mediate the effects of GH by the endocrine and paracrine (autocrine) way, the effects of GH and IGF-1 are not all the same in controlling postnatal growth. In terms of growth physiology, the GH and IGF-1 signaling pathways serve both independent and overlapping functions. With comparative analysis of growth of mouse mutants lacking either GH receptor or IGF-1, or both, Lupu (2001) estimated that in normal conditions, approximately 17% of body weight gained in the course of growth is unrelated to the GH/IGF-1 axis, that IGF-1 makes by itself a more significant contribution than GH acting alone (35% vs. 14%), and that the overlapping GH/IGF-1 function makes a major contribution to total weight (34%). Therefore, the independent and overlapping of GH and IGF-1 signaling pathways may also exist in adipose tissue. In the present study, we found the expression of cGHR mRNA was reduced with CLA treatment. However, the cIGF-1 mRNA and cIGF-IR mRNA expression in abdominal adipose tissue indicated no difference in CLA-treated group and control group. We concluded that CLA's inhibition of abdominal fat deposition in broiler chicken may be dependent only on decreasing the GHR available for GH, but independent of IGF and (or) GH-IGF pathway.

Although CLA isomers are weak ligands for PPARγ, this receptor is essential for the fatty acids to regulate gene expression in the adipocyte (Desvergne and Wahli, 1999). Dietary CLA lowered PPARγ mRNA levels in white adipose tissues of the mouse (Tsuboyama-Kasaoka et al., 2000). A CLA-dependent decrease in mRNA levels of PPARγ was seen in interscapular brown adipose tissue of both strains and in white adipose tissue of mice (Takahashi et al., 2002). Trans-10, cis-12 CLA inhibited differentiation of 3T3-L1 adipocytes and decreased PPARγ expression (Kang et al., 2003). Consistent with these observations, dietary CLA decreased PPARγ mRNA levels in white adipose tissue in the present study. Together, these studies have indicated that CLA reduced body fat gain as a possible mechanism by which CLA can inhibit the expression of PPARγ and its downstream target lipoprotein lipase in adipose tissues. These observations suggest that CLA decreases body fat gain in part by inhibiting the differentiation of preadipocytes.

The Yellow-feather broiler chicken was one of Chinese local strain. Compared with faster growing meat strains, it was a slower growing but higher quality meat strain, which was popular in China. The present study showed feeding the Yellow-feather broiler chicken the diet supplemented with CLA resulted in reduction of body fat gain, increase of serum leptin levels, no significant change in the secretion of adiponectin, adiponectin mRNA and its receptor mRNA expression in adipose tissue. The effects of CLA on both male and female broiler chickens were the same. But the above data was not consistent with the findings in the following literature related to rodent experiments. The t10, c12-CLA reduced leptin secretion from 3T3-L1 adipocytes (Kang and Pariza, 2001). In mice, t10, c12-CLA decreased mRNA expression of leptin and adiponectin (Warren et al., 2003). CLA-feeding decreased the levels of leptin and adiponectin, especially in obese/diabetic mice (Ohashi et al., 2004). A diet containing 1.0% CLA, mainly composed of c9, t11- CLA and t10, c12-CLA equivalent amounts, greatly decreased adipose tissue weight and serum concentrations of leptin and adiponectin and was accompanied by a down-regulation of the expression of various adipocyte-abundant genes in adipose tissue of mice (Ide, 2005). Our study implies that the effects of CLA on adiponectin and its receptor expression depend on the species of animal. In contrast to mammals, CLA's inhibition of fat deposition in broiler chickens may be independent of adiponectin action.

IMPLICATIONS

In conclusion, the mechanisms by which conjugated linoleic acid reduces adiposity in the broiler chicken remain unclear. Results of this study demonstrated that the CLA's decreasing fat gain is caused at least by inverse regulation of GH-R and PPARγ expression respectively in abdominal adipose tissue. The observed effects of CLA seem to depend on target mechanism of GH and inhibition of the differentiation of preadipocytes.

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