JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Green Tea Polyphenols Alleviate Obesity in Broiler Chickens through the Regulation of Lipid-Metabolism-Related Genes and Transcription Factor Expression

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ABSTRACT: The current study investigated the effects of green tea polyphenols (GTPs) on lipid metabolism and its mechanisms using broiler chickens (*Gallus gallus domesticus*). A total of 36 male chickens (35 days old) had been subjected to an oral administration of GTPs at a dosage of 0, 50 (low), and 100 (high) mg/kg of body weight for 20 days. Our results showed that GTPs significantly decreased the abdominal and subcutaneous fat masses of broilers and reduced the serum triglyceride, total cholesterol, and low-density lipoprotein cholesterol levels compared to those of the control. Furthermore, the expression levels for lipid anabolism genes were significantly downregulated, while the expression levels of fat transportation and catabolism-related genes, carnitine palmitoyl transferase I (CPT-I), acyl-CoA oxidase 1 (ACOX1), and peroxisome proliferator-activated receptor-α (PPARα) in liver, adipose triglyceride lipase (ATGL) in abdominal fat, and lipoprotein lipase (LPL) in skeletal muscles, were notably upregulated. Our data have revealed that GTPs alleviate obesity and serum lipid levels in broiler chickens by suppressing fatty acid synthesis and stimulating lipolysis.

KEYWORDS: *Chicken, green tea polyphenols, lipid-lowering effect, real-time quantitative PCR*

■ INTRODUCTION

During the past few decades, the broiler industry has witnessed a great improvement in growth rate and feed conversation efficiency.[1] However, this breeding strategy only targets growth speed and feed conversation rate, which has led to inevitable problems, such as the decline of meat quality and increased waste caused by the excessive deposition of abdominal and subcutaneous fat. For these reasons, adding specific lipid-lowering natural compounds to the feed has been shown to be an effective method to overcome these problems and, thus, has been reported.[2−6]

Green tea, derived from the tea plant (*Camellia sinensis*), is a widely consumed popular beverage, which contains high levels of polyphenols. The major group of polyphenols in green tea is the catechins, which constitute about one-third of its total dry weight.[7] Researches have reported that green tea and its catechins possess antioxidant, antiatherogenic, anti-inflammatory, and anticarcinogenic properties using *in vitro* cell culture and *in vivo* animal studies.[8−10] In addition, adding green tea extract to animal feeds also has potential benefits that may improve the quality of animal-derived products.[11,12] Cho et al.[13] added 5% of various green teas to the feed of juvenile olive flounders to improve growth, body composition, and blood chemistry. Among the potential health benefits of green tea, its lipid-lowering effect has been frequently investigated in rodent obese models and other non-mammals.[14,15] Wolfram et al.[16] showed that C57BL/6J mice fed a high-fat diet containing epigallocatechin gallate (EGCG) at 1.0% significantly decreased the plasma triglycerides and adipose tissue mass. Biswas et al.[17] found that 0.5−1.5% dietary Japanese green tea powder supplementations for 35 days significantly decreased AFR and the serum total cholesterol levels in boiler chickens. It was also reported that supplementation of feeds with 1−4% (w/w) of green tea powders for 2 weeks did not affect the performance parameters of broilers.[18]

Although the lipid-lowering effects of tea polyphenols have been reported for *in vitro* and *in vivo* animal models, the mechanisms of its anti-obesity properties were largely unknown. In addition, the effects of tea polyphenols on lipid metabolism of domestic birds were relatively less investigated, and the underlying molecular mechanisms relating to the anti-obesity action of tea polyphenols in chickens remains elusive.

In this study, therefore, we investigated the effects of an oral administration of green tea polyphenols (GTPs) using the broiler chickens as our animal model. We measured serum and body fat levels, the mRNA expression of key lipid-metabolism-related enzymes, and transcription factors in broilers to elucidate the mechanism of the anti-obesity actions of green tea *in vivo*.

■ MATERIALS AND METHODS

Ethics Statement. All animal experiments were performed in accordance with the "Guidelines for Experimental Animals" at the Ministry of Science and Technology (Beijing, China). All dissections were performed according to recommendations proposed by the European Commission, and all efforts were made to minimize suffering in our animals.

Received: May 8, 2013
Revised: August 12, 2013
Accepted: August 15, 2013
Published: August 15, 2013

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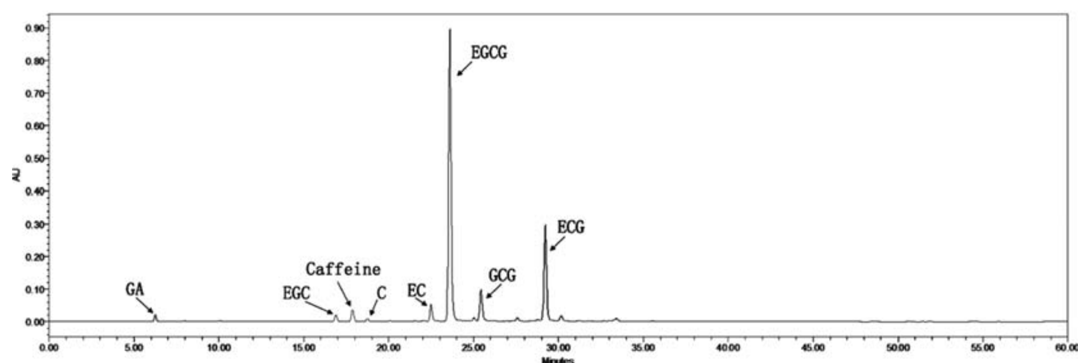

Figure 1. HPLC spectrogram of the main component in GTPs used in this experiment.

Table 1. Primers Used for Real-Time PCR[a]

GenBank ID	gene name	primer	product length
NM_205518	β-actin	forward: CTGTGCCCATCTATGAAGGCTA reverse: ATTTCTCTCTCGGCTGTGGTG	139
NM_204305	GAPDH	forward: TGCTAAGGCTGTGGGGAAAG reverse: CAGCAGCCTTCACTACCCTC	158
NM_205155	FAS	forward: GCAGCTTCGGTGCCTGTGGTT reverse: GCTGCTTGGCCCACACCTCC	119
NM_205505	ACC	forward: AACGAGTCGGGCTACTACCT reverse: ATCAGCATCCCGTGAAGTGG	119
NM_204303	ME	forward: TGCCAGCATTACGGTTTAGC reverse: CCATTCCATAACAGCCAAGGTC	175
NM_204542	LXRα	forward: GTGCAGAGAGTGACGAGCTT reverse: AGAGGTTTACGTGCGTGGAG	175
NM_204485	HMGR	forward: GCTGCCCTCTGCTTGCCAGG reverse: GCGGGCAAACCTACTTGTGCTG	106
NM_001012898	CPT-I	forward: TCGTCTTGCCATGACTGGTG reverse: GCTGTGGTGTCTGACTCGTT	143
NM_001006205	ACOX1	forward: ATGTCACGTTCACCCCATCC reverse: AGGTAGGAGACCATGCCAGT	133
NM_001001464	PPARα	forward: TGTGGAGATCGTCCTGGTCT reverse: CGTCAGGATGGTTGGTTTGC	103
NM_001113291	ATGL	forward: TCACCTTCAGCGTCCAAGTC reverse: GCACATGCCTCCAAAAGAGC	186
NM_205282	LPL	forward: ACTTGAAGACCCGTGCTCAG reverse: GGCTGGTCTACCTTGGTCAC	97
NM_001001460	PPARγ	forward: CAGTGGATCTGTCTGCGATG reverse: CTTTGGCAATCCTGGAGCTTG	172

[a]GAPDH, glyceraldehyde 3-phosphate dehydrogenase; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; ME, malic enzyme; LXRα, liver X receptors-α; HMGR, HMG-CoA reductase; CPT-I, carnitine palmitoyl transferase I; ACOX1, acyl-CoA oxidase 1; PPARα, peroxisome proliferator-activated receptor-α; ATGL, adipose triglyceride lipase; LPL, lipoprotein lipase; and PPARγ, peroxisome proliferator-activated receptor-γ.

Reagents. GTPs (98.92% of purity) (lot HTP98120921, QB2154-95) were purchased from Wuhu Tianyuan Science and Technology Development Co., Ltd. (Anhui province, China), which contained 52.22% EGCG, 7.58% epigallocatechin (EGC), 11.49% epicatechin gallate (ECG), 6.13% gallocatechin gallate (GCG), 4.71% epicatechin (EC), 1.10% catechin (C), 0.90% caffeine, and 0.66% gallic acid (GA), detected by Waters 600E series high-performance liquid chromatography (HPLC) equipped with a quaternary pump, a 2475 fluorescence detector, and a 2489 ultraviolet−visible (UV−vis) detector (Figure 1). All other reagents used in this experiment were analysis-grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Animal Handling and Sample Collection. Male broiler chicks (Ross 308, *Gallus gallus domesticus*) were obtained from a local hatchery at 1 day old and subsequently raised in a standard chicken facility. The breeding room temperature was maintained at 35 °C [50% relative humidity (RH)] for the first 3 days and then was gradually decreased to 21 °C (45% RH) until 28 days of age and maintained at such conditions until the end of the experiment. All chickens were provided a starter diet with 21% crude protein and 12.56 MJ/kg metabolizable energy until day 21, after that, they received a grower diet with 19% crude protein and 12.98 MJ/kg metabolizable energy. All birds were given *ad libitum* access to food and water during the entire rearing period.

At 35 days of age, 36 broilers with similar body weight (BM) (1.29 ± 0.02 kg) were selected and raised in wire cages (two chickens per cage). These broilers were randomly allocated into 3 groups, with each group containing 12 chickens. Each of the 3 groups was subjected to a daily oral administration of a low dose of GTPs (50 mg/kg of body weight), a high dose of GTPs (100 mg/kg of body weight), or the same volume of water (control group) at 8:00 a.m. continuously for 20 days. During the experimental period, all birds were individually weighed every 5 days to measure the change in body weight. At 55 days of age (20 day treatment), 6 chickens were randomly selected

Table 2. Time-Dependent Effect of GTPs on Body Weight Gain (g) of Broiler Chickens[a]

	CK	L-TP	H-TP	probability
5 day	105.83 ± 1.87	100.75 ± 2.89	97.75 ± 2.25	NS[b]
10 day	229.42 ± 3.83	220.92 ± 7.01	227.75 ± 6.25	NS
15 day	467.92 ± 10.18	460.42 ± 15.48	480.00 ± 15.32	NS
20 day	771.67 ± 18.42	758.75 ± 14.95	797.42 ± 42.44	NS

[a]Values are the mean ± SEM (n = 12). The mean initial body weight of experimental chickens was 1294.02 ± 15.01 g. The difference significance was tested by one-way ANOVA. [b]NS = not significant.

Table 3. Effect of GTPs on Body Lipid Index and Serum Biochemical Parameters[a]

	CK	L-TP	H-TP	probability
		Body		
AFR (%)	1.550 ± 0.161	0.846 ± 0.087[b]	0.736 ± 0.066[b]	<0.001
SFT (cm)	0.840 ± 0.014	0.738 ± 0.038[b]	0.567 ± 0.023[b]	<0.001
		Serum		
TG (mmol/L)	0.256 ± 0.007	0.252 ± 0.005	0.159 ± 0.008[b]	<0.001
TC (mmol/L)	2.974 ± 0.060	3.027 ± 0.045	2.786 ± 0.070[c]	0.028
HDL-C (mmol/L)	0.665 ± 0.018	0.738 ± 0.011[c]	0.681 ± 0.027	0.045
LDL-C (mmol/L)	1.710 ± 0.056	1.603 ± 0.027	1.352 ± 0.044[b]	<0.001
ALT (IU/L)	21.16 ± 0.638	21.23 ± 0.678	20.76 ± 0.820	NS
AST (IU/L)	166.09 ± 1.340	170.27 ± 3.521	169.32 ± 2.549	NS

[a]AFR, abdominal fat rate, which was calculated as the percentage of live weight; SFT, subcutaneous fat thickness, which was measured at the caudal spondyle including the skin and fat width with a vernier caliper; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, activity of alanine aminotransferase; and AST, activity of aspartate aminotransferase. Values are represented as the mean ± SEM (n = 6). [b]$p < 0.001$ represents significant difference compared to the control (CK), by ANOVA. [c]$p < 0.05$ represents significant difference compared to the control (CK), by ANOVA.

from each group. All chickens selected were sampled in a fasting (12 h food withdrawal) condition. Blood samples were obtained with a sterilized syringe within 30 s from a wing vein and collected. Blood was incubated in a water bath for 1 h at 37 °C and centrifuged at 400*g* for 10 min at 4 °C, and the last serum was obtained and stored at −80 °C for further analysis. After blood sampling, the chickens were killed by cervical dislocation and then exsanguinated.[19] Immediately after death, the abdominal and thoracic cavities were opened; liver, abdominal fat, breast muscle, and thigh muscle were harvested, weighed, and expressed as a percentage of the body weight (%). Tissue samples were collected from liver, abdominal adipose, breast muscle, and thigh muscle. Small parts of liver samples from the three groups were fixed in 10% formaldehyde and used for histochemical analysis. The remaining tissue samples were then washed with ice-cold sterilized saline, frozen in liquid nitrogen, and stored at −80 °C for further analysis of lipid-metabolism-related key gene expression.

Evaluation of Body Fat. The body fat level of birds in this experiment was evaluated using two indicators, abdominal fat rate (AFR) and subcutaneous fat thickness (SFT). The AFR was calculated as the percentage of live weight. The SFT was measured at the caudal spondyle including the skin and fat width with a vernier caliper following the method by Zhang et al.[20]

Measurement of Serum Biochemical Parameters. Assay kits (Changchun Huili Biotech. Co., Ltd., China) were used to determine the serum biochemical parameters. The triglyceride (A035), total cholesterol (A030), high-density lipoprotein cholesterol (A029), low-density lipoprotein cholesterol (A028), and activity of ALT (A001) and AST (B014) were measured using a Multi-Mode Microplate Reader (Spectra Max 190, Molecular Devices, LLC, Sunnyvale, CA). The quality controls, calibrations, and experimental procedures were carried out according to the instructions of the suppliers.

Histochemical Analysis. Livers were fixed overnight in 10% phosphate-buffered formalin and processed for paraffin embedding, and cross-sections were cut into 6 μm sections and placed onto glass slides. The slides were dewaxed in xylene and rehydrated through gradient ethanol washes. Hematoxylin and eosin staining was performed using standard procedures.

Real-Time Polymerase Chain Reaction (PCR) Analysis. The expression of genes in liver, adipose tissue, thigh muscle, and breast muscle was measured using real-time quantitative PCR (RT-qPCR) with SYBR Green I labeling.

Total RNA from tissue samples was isolated using the reagent Trizol (Invitrogen, San Diego, CA) and purified with RNAprep pure tissue kit (Tiangen, Beijing, China). The quality of the RNA was tested by electrophoresis on an agarose gel, and the quantity of the RNA was measured with a spectrophotometer (Nanodrop 1000, Thermo Scientific, Waltham, MA).

Reverse transcription was performed immediately following the total RNA isolation using the Primescript II first strand cDNA synthesis kit (Takara, Dalian, China). RT-qPCR was performed using a CFX96 real-time system (Bio-Rad, Hercules, CA). RT-qPCRs were performed at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. SYBR green fluorescence was detected at the end of each cycle to monitor the amount of PCR product. A standard curve was created using a template of 10-fold series dilutions, and its slope was used to calculate the efficiency of the qPCR primers. Primer sequences are listed in Table 1.

The relative amount of a target gene mRNA was calculated according to the method by Livak and Schmitten.[21] The expression level of a target gene mRNA was normalized to the mRNA level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β-actin. The $\Delta\Delta C_T$ was calibrated against an average from the control group. The linear amount of the target gene expression to the calibrator was calculated by $2^{-\Delta\Delta C_T}$. Therefore, all gene expression results are reported as the fold difference between the treated and control groups. The specificity of the real-time PCR product was verified using the melting curve and DNA sequencing.

Statistical Analysis. Data are expressed as the mean ± standard error of the mean (SEM). All data were analyzed using one-way analysis of variation (ANOVA) by the SPSS software package (SPSS 13.0 for Windows, SPSS, Inc., Chicago, IL) to evaluate the main effect of the GTP treatment. When the main effect of the treatment was significant, differences between means were assessed using Duncan's multiple range analysis. Differences were considered significant at a probability value of <0.05, 0.01, and 0.001.

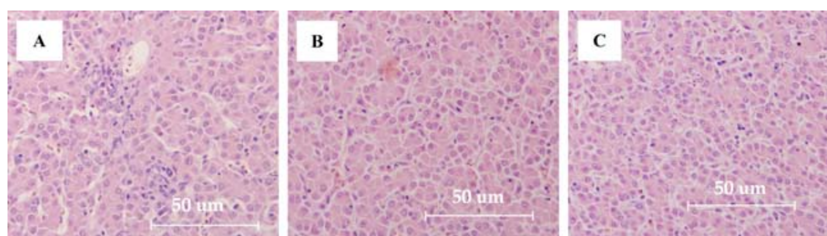

Figure 2. Liver paraffin slices of broilers treated with GTPs. Livers were fixed overnight in 10% phosphate-buffered formalin, and the tissue sections were prepared for hematoxylin and eosin staining. Representative images were captured by a microscope (Olympus BX51, Japan) at 200×: (A) daily oral administration of the same volume of distilled water as treated groups, (B) 50 mg/kg GTP-treated group, and (C) 100 mg/kg GTP-treated group.

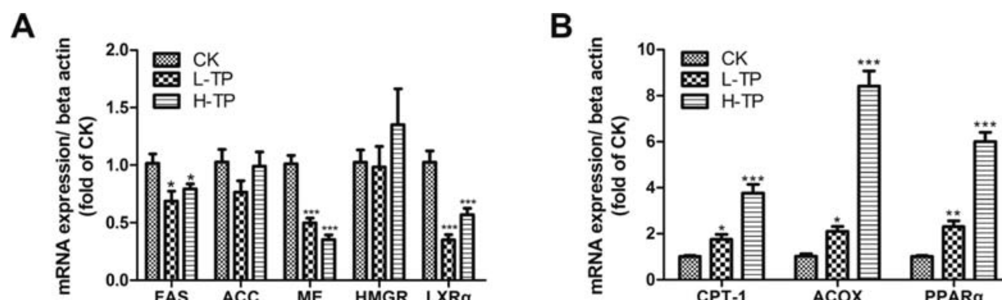

Figure 3. Some key gene mRNA expression levels in the liver of broilers treated with GTPs or the control group. Panels A and B show mRNA expression levels of lipid-metabolism-related key genes in the liver. CK, oral administration of the same amount of distilled water as the treated groups; L-TP, 50 mg/kg GTP-treated group; and H-TP, 100 mg/kg GTP-treated group. The values are the mean ± SEM ($n = 6$). (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ represent significant difference between treated groups and the control.

RESULTS

GTPs Have No Influence on the Body Weight Gain and Hepatic Function of Chickens. During the breeding period, the body weight of all experimental birds increased progressively. In addition, with the experiment carrying on, the body weight gain among different oral incubation intervals enhanced, complying with the broiler growth law. However, in comparison to the control, the body weight gain using different doses of GTP treatments had no significant difference (Table 2).

In comparison to the control group, there were also no significant alterations in the activity of serum ALT and AST from broilers treated with GTPs (Table 3). Histological studies showed that the hepatic cell structure of all groups was normal, and no degeneration, necrosis, and inflammatory cell infiltration were found (Figure 2).

GTPs Decrease the Body and Serum Lipid Levels. The body and serum lipid parameters were shown in Table 3. Both body and serum lipid levels from GTP-treated groups were significantly decreased in comparison to that of the control group. The AFR of broilers treated with 50 or 100 mg/kg GTPs was dramatically reduced by 45.42% ($p < 0.001$) and 52.52% ($p < 0.001$), respectively. However, no significant difference was found between 50 and 100 mg/kg polyphenol-treated groups. The broilers treated with 50 or 100 mg/kg of polyphenols significantly decreased the SFT by 12.14% ($p < 0.001$) or 32.50% ($p < 0.001$), respectively, compared to that of the control group, and a high dosage of polyphenols (100 mg/kg) had a stronger effect to decrease the SFT than a lower dosage of polyphenols.

The serum triglyceride, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) levels were significantly decreased by 37.89% ($p < 0.001$), 6.32% ($p < 0.05$), and 20.94% ($p < 0.001$), respectively, from 100 mg/kg GTP-treated broilers compared to control broilers. However, these three serum parameters were not altered by the 50 mg/kg GTP treatment compared to the control group. Furthermore, the serum high-density lipoprotein cholesterol (HDL-C) level was elevated in the chickens with both dosages of GTP treatments.

GTPs Regulate the Expression of Lipid-Metabolic-Related Genes. To investigate the mechanisms of the lipid lowering effects of GTPs, the mRNA expression levels of some key genes and transcription factors related to lipid metabolic pathways in liver, adipose tissue, thigh muscle, and breast muscle were detected using RT-qPCR.

The mRNA level of fatty acid synthase (FAS) in the liver of broilers treated by 50 or 100 mg/kg of GTPs was significantly decreased ($p < 0.05$) compared to the control. In addition, GTP treatments did not affect the acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGR) gene expressions of the liver. Whereas, the mRNA level of malic enzyme (ME) in the liver was significantly downregulated dramatically by 50.86% ($p < 0.001$) or 65.11% ($p < 0.001$) with 50 or 100 mg/kg GTP treatment, respectively. Furthermore, the mRNA expression of liver X receptors-α (LXRα) in the liver was also significantly decreased by 65.95% ($p < 0.001$) and 44.64% ($p < 0.001$) with 50 or 100 mg/kg GTP treatment, respectively (Figure 3A).

On the other hand, the expression levels of hepatic lipid-β-oxidation-related genes and transcription factors were significantly increased by the treatment of GTPs (Figure 3B). The carnitine palmitoyl transferase I (CPT-I) mRNA expression in liver was significantly increased by 1.75 ($p < 0.05$) and 3.76 ($p < 0.001$) fold in 50 and 100 mg/kg GTP-treated broilers, respectively, compared to that of the control group. We also observed a 2.10 ($p < 0.05$) and 8.42 ($p < 0.001$) fold upregulation in acyl-CoA oxidase 1 (ACOX) gene expression of broilers treated with 50 and 100 mg/kg GTPs, respectively.

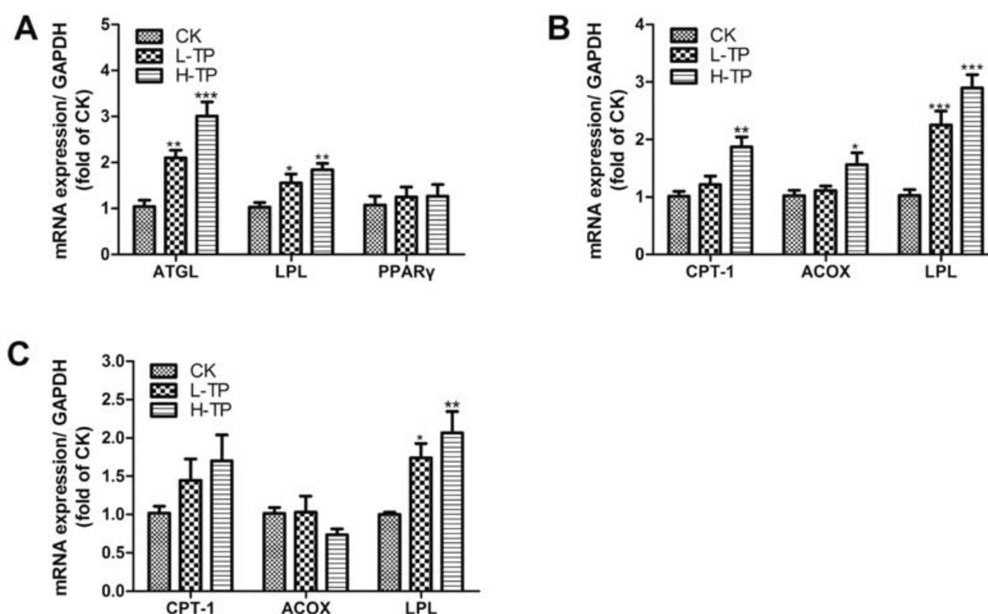

Figure 4. Some key gene mRNA expression levels in the abdominal tissue and muscles of broilers treated with GTPs or the control group. Panels A−C show mRNA expression levels of the lipid-metabolism-related key genes in different tissues: (A) abdominal adipose tissue, (B) breast muscle, and (C) thigh muscle. The values are the mean ± SEM ($n = 6$). (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ represent significant difference between treated groups and the control.

Furthermore, the mRNA expression of peroxisome proliferator-activated receptor-α (PPARα), an important nuclear transcription factor, was obviously elevated in the liver of broilers treated with GTPs compared to the control. Furthermore, the 100 mg/kg GTP treatment potentiated more PPARα mRNA expression than the 50 mg/kg treatment.

In the abdominal adipose tissue, the mRNA expression of ATGL, a key lipid-degrading enzyme, was significantly elevated with 50 and 100 mg/kg GTP treatments by 2.10 ($p < 0.01$) and 3.01 ($p < 0.001$) fold, respectively, compared to the control. A similar result was found regarding lipoprotein lipase (LPL) mRNA expression. However, GTP treatments did not alter peroxisome proliferator-activated receptor-γ (PPARγ) mRNA expression (Figure 4A).

LPL mRNA expression in both thigh muscle and breast muscle of both dosage-treated broilers were significantly increased compared to the control. Additionally, in the breast muscle of treated chickens, the mRNA levels of CPT-I and ACOX1 were increased only in response to the 100 mg/kg GTP treatment. No alteration of the mRNA expression of these two genes was found regarding the 50 mg/kg dosage treatment. Furthermore, GTP treatments did not significantly alter the CPT-I and ACOX1 mRNA expression in the thigh muscle compared to that of the control (panels C and B of Figure 4).

■ DISCUSSION

The present study has clearly revealed that an oral administration of GTPs has significantly decreased abdominal fat mass as well as SFT. It has also decreased the expression of key lipid-synthesis-related genes and increased lipid-β-oxidation-related genes. Our new set of data has demonstrated the anti-obesity properties of green tea and elucidated its mechanism in broiler chickens.

In our current research, experimental dosages of GTPs did not alter the liver function of boilers and had no hepatic toxicity, through serum aminotransferase concentration and liver histochemical analysis.

Body weight, abdominal fat mass, and SFT are an index widely used to measure the degree of obesity in the broiler.[22,23] Serum triglyceride, total cholesterols, LDL-C, and HDL-C are also the crucial biochemical parameters reflecting the status or rate of lipid metabolism. Present studies have shown that an oral administration of GTPs significantly suppressed these body and serum lipid indicators in experimental birds. However, the body weight gain of broilers was not reduced by experimental dosages of GTPs. This result is in agreement with the findings by Biswas and Wakita,[17] which showed that 0.5−1.5% dietary Japanese green tea powder supplementations for 35 days did not alter the body weight of boilers, while AFR and serum total cholesterols in these chickens were decreased significantly. Similar lipid-lowering effects of green tea, tea polyphenols, or EGCG were also found in rats or mice.[24−31] However, Shomali et al.[18] recently reported that 2 weeks of dietary supplementation with green tea powder did not affect the performance and serum concentration of triglycerides, total cholesterol, LDL-C, and HDL-C in broiler chickens. The main difference between our study and the experiment by Shomali et al. is the intervention period. A total of 2 weeks of dietary supplementation with green tea powder may not be long enough to see its lipid-lowering effects.

In avian species, the liver is a primary organ for lipogenesis and accounts for approximately 95% of the *de novo* fatty acid synthesis. It is generally assumed that almost all fat, accumulating in broiler adipose tissue, is synthesized in the liver or derived from the diet.[32] In addition, the liver is the most important organ for the intermediary metabolism of lipids and energy. Therefore, regulation of hepatic gene expression may play a central role in the adaptive response to alter digestion by changing the capacity of enzymes in relevant metabolic pathways.[33] Huang et al. reported that the mRNA expression levels of fatty acid *de novo* synthesis key enzymes (FAS, ACC, and ME) and transcription factors [sterol regulatory element binding protein-1 (SREBP-1)] of Arbor Acres (AA) chicks fed with corn−soybean basal diets containing 0.5−2% soy lecithin

were significantly altered and their body fat composition and serum lipid biochemical parameters were also changed.[6] Another group reported that dexamethasone (2 mg/kg of body weight) administration to male AA chicks for 3 days significantly increased the liver and cervical adipose tissue mass and plasma triglyceride level and the FAS and ACC gene expression levels were upregulated simultaneously.[34] The present study demonstrated that the mRNA levels of hepatic FAS and ME were significantly downregulated by GTPs, while fatty acid β-oxidation velocity-controlling enzymes (CPT-I and ACOX1) were drastically increased. Liver X receptor α (LXRα), which is a member of the nuclear receptor family, has been proven to have a critical role in the regulation of cholesterol and lipid metabolism. LXRα was the prime mover of hepatic lipid metabolism, and the activation of LXRα altered the mRNA expression of genes involved in lipogenesis.[35,36] The present study showed that the mRNA expression level of LXRα was significantly decreased by GTPs. Peroxisome proliferator-activated receptor α (PPARα) is another member of the nuclear receptor superfamily, which has an important role in regulating lipid metabolism. Barger and Kelly reported that the activation of PPARα enhanced the fatty-acid-oxidation-related gene (CPT, ACS, and ACOX) expression in the liver. The activation of PPARα also increased in the hepatic fatty acid oxidation in our animal model.[37] Our data showed that increased expressions of PPARα as well as CPT-I and ACOX1 were found in broilers administrated with 100 mg/kg of GTPs. In summary, our data have clearly shown that an oral administration of GTPs decreased the expression of some key genes related to fatty acid *de novo* synthesis and increased the expression of some key genes functioning in fatty acid β-oxidation in the liver of broilers. Similar results were also reported in mice and rats subjected to green tea, green tea extract, tea polyphenols, or EGCG.[25,28−30]

Adipose tissue and muscle are important organs related to lipid metabolism and energy consumption. Therefore, regulation of lipid metabolism key gene expression by GTPs in these two tissues is of great interest. Our data showed that chickens treated with GTPs have less abdominal fat and subcutaneous fat mass. The results are in agreement with previous reports.[24,29] Accumulation of adipose tissue mass specifically depends upon the balance of fat anabolism and catabolism. Fat anabolism depends upon the availability of plasma triglycerides that are hydrolyzed prior to uptake by adipocyte and fatty acid *de novo* synthesis.[38] On the other hand, fat catabolism mostly depends upon the activity of triglyceride catabolic key enzymes. LPL is the most abundant lipase in adipose tissue and skeletal muscle. LPL catalyzes the rate-limiting step in the hydrolysis of triglycerides from circulating chylomicrons and very low-density lipoprotein (VLDL).[39] The function of LPL is tissue-specific. Increasing the adipose LPL activity can lead to fat deposition, while the elevation of enzyme activity in skeletal muscle can enhance fat catabolism.[40] Adipose triglyceride lipase (ATGL) is a key lipid-degrading enzyme, which catalyzes the first step of the catabolic process of triglycerides. The substrate specificity of ATGL to triglycerides is 10-fold higher than that to diglycerides.[41] Our data demonstrated that mRNA expressions of LPL and ATGL were significantly upregulated in abdominal adipose tissue by GTPs, while the serum triglyceride concentration was significantly decreased by treatment of GTPs. In summary, the present study demonstrated that the reduced abdominal fat mass in GTP-treated broilers is attributed to its promoting actions on catabolism in adipose tissue and suppressed effects of chylomicrons in the liver.

Skeletal muscle is a mitochondria-rich tissue, and it requires sufficient energy derived from carbohydrates and fat to maintain its movement function. In muscle tissue, LPL is secreted into capillaries, regulating the absorption of serum triglyceride of the skeletal muscle. The present data showed that GTPs elevated the LPL mRNA level in both breast and thigh muscles, and GTPs increased the expression of CPT-I and ACOX1 in breast muscle. Consequently, GTPs may increase the absorption of triglycerides and increase the β-oxidation of fatty acids in skeletal muscle.

The question remains, however, as to why the lipid metabolism genes in different tissues of the birds were modulated by GTPs in such a coordinated way. There may be a metabolic regulator involved in producing these effects. AMP-activated protein kinase (AMPK) is a serine/threonine kinase that is implicated in the control of energy metabolism at both the cellular and whole-organ levels.[42] AMPK activation results in the phosphorylation and inhibition of ACC, and the loss of inhibition of CPT-I by decreasing the concentration of malonyl-CoA leads to the increased fatty acid oxidation. Murase et al. examined the effects of green tea catechins on the AMPK signaling pathway in cultured cells (Hepa 1−6, L6, and 3T3-L1) and in mice. They demonstrated that catechins with a gallocatechin moiety or a galloyl residue activate LKB1/AMPK in cultured cells and oral administration of EGCG in mice stimulates energy expenditure, concomitant with the upregulation of AMPKα phosphorylation and AMPKα activity in the liver.[43] The modulation pattern of gene expression in our present study was similar in the regulating mode with the AMPK pathway; therefore, it may be AMPK that acts as the role of master metabolic regulator.

In conclusion, the present study demonstrated that abdominal and subcutaneous fat masses were significantly decreased in the GTP-treated groups and the concentration of serum triglyceride, total cholesterol, and LDL-C levels was also decreased in the serum of GTP-treated broilers. Our data further revealed that GTP administration suppressed the expression of some key genes related to fatty acid *de novo* lipogenesis, while elevated expressions of some key genes were related to fatty acid β-oxidation in the liver. Incubation of GTPs promoted the catabolism in abdominal adipose tissue and increased the lipid metabolic usage in skeletal muscle.

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Funding

This work was supported by the Agricultural Science and Technology Achievements Transformation Project (2013GB2C300220, the Ministry of Science and Technology of People's Republic of China) and the Earmarked Fund for Modern Agro-industry Technology Research System in Tea Industry (nycytx-26, the Ministry of Agriculture of People's Republic of China).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Anhui Feixi Chicken Agri-Husbandry Co., Ltd. (Hefei, China) for providing the breeding rooms.

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