《Research Note》

Expression of Lipid Metabolism-Associated Genes in Male and Female White Feather Chicken

Yunjie Tu, Yijun Su, Guohui Li, Xueyu Zhang and Haibing Tong

Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou, Jiangsu, 225125, China

Differential lipid metabolic requirements of sexually-mature males and females may influence the regulation of lipid metabolism-associated genes and hence the content of adipose tissue. We measured the expression of eight lipid metabolism-associated genes (fatty acid synthase, *FASN*; acylglycerol- 3- phosphate O-acyltransferase 9, *AGPAT9*; peroxisomal proliferator-activated receptor γ , *PPAR γ* ; lipoprotein lipase, *LPL*; carnitine palmitoyl transferase 1 A, *CPT1A*; carnitine palmitoyl transferase 1 B, *CPT1B*; acyl-CoA dehydrogenase long chain, *ACADL*; monoglyceride lipase, *MGL*) in eight tissues (hypothalamus, HYP; liver; heart; pectoralis major muscle, PM; gastrocnemius muscle, GAS; abdominal fat, AF; clavicular fat, CF; subcutaneous fat, SF) of five male and five female white feather chickens using real time PCR at 217 d (when the females were at peak egg production). There were no difference between sexes, nor were there sex by tissue interactions for *CPT1A* and *MGL*. In both cases expression was greater for liver than the other tissues. When interactions of sex by tissue were significant, the *FASN* mRNA abundance in HYP, liver, and PM was greater for females than males. There was no sexual dimorphism for any tissue for *PPAR γ* . Overall values were greater for adipose depots than HYP and liver with muscles intermediate for *AGPAT9*. *LPL* mRNA abundance in PM and AF was greater for females than males, with the pattern reversed for heart and SF. *CPT1B* mRNA abundance in GAS and CF was greater for females than males, with the relationship reversed for liver. *ACADL* mRNA abundance in HYP, liver, and GAS was greater for females than males, and lower in PM than males. The results demonstrated that expression of lipid metabolism-associated genes varies among sexes in mature chickens depending on the gene and the tissue.

Key words: chickens, gene expression, lipid metabolism-associated genes, metabolism path way, real time PCR

J. Poult. Sci., 53: 118–123, 2016

Introduction

The triglyceride content of a cell is regulated by the balance between fat deposition (lipogenesis) and hydrolysis (lipolysis). Accumulation of adipocytes through enhanced rates of cellular differentiation, a process known as adipogenesis, also influences the neutral lipid content of a tissue. Lipid-metabolic genes, including those involved in lipogenesis, can influence the rate of these processes and hence the content of tissue fat. Because of high growth rate and large deposition of fat in the abdomen, the chicken has been used as a model organism for understanding lipid metabolism, fattening and growth (Stern, 2005; Lee *et al.*, 2008). Because egg production causes major changes in the metabolism of lipids to meet the demands of yolk formation (Richards *et al.*, 2003), it is important to understand underlying genetic mechanisms governing lipid metabolism

especially at the peak phase of egg production. Birds have the ability to store large quantities of excess energy (in the form of triglycerides) in the liver, adipose tissue and in the yolk of developing oocytes (Hermier, 1997). Lipogenesis takes place primarily in the liver of birds (Leveille *et al.*, 1975) and involves a series of linked enzyme catalyzed reactions including glycolysis, the citric acid cycle and fatty acid synthesis. Fatty acid synthase (*FASN*) has synthesis of long-chain fatty acids functions in lipid metabolism pathways. In chickens, like in humans (Dorn *et al.*, 2010), *FASN* primarily occurs in the liver (Demeure *et al.*, 2009). Fatty acid esterification includes some holding functional genes such as acylglycerolphosphate acyltransferase 9 (*AGPAT9*) (Bitou *et al.*, 1999), and peroxisomal proliferator-activated receptor γ (*PPAR γ*) (Wang *et al.*, 2008). Among genes involved in lipid uptake, lipoprotein lipase (*LPL*) has an important role in chicken and mice (Merkel *et al.*, 1998). Carnitine palmitoyl transferase 1A (*CPT1A*), carnitine palmitoyl transferase 1B (*CPT1B*), and acyl-CoA dehydrogenase long chain (*ACADL*) have fatty acid oxidation functions of skeletal muscle in chicken (He *et al.*, 1992; Wu *et al.*,

Received: May 4, 2014, Accepted: September 28, 2015

Released Online Advance Publication: November 25, 2015

Correspondence: Dr. Y Tu, Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou, 225125, China. (E-mail: tyj3030@126.com)

2012; Lorzadeh *et al.*, 2013), and monoglyceride lipase (*MGL*) has a role in lipolysis of adipose tissue in chicken and mice (Tashler *et al.*, 2011). Differential metabolic requirements of sexually-mature males and females may influence the regulation of these genes and body composition in the rat (Kushlan *et al.*, 1981; Capel and Smallwood, 1983), fish (Shrestha *et al.*, 2013), and hens (Moradi *et al.*, 2013). Although there are reports on lipid metabolism-associated gene expression of some tissues in chickens (Karpe *et al.*, 1998; Meng *et al.*, 2005; Lorzadeh *et al.*, 2013), data for sexually-mature males and females is scarce. In this study we investigated expression of lipid metabolism-associated genes in different tissues of white feather chicken at the time of peak egg production, a time of considerable lipid activity.

Materials and Methods

Chickens and Husbandry

In 1999 a white feather chicken population of 2,800 females and 800 males was introduced from the SASSO Company in France by the Poultry Institute, Chinese Academy of Agricultural Sciences. Since then it has been maintained as a closed population conserved in National Chicken Genetic Resources (NCGR). It belongs to a white feather broiler, and was bred from White Plymouth Rocks. The plumage is recessive white with light yellow skin and shank colors. Eggshell color is a pale brown, and the population is segregating for early and late feathering. The average BW of males and females at 42 d is approximately 1,015 and 865 g, respectively, and at 84 d is approximately 2,340 and 1,891 g, respectively, and they are consistent with those for the population as reported by Wang *et al.* (2006). The flock of egg production was at 80% when sampled at 217 d. Egg number to 63 w is 175. The white feather chicken has been used as a parent of quality chicken breeding in China.

The procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Chinese Academy of Agricultural Sciences. Management requirements were in accordance with the white feather chicken meat Breeder Management Guidelines. Chicks were reared in sex-intermingled flocks in floor pens with wood shaving for litter. They were weighed, sexed, and moved to separate floor pens with wood shavings litter at 42 d. Chickens were transferred to individual cages at 140 d.

Diets, fed in mash form, were 20% crude protein (CP), 12.12 MJ/kg metabolically energy (ME), 1.0% Ca, 0.5% P, 0.44% Met, and 0.95% Lys from hatch to 42 d, and 14% CP, 11.5 MJ/kg ME, 1.0% Ca, 0.45% P, 0.34% Met, and 0.70% Lys from 42 d to 140 d. Thereafter, the diet consisted of 15% CP, 11.5 MJ/kg ME, 3.2% Ca, 0.45% P, 0.37% Met, and 0.90% Lys. These diets have remained constant during selection and are free of antibiotics.

In chickens, after hatch a dramatic increase in fatty acid oxidation occurs in the heart, which has been attributed to an increase in L-carnitine levels and a switch from the liver to muscle (Lorzadeh *et al.*, 2013). The liver is the major tissue of lipid synthesis, and the lipid is transported to adipose tissue, the major tissue of lipid storage (Demeure *et al.*,

2009). The hypothalamus has the capacity to “talk” with adipose tissue to regulation of energy metabolism (Zhang *et al.*, 2014). Muscle is an important part of the chicken and relevant part thereafter life. Therefore, we chose these 8 kinds of tissue: liver, hypothalamus (HYP), heart, pectoralis major (PM), gastrocnemius muscle (GAS), abdominal fat (AF); clavicular fat (CF), and subcutaneous fat (SF) to investigate the genes expression.

At 217 d, 5 males and 5 females were randomly selected and their BW (g) recorded at 4 h post feed-withdrawal. After cervical dislocation and decapitation, the HYP was isolated following the description of Xu *et al.* (2011). It was wrapped in aluminum foil, placed in liquid nitrogen and transferred to -80°C for storage until RNA isolation. The body cavity was opened and a 300 mg aliquot of the liver (left lobe), heart, PM, GAS, AF, CF, and SF was removed, immediately frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. CF was the fat above the left side of the clavicle, and SF was obtained from the left side of the chicken from the skin of the peritoneum. The PM was removed from the left side of the keel as a longitudinal strip, while the GAS was isolated from the left leg.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from each of the tissues sampled on individual chickens (300 mg of adipose tissue; about 100 mg of all other tissue types) using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA quality was determined by UV absorbance ratio at 260 and 280 nm. Extracted RNA samples were stored at -80°C .

The complementary DNA was synthesized from 20 ng/ μL of total RNA by PrimeScript[®] 125 reverse transcription Master Mix 126 Perfect Real Time (Takara, Dalian, China) according to the manufacturer's protocol. The reaction volume was 20 μL , and contained 2 μL reverse transcription buffer, 2 μL Random primers, 4 μL dNTP at 2.5 mM, 1 μL reverse transcriptase, and 11 μL RNA (20 ng/ μL). Reaction conditions for reverse transcription were as follows: 25°C for 10 min, 37°C for 120 min, 85°C for 5 s, and the kept at 4°C . The cDNA sample was then diluted 8-fold with nuclease-free water and stored at -20°C before proceeding with real time PCR.

Primers

The chicken-specific primers were designed with Primer Express software (Table 1), for lipid metabolism-associated genes including *FASN*, *PPAR γ* , *AGPAT9*, *LPL*, *CPT1A*, *CPT1B*, *ACADL*, and *MGL*. Primers were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Primer amplification efficiency was validated for β -actin and all target genes, with efficiencies that ranged from 95 to 105%. Specificities were confirmed by melting curve analyses (60°C) performed on each sample. β -actin was chosen as the house-keeping gene across tissues and between sexes.

Real Time PCR

Real time PCR was performed with Fast SYBR Green Master Mix (ABI 4385612, USA), 96-well thin-walled plates (ABI 4346906, USA), and ABI Prism optical seals (ABI

Table 1. Primers for real time PCR¹

Gene symbol	Sequence 5' → 3'	Amplicon size/ bp	Location	Genbank ID
<i>FASN</i>	F: CCATTGCACCAGCACTACTCA R: ACGAGGCTTAGGGTGTGGAA	59	785-805 824-843	NM_205155
<i>PPARγ</i>	F: CACTGCAGGAACAGAACAAAGAA R: TCCACAGAGCGAACTGACATC	67	806-828 851-872	NM_001001460
<i>AGPAT9</i>	F: CCCATAGATGCGATCATTTTGA R: CGTGAACCTGGCCAACCAT	61	787-808 829-847	NM_001031145
<i>LPL</i>	F: GACAGCTTGGCACAGTGCAA R: CACCCATGGATCACCACAAA	62	298-317 340-359	NM_205282
<i>CPT1A</i>	F: GCCCTGATGCCTTCATTCAA R: ATTTTCCCATGTCTCGGTAGTGA	60	1807-1826 1844-1866	NM_001012898
<i>CPT1B</i>	F: TGCTGCTTCCAATTCGACG R: TGCAGCGCGATCTGAATG	68	72-90 140-157	DQ314726
<i>ACADL</i>	F: GACATCGGCACTCGGAAGA R: CCTGGTGTCTCCCTGAAGA	60	148-166 188-207	NM_001006511
<i>MGL</i>	F: GCGGACGAGCGTAGACTCA R: GCGAATAGCCTGGTTTGCAA	55	1597-1615 1631-1651	XM_414365
β -actin	F: GTCCACCGCAAATGCTTCTAA R: TGCGCATTTATGGGTTTGT	58	1176-1196 1233-1253	NM_205518.1

¹ F: Forward; R: Reverse.

FASN=fatty acid synthase, *PPAR γ* =peroxisomal proliferator-activated receptor γ , *AGPAT9*=acylglycerol-3-phosphate O-acyltransferase 9, *LPL*=lipoprotein lipase, *CPT1A*=carnitine palmitoyl transferase 1 A, *CPT1B*=carnitine palmitoyl transferase 1 B, *ACADL*=acyl-CoA dehydrogenase long chain, *MGL*=monoglyceride lipase.

4311971, USA) using a 7500 FAST system. Reactions were performed in duplicate for each sample in 10 μ L volumes, including 5 μ L Fast SYBR Green Master Mix, 0.25 μ L forward and reverse primers (5 μ M stocks), respectively, 3 μ L of diluted cDNA, and 1.5 μ L of nuclease free water. The real time PCR conditions were 95°C for 20 s, followed by 40 cycles at 95°C for 30 s, 60°C for 30 s, and final steps of 95°C 15 s, 60°C 1 min, 95°C 15 s for melting curve analysis.

Statistical Analysis

The average of the duplicate Ct values were analyzed using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen, 2001), where beta actin Ct data were used to calculate ΔC_t ($C_{t_{\text{target}}} - C_{t_{\text{beta actin}}}$) with the mean of liver for males used as the calibrator sample to calculate $\Delta\Delta C_t$ ($\Delta C_{t_{\text{X group}}} - \Delta C_{t_{\text{calibrator}}}$) within a gene. Relative quantity (RQ) was calculated ($2^{-\Delta\Delta C_t}$).

Data were analyzed using ANOVA and JMP Version 9.2 (SAS Institute, Inc., Cary, NC, USA). The statistical model included the main effects of tissue, sex, and the interaction between them. The statistical model was $y_{ij} = \mu + S_i + T_j + (ST)_{ij} + \epsilon_{ij}$, where y_{ij} was the dependent variable of gene mRNA abundance, S_i , T_j and $(ST)_{ij}$ were the fixed effect of sex, tissues, and the two way interaction, respectively, with ϵ_{ij} as the random error. Tukey's test was used as a post-hoc test for pairwise comparisons and differences considered significant at $P < 0.05$. Body weights were compared by one-way ANOVA with sex as the main variable. When tissue by sex interactions was significant comparisons were by the Paired sample T-test using SPSS 16.0 version. All values were shown as means $\pm$ SEM.

Results

Body Weights

There was sexual dimorphism for BW ($P < 0.05$) at 217 d. Means and SEM for BW at 217 d were $3,009 \pm 150$ g and $2,257 \pm 180$ g for males and females, respectively.

Lipid Metabolism-associated Factor mRNA Abundance in Different Tissues of Males and Females

The UV absorbance ratio at 260 and 280 nm of total RNA was 1.8–2.0. Of the eight genes studied there were significant sex by tissue interactions for all but *CPT1A* and *MGL*. For these two genes, values were similar for males and females (Table 2). Among tissues, *CPT1A* values for liver were significantly higher than those for the other tissues which did not differ and for *MGL* value for heart was highest (Table 2).

When interactions of sex by tissue were significant (Table 3) the *FASN* mRNA abundance in HYP, liver, and PM was greater ($P < 0.05$) for females than males, with no difference between sexes for any of the other tissues. Within males there was consider overlapping among tissues with a clear significance of HYP > PM. In contrast for females there were clear differences with liver > HYP > the remaining tissues.

Although there was no sexual dimorphism for any tissue for *PPAR γ* , the pattern for males was greater expression in adipose than the other tissues. Although this general pattern existed for females the overlap was greater than for males. As with *PPAR γ* , there was a lack of sexual dimorphism for *AGPAT9*. Overall values were greater for the adipose depots

Table 2. Least squares means $\pm$ SEM in mRNA abundance of genes in tissues where there were no sexual dimorphism or sex by tissue interactions

Tissue	Gene	
	Carnitine palmitoyl transferase 1 A	Monoglyceride lipase
Hypothalamus	0.25 ^b	0.35 ^b
Liver	2.09 ^a	1.28 ^a
Heart	0.78 ^b	1.38 ^a
Pectoralis major muscle	0.39 ^b	0.17 ^b
Gastrocnemius muscle	0.34 ^b	0.17 ^b
Abdominal fat	0.27 ^b	0.07 ^b
Clavicular fat	0.28 ^b	0.13 ^b
Subcutaneous fat	0.45 ^b	0.27 ^b
SEM	0.12	0.11

^{a, b} Different lower case letters within a column indicates a significant difference between tissues ($P < 0.05$).

Table 3. Least squares means $\pm$ SEM of mRNA abundance of genes where tissue by sex interactions were significant

Tissue	Fatty acid synthase		Peroxisomal proliferator-activated receptor γ		Acylglycerol- 3-phosphate O-acyltransferase 9		Lipoprotein lipase		Carnitine palmitoyl transferase 1 B		Acyl-CoA dehydrogenase long chain	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Hypothalamus	1.25 ^{a*}	2.90 ^b	0.14 ^c	0.45 ^{bc}	0.71 ^c	0.84 ^c	0.05 ^c	0.12 ^d	5.60 ^c	9.63 ^b	1.56 ^{cd*}	5.99 ^b
Liver	0.76 ^{abc*}	7.72 ^a	0.43 ^c	0.49 ^{bc}	1.22 ^{bc}	0.64 ^c	0.37 ^c	0.24 ^{cd}	3.85 ^{c*}	0.06 ^c	3.71 ^{bc*}	7.56 ^b
Heart	1.01 ^{ab}	0.93 ^c	1.39 ^c	0.39 ^c	1.39 ^{bc}	1.18 ^{bc}	11.69 ^{a*}	6.23 ^a	59.50 ^a	73.46 ^a	5.60 ^b	2.49 ^c
Pectoralis major muscle	0.04 ^{c*}	0.24 ^c	0.32 ^c	0.73 ^{bc}	3.30 ^{ab}	1.57 ^{bc}	0.04 ^{c*}	0.30 ^d	46.24 ^{ab}	68.30 ^a	1.18 ^{cd*}	0.23 ^c
Gastrocnemius muscle	0.99 ^{ab}	0.52 ^c	4.72 ^{bc}	1.89 ^{bc}	1.60 ^{bc}	1.56 ^{bc}	0.49 ^c	0.28 ^{cd}	45.21 ^{ab*}	534.47 ^b	9.48 ^{a*}	15.01 ^a
Abdominal fat	0.35 ^{bc}	0.89 ^c	7.54 ^{ab}	3.63 ^{bc}	4.96 ^a	7.23 ^a	0.49 ^{c*}	2.45 ^{bc}	23.67 ^{abc}	16.42 ^b	0.60 ^{cd}	0.44 ^c
Clavicular fat	0.70 ^{abc}	1.12 ^c	11.78 ^a	18.00 ^a	1.91 ^{bc}	3.44 ^{bc}	4.59 ^b	3.42 ^b	10.10 ^{bc*}	23.04 ^b	0.94 ^d	1.84 ^c
Subcutaneous fat	0.63 ^{abc}	0.56 ^c	7.29 ^{ab}	6.29 ^b	3.35 ^{ab}	3.81 ^b	0.57 ^{c*}	0.19 ^d	35.70 ^{abc}	40.65 ^b	2.43 ^{cd}	1.80 ^c
SEM	0.18	0.30	1.13	1.27	0.51	0.62	0.75	0.49	8.00	26.69	0.65	0.76

^{a, b, c, d} Values within a column with different superscripts differ significantly ($P < 0.05$).

* Significant difference ($P < 0.05$) between columns.

than HYP and liver with muscles intermediate.

LPL mRNA abundance in PM and AF was greater ($P < 0.05$) for females than males, with the pattern reversed for heart and SF. Within males the pattern was heart > CF > the other tissues, although for females general pattern was the same differences were not as delineated. *CPT1B* mRNA abundance in GAS and CF was greater ($P < 0.05$) for females than males, with the relationship reversed for liver. Within males abundance was greater for heart, PM, GAS than HYP and liver with adipose tissues intermediate. For females the ordering was GAS > heart, PM, SF, CF, AF, HYP > liver.

Expression of *ACADL* mRNA abundance was greater in the GAS than any of the other tissues. Although *ACADL* mRNA abundance in HYP, liver, and GAS was greater ($P < 0.05$) for females than males, in PM it was lower in females than males. For other tissues, males and females were similar. Within males values were greatest for GAS and least for AF with overlapping. In contrast for females the differences were clear with GAS > liver, HYP > the other tissues.

Discussion

In this study the mRNA abundance of eight lipid metabolic genes in eight tissues were compared for males and females, a time when the later were at peak production and lipid metabolism should be great, due to yolk production. *FASN* mRNA abundance in HYP, liver, and PM was greater for females than males. *FASN* catalyzes the last step in fatty acid biosynthesis, it is a major determinant of the maximal hepatic capacity to generate fatty acids by *de novo* lipogenesis (Kusakabe *et al.*, 2000). This is especially relevant in chickens where the liver is the major site of *de novo* lipogenesis (Demeure *et al.*, 2009), along with a sizable contribution from bone (Yalcin *et al.*, 2012). Consistent with those findings, we observed at peak lay that *FASN* mRNA abundance in liver was greater for females than males, and with expression in the liver was greater than in the other tissues. While for males, *FASN* is expressed in HYP, heart as much as in liver. The metabolic requirements of females and males are thus different, and by this age excess fat deposition is essential to support reproduction during peak lay.

PPAR γ is a ligand-activated transcription factor that belongs to the steroid receptor superfamily (Tontonoz and Spiegelman, 2008) and is predominantly expressed in adipose tissue where it is a major inducer of differentiation and adipogenesis (Meng *et al.*, 2005; Kilroy *et al.*, 2012). Thus, it is not surprising that its mRNA was relatively more abundant in adipose than other tissues.

Fatty acid esterification enhances TG synthesis and may be associated with increased content of muscle fat (Ji *et al.*, 2012). Lysophosphatidic acid is further acylated at the sn-2 position by *AGPAT* to form phosphatidic acid (Bitou *et al.*, 1999). In the next step, the phosphate group is removed by phosphatidate phosphohydrolase to produce diacylglycerol. Accordingly, in females *AGPAT9* mRNA abundance in adipose depots was greater than in the other tissues measured.

Among lipid uptake genes, the mRNA abundance of the *LPL* gene was highly expressed in the hearts of males. Lipoprotein lipase is a rate-limiting enzyme for the hydrolysis of the triacylglycerol (TG) core of circulating TG-rich lipoproteins, chylomicrons, and very-low-density lipoprotein (VLDL). *LPL*-catalyzed reaction products, fatty acids, and monoacylglycerol are transported into adipose tissue and skeletal muscle and stored as neutral lipids (Wang and Eckel, 2009). Our results that abundance was greatest in expression of *LPL* in heart were consistent with previous reports (Karpe *et al.*, 1998), although our values were lower in the other tissues.

The two main isoforms of *CPTI* are the liver isoform (*CPTIA*) and the muscle isoform (*CPTIB*) (Park *et al.*, 1998). *CPTIA* is expressed in most tissues, but not in skeletal muscle, whereas *CPTIB* is expressed in muscles and adipocytes (Cook and Park, 1999; Jackson-Hayes *et al.*, 2003). That mRNA abundance of the *CPTIA* gene was more strongly expressed in liver was consistent with previous

reports. Our results that *CPTIB* and *ACADL* mRNA abundance was greatest expression in GAS of females than the other sex and tissue combinations suggest that the subsequent increased fatty acid oxidation may contribute to decreasing lipid deposition in the chicken. *CPTIB* and *ACADL* were differentially expressed in GAS and PM. The increased expression in GAS and reduced expression in PM is consistent with an increased food intake in the females and at the same time reduced fatty acid oxidation in PM yielding a net accumulation of fat storage in PM during peak lay. The results presented in this study were consistent with Ka *et al.* (2013).

Within cells monoglycerides are derived from the hydrolysis of glycerophospholipids or TG. Glycerophospholipids may be degraded by phospholipase C generating sn-1, 2-diacylglycerols, which are further hydrolyzed by sn-1-specific diacylglycerols lipase resulting in the formation of 2-monoglyceride (Gao *et al.*, 2010). The enzyme is expressed in most cell types and is considered the rate-limiting enzyme in the degradation of monoglycerides (Karlsson *et al.*, 2000). Hence, *MGL* mRNA abundance in liver was greater than all the other tissues except for heart.

In conclusion, our results suggested that *FASN*, *PPAR γ* , *AGPAT9*, *LPL*, *CPTIB*, and *ACADL* mRNA abundance was influenced by sex and tissue combinations when hens are at peak lay. Liver *FASN* plays an important role in the synthesis of fatty acid, and GAS of *CPTIB* and *ACADL* are important to fatty oxidation, particularly in laying females.

Acknowledgments

The current research was funded in part by the National Natural Science Foundation of China (31402032), Sixth talent peaks project in Jiangsu Province (2014-NY-004), the National 863 project in China (2011AA100305-J-09), National agricultural science and technology achievement transformation project funds (2011GB2C100006), the National Natural Science Foundation of China (31372277).

References

- Bitou N, Ninomiya M, Tsujita T and Okuda H. Screening of lipase inhibitors from marine algae. *Lipids*, 34: 441–445. 1999.
- Capel ID and Smallwood AE. Sex differences in the glutathione peroxidase activity of various tissues of the rat. *Research Communications in Chemical Pathology and Pharmacology*, 40: 367–378. 1983.
- Cook GA and Park EA. Expression and regulation of carnitine palmitoyltransferase-1 α and -1 β genes. *Aminal Journal of Medicine Sciences*, 318: 43–48. 1999.
- Demeure O, Duby C, Desart C, Assaf S, Hazard D, Guillou H and Lagarrigue S. Liver X receptor α regulates fatty acid synthase expression in chickens. *Poultry Science*, 88: 2628–2635. 2009.
- Dorn C, Riener MO, Kirovski G, Saugspier M, Steib K, Weiss TS, Gäbele E, Kristiansen GK, Hartmann A and Hellerbrand C. Expression of fatty acid synthase in nonalcoholic fatty liver disease. *International Journal of Clinical and Experimental Pathology*, 3: 505–514. 2010.
- Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, Shen R, Zhang MY, Strassle BW, Lu P, Mark L,

- Piesla MJ, Deng K, Kouranova EV, Ring RH, Whiteside GT, Bates B, Walsh FS, Williams G, Pangalos MN, Samad TA and Doherty P. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *Journal of Neuroscience*, 30: 2017–2024. 2010.
- He XY, Yang SY and Schulz H. Inhibition of enoyl-CoA hydratase by long-chain L-3-hydroxyacyl-CoA and its possible effect on fatty acid oxidation. *Archives of Biochemistry and Biophysics*, 298: 527–531. 1992.
- Hermier D. Lipoprotein metabolism and fattening in poultry. *Journal of Nutrition*, 127: 805S–808S. 1997.
- Jackson-Hayes L, Song S, Lavrentyev EN, Jansen MS, Hillgartner FB, Tian L, Wood PA, Cook GA and Park EA. A thyroid hormone response unit formed between the promoter and first intron of the carnitine palmitoyltransferase-I α gene mediates the liver-specific induction by thyroid hormone. *Journal of Biological Chemistry*, 278: 7964–7972. 2003.
- Ji B, Ernest B, Gooding JR, Das S, Saxton AM, Simon J, Dupont J, Metayer-Coustard S, Campagna SR and Voy BH. Transcriptomic and metabolomic profiling of chicken adipose tissue in response to insulin neutralization and fasting. *BMC Genomics*, 13: 441. 2012.
- Ka S, Markljung E, Ring H, Albert FW, Harun-Or-Rashid M, Wahlberg P, Garcia-Roves PM, Zierath JR, Denbow DM, Pääbo S, Siegel PB, Andersson L and Hallböök F. Expression of carnitine palmitoyl-CoA transferase-1B is influenced by a cis-acting eQTL in two chicken lines selected for high and low body weight. *Physiological Genomics*, 45: 367–76. 2013.
- Karlsson M, Tornqvist H and Holm C. Expression, purification, and characterization of histidine-tagged mouse monoglyceride lipase from baculovirus-infected insect cells. *Protein Expression and Purification*, 18: 286–292. 2000.
- Karpe F, Olivercrona T, Olivercrona G, Samra JS, Summers LKM, Humphreys SM and Frayn KN. Lipoprotein lipase transport in plasma: role of muscle and adipose tissues in regulation of plasma lipoprotein lipase concentrations. *Journal of Lipid Research*, 39: 2387–2393. 1998.
- Kilroy G, Kirk-Ballard H, Carter LE and Floyd ZE. The ubiquitin ligase *siah2* regulates *PPAR γ* activity in adipocytes. *Endocrinology*, 153: 1206–1218. 2012.
- Kusakabe T, Maeda M, Hoshi N, Sugino T, Watanabe K, Fukuda T and Suzuki T. Fatty acid synthase is expressed mainly in adult hormone-sensitive cells or cells with high lipid metabolism and in proliferating fetal cells. *Journal of Histochemistry and Cytochemistry*, 48: 613–622. 2000.
- Kushlan MC, Gollan JL, Ma WL and Ocher RK. Sex differences in hepatic uptake of long chain fatty acids in single-pass perfused rat liver. *Journal of Lipid Research*, 22: 431–436. 1981.
- Lee KY, Jung KC, Jang BG, Choi D, Jeon JT and Lee JH. Identification of differentially expressed proteins at four growing stages in chicken liver. *Asian Australia Journal of Animal Sciences*, 10: 1383–1388. 2008.
- Leveille GA, Romsos DR, Yeh Y and O’Hea EK. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. *Poultry Science*, 54: 1075–1093. 1975.
- Livak KJ and Schmittgen TD. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, 25: 402–408. 2001.
- Lorzadeh S, Doosti A, Nilchian S, Doosti A and Kheiri F. Evaluation of CPT-1 Gene expression with treatment diet of L-Carnitine on broiler chicks using real-time PCR assay. *Bulgarian Journal of Agricultural Science*, 19: 601–605. 2013.
- Meng H, Li H, Zhao JG and Gu ZL. Differential expression of peroxisome proliferator-activated receptors α and γ gene in various chicken tissues. *Domestic Animal Endocrinology*, 1: 105–110. 2005.
- Merkel M, Kako Y, Radner H, Cho IS, Ramasamy R, Brunzell JD, Goldberg IJ and Breslow JL. Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: Direct evidence that lipoprotein lipase bridging occurs in vivo. *Journal of Biological Chemistry*, 95: 13841–13846. 1998.
- Moradi S, Zaghari M, Shivazad M, Osfoori R and Mardi M. The effect of increasing feeding frequency on performance, plasma hormones and metabolites, and hepatic lipid metabolism of broiler breeder hens. *Poultry Science*, 92: 1227–1237. 2013.
- Park EA, Steffen ML, Song S, Park VM and Cook GA. Cloning and characterization of the promoter for the liver isoform of the rat carnitine palmitoyltransferase I (L-CPT I) gene. *Biochemical Journal*, 330: 217–224. 1998.
- Richards MP, Poch SM, Coon CN, Rosebrough RW, Ashwell CM and McMurtry JP. Feed restriction significantly alters lipogenic gene expression in broiler breeder chickens. *Journal of Nutrition*, 133: 707–715. 2003.
- Shrestha B, Javonillo R, Burns JR, Pirger Z and Vertes A. Comparative local analysis of metabolites, lipids and proteins in intact fish tissues by LAESI mass spectrometry. *Analyst*, 138: 3444–3449. 2013.
- Stern CD. The chick: A great model system becomes even greater. *Development of Cell*, 8: 9–17. 2005.
- Tashler U, Radner FPW, Heier C, Schreiber R, Schweiger M, Schoiswohl G, Preiss-Landl K, Jaeger D, Reiter B, Koefeler HC, Wojciechowski J, Theussl C, Penninger JM, Lass A, Haemmerle G, Zechner R and Zimmermann R. Monoglyceride lipase deficiency in mice impairs lipolysis and attenuates diet-induced insulin resistance. *Journal of Biological Chemistry*, 10: 17467–17477. 2011.
- Tontonoz P and Spiegelman BM. Fat and beyond: the diverse biology of *PPAR γ* . *Annual Review of Biochemistry*, 77: 289–312. 2008.
- Wang Y, Mu Y, Li H, Ding N, Wang Q, Wang Y, Wang S and Wang N. Peroxisome Proliferator-Activated Receptor- γ Gene: A key regulator of adipocyte differentiation in chickens. *Poultry Science*, 87: 226–232. 2008.
- Wang H and Eckel RH. Lipoprotein lipase: from gene to obesity. *Endocrinology and Metabolism: American Journal of Physiology*, 297: E271–E288. 2009.
- Wang KH, Gao YS, Lu JX and Tong HB. Early development rule of Recessive White chicken. *China Poultry*, 28: 20–22. 2006.
- Wu J, Fu W, Huang Y and Ni Y. Effects of kisspeptin-10 on lipid metabolism in cultured chicken hepatocytes. *Asian Australasian Journal of Animal Sciences*, 25: 1229–1236. 2012.
- Xu PW, Siegel PB and Denbow DM. Genetic selection for body weight in chickens has altered responses of the brain’s AMPK system to food intake regulation effect of ghrelin, but not obestatin. *Behavioural Brain Research*, 221: 216–226. 2011.
- Yalcin S, Bagdatlioglu N, Yenisey C, Siegel PB, Özkan S and Aksit M. Effect of manipulation of incubation temperature on fatty acid profiles and antioxidant enzyme activities in meat-type chicken embryos. *Poultry Science*, 91: 3260–3270. 2012.
- Zhang W, Cline MA and Gilbert ER. Hypothalamus-adipose tissue crosstalk: neuropeptide Y and the regulation of energy metabolism. *Nutrition and Metabolism*, 11: 4–12. 2014.