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Identification of the transcript isoforms and expression characteristics for chicken *Lpin1*

X. K. Wang[1*], W. Chen[1*], Y. Q. Huang[1†], X. T. Kang[1], J. P. Wang[2], G. X. Li[1] and R. R. Jiang[1]

[1]*College of Livestock Husbandry and Veterinary Engineering, Henan Agricultural University, No. 95 Wenhua Road, Zhengzhou, Henan 450002, P. R. China;*
[2]*Department of Animal Resource & Science, Dankook University, Anseodong, Cheonan, Choongnam 330-714, South Korea*

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Lpin1 *was a gene with important effects on controlling lipid/energy metabolism in humans and mice. However, little was known about chicken* Lpin1 *gene. In the present study, two transcript isoforms of chicken* Lpin1 *were identified.* Lpin1-α *was predicted encoding one 902 amino acid protein, whereas* Lpin1-δ *was predicted encoding one 918 amino acid protein with an insertion of 48-bp fragment from intron 12 of chicken* Lpin1-α*, and a conservative element was found to be located in intron 12 of chicken* Lpin1-α *genomic sequence. Ten variants were identified from chicken* Lpin1-α *coding sequence, and two missense mutations were predicted to affect the protein function of* Lpin1*. Reverse transcription PCR (RT-PCR) analysis revealed that chicken total* Lpin1*,* Lpin1-α *and* Lpin1-δ *were expressed in all analyzed tissues, and presented clear tissue expression differences. Real-time quantitative RT-PCR revealed that 30% energy restriction significantly elevated the total* Lpin1 *mRNA expression level in hepatic* ($P < 0.01$) *and adipose* ($P < 0.01$) *tissues of birds. Chicken total* Lpin1 *gene mRNA expression level presented a significantly inverse correlation with some traits including abdominal fat rate* ($P < 0.01$)*, serum high-density lipoprotein* ($P < 0.05$) *and total cholesterol* ($P < 0.05$)*, which would make a foundation for the further study on chicken* Lpin1 *gene function.*

Keywords: *Lpin1*, chicken, cloning, expression pattern

Implications

In this study, we identify the basic expression characteristics of chicken *Lpin1*, including its two transcript isoforms *Lpin1-*α and *Lpin1-*δ, the tissue expression pattern and the effect of energy restriction on gene expression, which would make a foundation for further learning about the gene function of chicken *Lpin1* and conducting the molecular selection or molecular regulation of chicken-related traits.

Introduction

Lpin1 was discovered as a mutated gene in a fatty liver dystrophic mouse using a positional cloning approach (Peterfy *et al.*, 2001). It was demonstrated that modulation of *Lpin1* expression level (i.e. null, normal and elevated specifically in adipose tissue or skeletal muscle) led to dramatic alterations in mouse adiposity, ranging from 10% to triple the normal amount of adipose tissue mass (Phan *et al.*, 2004; Phan and Reue, 2005; Reitman, 2005; Yao-Borengasser *et al.*, 2006; van Harmelen *et al.*, 2007). The overexpression of *Lpin1* in skeletal muscle or adipose tissue influences fat deposition through diverse mechanisms (Phan and Reue, 2005). Unlike many obesity-related genes, *Lpin1* was predominantly expressed in and exerts its actions on peripheral tissues, and thus may be a novel peripheral target gene for the treatment of obesity and/or lipodystrophy (Phan and Reue, 2005; Reitman, 2005).

Recent studies have revealed two molecular functions of *Lpin1*. First, LPIN1, as well as the related LPIN2 and LPIN3 proteins, acts as Mg^{2+}-dependent phosphatidate phosphatase type-1 (PAP1) enzymes, which catalyze a key step in the synthesis of glycerolipids (Han *et al.*, 2006; Donkor *et al.*, 2007; Harris *et al.*, 2007). Second, *Lpin1* can also act as a transcriptional coactivator. Through direct interaction with *PPAR*α and *PCG-1*α, *Lpin1* mediates enhanced expression of *PPAR*α target genes involved in fatty acid oxidation in mouse liver (Donkor *et al.*, 2009).

Mutations in *Lpin1* gene cause mouse lipodystrophy (Peterfy *et al.*, 2001) and human myoglobinuria (Zeharia *et al.*, 2008; Michot *et al.*, 2010). In addition, human *Lpin1* polymorphisms have been associated with numerous metabolic traits including insulin and/or glucose levels (Suviolahti *et al.*, 2006; Loos *et al.*, 2007; Wiedmann *et al.*, 2008), resting metabolic rate (Loos *et al.*, 2007) and systolic blood

* These authors contributed equally to this work.
† E-mail: hyanqun@yahoo.com.cn

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pressure (Ong *et al*., 2008). Gowri *et al*. (2007) suggested that lipid-regulating phosphatase not only had an important role in central metabolic regulation, but also had a new role in uterine function and reproductive biology. The different genotypes of the pig *Lpin1* were found to be associated with percentage of pig leaf fat and intramuscular fat (He *et al*., 2009). However, little is known about *Lpin1* gene in chicken at present. Here we cloned chicken *Lpin1* coding sequence, studied the tissue expression of chicken *Lpin1*, evaluated the regulation of *Lpin1* expression by energy restriction and conducted the correlation analysis of *Lpin1* expression levels with some phenotypic, physiological and biochemical indicators of broilers, which would make a foundation for the further study on the function of chicken *Lpin1*.

Material and methods

Samples

The tissue samples including lung, spleen, abdominal fat, heart, pectoralis major, brain, liver, pancreas and cartilage tissues were collected from two 40-week-old healthy silkies (one male and one female), two 8-week-old hybrid meat chickens (one male and one female) for the identification of chicken *Lpin1* transcripts/tissue expression pattern analysis. The collected tissue samples were deep-frozen in liquid nitrogen, and then stored at −70°C until use. Chickens were from Henan Agricultural University Poultry Breed Resource Conservation Center.

The deposited RNA samples of hepatic and abdominal fat tissues from the 30% energy restriction population were used to study the effect of 30% energy restriction on chicken *Lpin1* expression by real-time quantitative reverse transcription PCR (qRT-PCR).

All birds used in this trial were handled in accordance with the guidelines for the Care and Use of Agricultural Animals in Research and Teaching, 3rd edition, 2010 (Association Headquarters, Champaign, IL 61822, USA), and was approved by Animal Care and Use Committee of Henan Agricultural University.

Establishment of 30% energy restriction population

The 30% energy restriction population was established as Chen *et al*. (2012) described. Briefly, 50 healthy female Arbor Acre broiler chickens with similar weight (average weight of female broilers 330 ± 30 g) were selected at 18 days from one commercial Arbor Acre broiler population. Chicks were randomly allocated to free feeding (FF, $n = 25$) group and energy restriction (ER, $n = 25$) group. Chicks were housed individually with 23 h of light per day. From 18 to 48 days, FF group broilers were fed *ad libitum* with control diet, whereas ER group broilers were only provided 70% energy intake with low energy diet on ratio; other nutrient ingredients' supply was the same as FF group broilers. The ingredients and nutrient levels of experimental diets were presented in Supplementary material 1. The control diet was prepared referring to 2004 Chinese Feed Standard of Chicken criterion.

Chicks were weighed and harvested at 49 days individually. Abdominal fat pad was excised and weighted. Abdominal fat rate (AFR = abdominal fat pad weight/BW) was calculated. Hepatic and adipose tissue RNA samples were collected and snap-frozen in liquid nitrogen, and then stored at −70°C. About 5 ml blood sample was collected to separate blood serum for the determination of following physiological and biochemical indicators including total cholesterol (CHO), high-density lipids (HDL), low-density lipids (LDL), triacylglycerols (TG), glucose (GLU; Nanjing Jiancheng Bioengineering Institute, Nanjing, P. R. China) using the Hitachi 911 analyzer (Roche, Basel, Switzerland), and insulin level (INS) was determined by the ELISA method with commercially available kits (CUSABIO Biotech, Wuhan, P. R. China).

Total RNA extraction and cDNA synthesis

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity and quality of the isolated RNA was determined by GeneQuant pro ultraviolet spectrophotometry (Amersham Pharmacia Ltd, Bucks, UK) and agarose gel electrophoresis, respectively. Isolated total RNAs were dissolved in diethyl pyrocarbonate water and kept at −70°C. one microgram total RNA was subsequently reversely transcribed with PrimeScript® RT reagent kit with gDNA Eraser (Takara, Dalian, P. R. China) according to the manufacturer's instructions. The synthesized first strand cDNA was stored at −20°C.

Cloning of chicken Lpin1 open reading frame (ORF)

The coding sequence of mouse *Lpin1-α* (NM_172950.3) was used to search for chicken *Lpin1*-related sequence information from chicken Refseq RNA database and EST database (http://www.ncbi.nlm.nih.gov/blast.cgi). On the basis of gained predicted mRNA sequence (XM_419957.2), eight primer pairs (LP1-8, Supplementary material 2) were designed to clone chicken *Lpin1* ORF and conduct sequence variation analysis with the hepatic tissues of two silkies and two hybrid meat chickens. A general touchdown thermal cycling protocol as follows was used to amplify chicken *Lpin1* with LP1-8, respectively: 5 min denaturation at 94°C followed by 3 cycles of 1 min at 94°C, 1 min at 65°C, 1 min at 72°C; the annealing temperature was then decreased by 5°C every third cycle and a further 25 cycles were carried out when the annealing temperature reached 55°C; the final extension step was 72°C for 10 min. The PCR products of LP1-8 primer sets were purified with DNA agarose gel purification system (QIAGEN, Hilden, Germany), and directly PCR sequenced by commercial service. RT-PCR amplification of LP9 primer set was finished with LongAmp Taq DNA polymerase (LongAmp Taq kit, NEB, Beijing, P. R. China) according to the manufacturer's instruction. The PCR condition for LP9 primer set was 5 min denaturation at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, 1 min at 72°C; the final extension step was 72°C for 10 min. The PCR amplification product of LP9 primer set was cloned into the pGEM-T vector (Invitrogen) and clones were sequenced by

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373A Automated Sequencer (Applied Biosystems, Foster City, CA, USA) with M13 primer.

Tissue expression profile analysis by RT-PCR

The tissue expression profiles of chicken total *Lpin1* and *Lpin1-α*/*Lpin1-δ* were detected by RT-PCR with LP7 and LP8 primer sets, respectively, the expression of *β-actin* (L08165.1) was used as a control, the primer sets were presented in Supplementary material 2. PCR amplification was done in a total volume of 15 μl containing 50 ng template cDNA, 1.0 μM of each primer and 1× Go Taq Green Master Mix (Promega, Beijing, P. R. China). A touchdown thermal cycling protocol as above was used to analyze the expression of chicken *Lpin1* and *β-actin* among tissues. Finally, 10 μl of each PCR product was electrophoresed on 1.5% agarose gel. The band intensities were determined using NIH Image 1.61 software (National Institute of Health, Bethesda, MD, USA).

The effect of 30% energy restriction on chicken total Lpin1 *expression*

First, the SYBR GREEN qRT-PCR method (relative quantitative) was used to detect the effect of 30% energy restriction on chicken total *Lpin1* relative expression in hepatic tissues and adipose tissues with LP6 primer set (Supplementary material 2). The *β-actin* was used as a control to normalize the differences among individual samples. Each sample had three replications for *β-actin* and objective gene, respectively. The qRT-PCR reactions were performed as two-step reactions in ABI 7700 (Applied Biosystems). It was done in a total volume of 30 μl containing 50 ng template cDNA, 0.4 μM of each primer, 2× Power SYBR GREEN PCR Master Mix (Applied Biosystems), following next parameters: one cycle of 94°C for 5 min, then followed by 40 cycles at 94°C for 30 s, 57°C for 25 s and 72°C for 30 s. The results of objective gene expression were normalized to the housekeeping gene *β-actin*. Gene expression results were calculated using the delta delta cycle threshold ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen, 2001).

Then TaqMan qRT-PCR methods (absolutely quantitative) were used to determine the copy number of total *Lpin1* in hepatic tissues of ER and FF chickens. The qRT-PCR primers and the reaction conditions were optimized according to the reference (Gangisetty and Reddy, 2009). The optimized primers were presented in Supplementary material 2. All reactions were carried out in triplicate with no template control and no-RT sample. The standard curve for each gene was constructed with purified PCR products generated using the specific primers of total *Lpin1* gene, template cDNA was quantified using spectrophotometer and serially diluted 10-fold to span the range of predicted CT values that would be obtained from experimental samples. The calculation method of copy number for each template was performed according to the reference (Gangisetty and Reddy, 2009). The qRT-PCR reactions were performed as two-step reactions in ABI 7700 (Applied Biosystems). qPCR amplification was done in a total volume of 25 μl containing 12.5 μl TaqMan Universal PCR Master mix (Applied Biosystems), 1.0 μl cDNA (1 μg/μl), 260 nM of each specific forward and reverse primer, 250 nM TaqMan™ fluorescent probe, the parameters were as follows: 5 min at 94°C for predegeneration, followed by 40 cycles at 94°C for 20 s, 60°C for 1 min.

Statistical analysis

Data were analyzed using SPSS statistics program (version 13.0, Chicago, IL, USA). The unpaired *t*-test was used to compare the effect of energy restriction on chicken total *Lpin1* expression; data were represented as means ± s.e.m. The Pearson correlation coefficients between *Lpin1* expression level (detected by TaqMan probe-based qPCR) in hepatic tissues and some traits (including AFR and some physiological and biochemical indicators) were computed by the bivariate correlations procedure with two-tailed test. $P < 0.05$ was considered significant.

Results

Cloning of chicken Lpin1 *ORF*

Blast searching chicken Refseq RNA database and EST database (http://www.ncbi.nlm.nih.gov/blast.cgi) with the encoding sequence of mouse *Lpin1-α* (NM_172950.3), a predicted chicken sequence XM_419957.2 (similar to KIAA0188: LOC421945) was gained. Comparing this predicted chicken sequence with the ORF of human/mouse *Lpin1-α*, it indicated that the translation start site of chicken *Lpin1* encoding sequence should be located at 1051 nt, whereas not at 1 nt of sequence XM_419957. The Chicken *Lpin1* coding sequence should be 2709 bp, from 1051 to 3759 nt, possessing 71.4% and 70.20% nucleotide identity with human (NM_145693.1) and mouse (NM_172950.3) *Lpin1-α*, respectively, 67.9% nucleotide identity with both human (d80010, BM786275.1) and mouse (NM_015763.4) *Lpin1-β*, respectively, which suggests that sequence XM_419957.2 should contain chicken *Lpin1*-α ORF. The RT-PCR amplification with eight overlapping primer sets (LP1-8, Supplementary material 2) gained the ORF of chicken *Lpin1-α* from the hepatic tissues of two silkies and two hybrid meat chickens, and the sequence from one silkie chicken was submitted to GenBank (GenBank accession no. HM473175). By long-range PCR amplification with LP9 primer set (spanning exon 1 and exon 19 of chicken *Lpin1-α*) and clone sequencing, we further confirmed the expression of chicken *Lpin1-α*.

Analysis indicated that chicken *Lpin1-α* ORF contained 19 exons, spanning about 31-kb genome, and the exon–intron boundary abided by GT–AG rule. It predicted encoding a 902 amino acids protein (ADK89033) with a calculated molecular mass of 99.69 kDa and an isoelectric point of 6.10. The multiple sequence alignment indicated that LPIN1 proteins were conservative among species. The predicted chicken Lpin1-α protein shared 74.42%, 73.68%, 71.09%, 71.31% and 74.59% identity with the counterpart of human-isoform-α (NP_663731.1), mouse-isoform-α (NP_766538.2), mouse-isoform-β (NP_056578.2), rat (NP_001012111.1) and pig (ACF16051.1) LPIN1 proteins, respectively (Figure 1). Some

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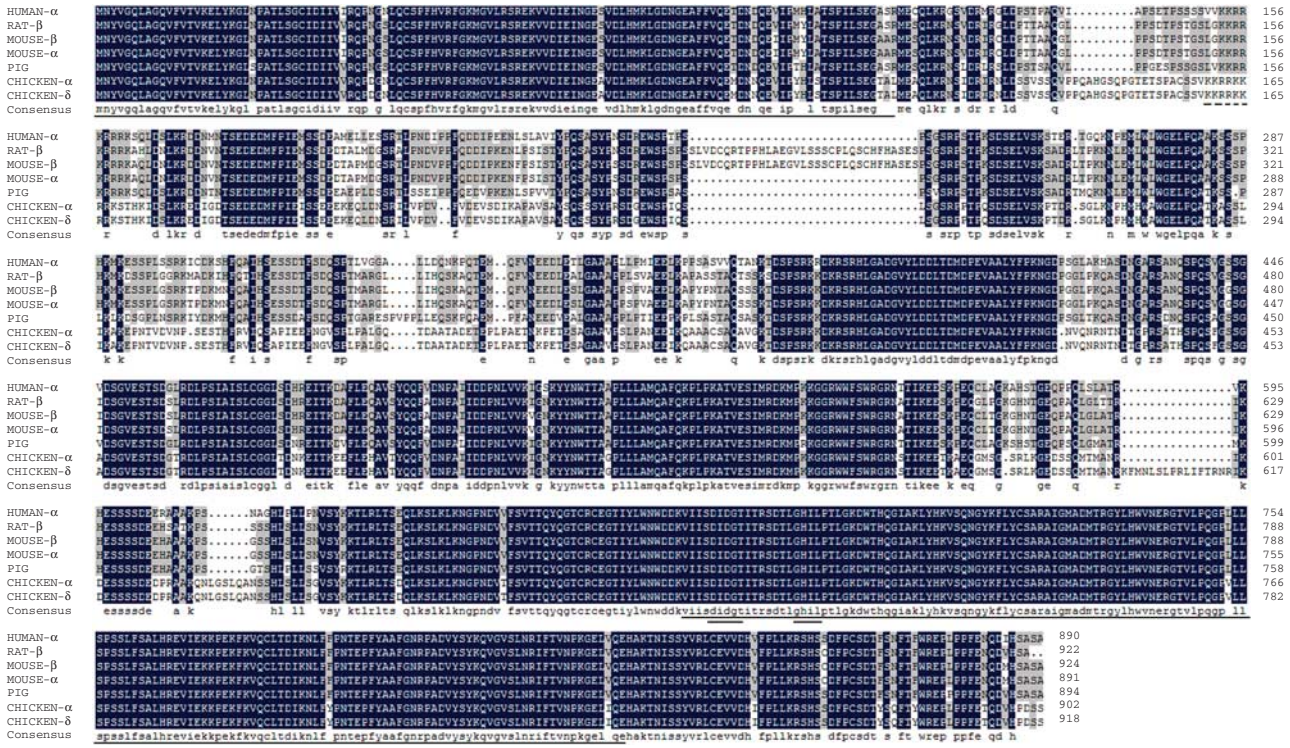


Figure 1 The deduced amino acid sequence of the chicken *Lpin1* gene was aligned to human-isoform-α (NP_663731.1), mouse-isoform-α (NP_766538.2), mouse-isoform-β (NP_056578.2), rat (NP_001012111.1) and pig (ACF16051.1) protein sequences. Identical acid residues among species were presented. The Lipin_N amino-terminal lipin domain and carboxy-terminal lipin domain were underlined with a uninterrupted line, nuclear localization signal domain was underlined with a dot line, DXDXT and LXXIL motifs were presented in double uninterrupted lines, respectively.

strongly conserved domains were revealed, including the amino-terminal lipin domain (NLIP), a nuclear localization signal and a carboxy-terminal lipin domain (CLIP) containing DXDXT and the LXXIL motif (Figure 1).

Identification of chicken transcript variant Lpin1-δ

In addition, one alternative splicing form of chicken *Lpin1* gene (named as chicken *Lpin1-δ*, GenBank accession no. JN007385) was obtained by RT-PCR with LP8 primer set from chicken hepatic tissues. With LP9 primer set spanning exon 1 and exon 19 of *Lpin1-α*, we also identified chicken *Lpin1-δ* by long-range PCR amplification and clone sequencing. *Lpin1-δ* isoform was caused by a 48-bp fragment retention of intron 12 in chicken *Lpin1-α*, which was 441 bp apart from the 3′ boundary of exon 12 and 1601 bp apart from the 5′ boundary of exon 13. Analysis showed that the sequence insertion in chicken *Lpin1-δ* do not affect the ORF of chicken *Lpin1*; two transcripts predicted encoding highly similar protein with length variation of 16 amino acids, predicted chicken *Lpin1-δ* protein (AEX31552.1) shared 98.25% identity with chicken-isoform-α (ADK89033), shared 72.02%, 69.49%, 67.09%, 69.46% and 71.65% identity with the counterpart of human-isoform-α (NP_663731.1), mouse-isoform-α (NP_766538.2), mouse-isoform-β (NP_056578.2), rat (NP_001012111.1) and pig (ACF16051.1) LPIN1 proteins, respectively. Searching EST database of human, mouse and rat, a similar alternative splice variant (CB997204.1) was revealed from the human EST database, which contained a 213-bp intron 12 fragment comparing with human *Lpin1-α* (NM_145693.1). Searching computational dataset with VISTA Enhancer Browser (http://enhancer.lbl.gov/), an approximately 180-bp conservative element among chicken, human, mouse, rat and dog was identified from intron 12 of chicken *Lpin1-α* (Supplementary material 3).

Detected coding sequence variation of chicken Lpin1 *and the potential effect*

Sequence comparisons among chicken *Lpin1* coding sequences of two silkies and two hybrid meat chickens revealed 10 coding single nucleotide polymorphisms (cSNPs; eight new cSNPs), which include five samesense mutations and five missense mutations. c.735C>T, c.1112A>G, c.1439C>T, c.1532A>C and c.1567T>C variation (reference to chicken coding sequence, HM473175) were conservative among five species (Table 1). These conservative variation sites may have potential important function. Sift program (http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html) predicted that c.1439C>T and c.1532A>C substitutions may affect the protein function (Kumar *et al.*, 2009; Table 1).

Expression profile of chicken Lpin1

LP6 and LP8 primer sets were used to conduct the tissue expression analysis of chicken total *Lpin1* and *Lpin1-α*/*Lpin1-δ* in four individuals, respectively. RT-PCR analysis revealed that the total *Lpin1*, *Lpin1-α* and *Lpin1-δ* were expressed in all analyzed tissues (Figure 2). Comparing with other analyzed

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Table 1 *The variation sites detected from chicken* Lpin1 *coding region*

Exon	Sites[a]	Amino acid change[b]	Human	Mouse	Pig	Rat	Sift score[c]
4	c.735C>T		c	c	c	c	
6	c.933T>A		t	g	g	c	
8	c.1362T>C		g	c	c	t	
11	c. 1689T>C		a	c	g	c	
16	c.2220A>T		g	g	g	g	
6	c.1112A>G	p.E371G	a	a	a	a	0.17
8	c.1310C>T	p.T437F	a	a	a	a	0.22
8	c.1439C>T	p.T480M	g	g	g	g	0.01*
9	c.1532A>C	p.N511T	a	a	a	a	0.02*
10	c.1567T>C	p.W523R	t	t	t	t	0.06

[a]Each coding sequence variant was named relative to the reference allele found in chicken *Lpin1* coding sequence (HM473175). Variants with underline were also detected from genome sequencing plan.
[b]Each amino acid variant was named relative to the reference allele found in predicted chicken *Lpin1* protein sequence (ADK89033).
[c]Asterisk represents the amino acid substitution was predicted to affect protein function by sift program.

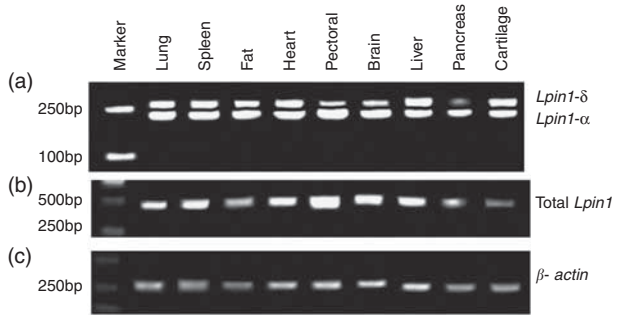


Figure 2 Tissue expression distributions of chicken *Lpin1* transcripts among tissues. (a) *Lpin1-δ*/*Lpin1-α*; (b) total *Lpin1*; (c) *β-actin*.

tissues, chicken total *Lpin1* mRNA had a relatively higher expression in the pectoralis major and a lower expression in the pancreas and cartilage (Figure 2b). LP8 primer set could detect the expression of two transcript isoforms *Lpin1-δ* and *Lpin1-α* at the same time. It showed that chicken *Lpin1-α* mRNA had a higher expression in the pectoralis major and a lower expression in the pancreas, whereas chicken *Lpin1-δ* mRNA had a higher expression in the hepatic and cartilaginous tissue and a lower expression in the pancreas (Figure 2a). Chicken *Lpin1-δ*/*Lpin1-α* ratios >1 in hepatic and cartilaginous tissue, whereas chicken *Lpin1-δ*/*Lpin1-α* <1 in other analyzed tissues, and chicken *Lpin1-δ*/*Lpin1-α* ratios were relatively lower in pancreas, brain and pectoralis major tissues (Figure 2a).

The effect of 30% energy restriction on chicken total Lpin1 *expression*

By SYBR GREEN qRT-PCR, it showed that the expression of *β-actin* between FF group and ER group had no significant difference and could be used as an internal control. The relative expression of chicken total *Lpin1* gene mRNA in ER group birds was significantly higher than that of FF group birds in hepatic tissue ($P < 0.01$) and adipose tissue ($P < 0.01$; Figure 3a). Absolute qPCR by TaqMan probe also revealed that energy restriction significantly upregulated chicken total *Lpin1* level by about fourfold in hepatic tissue ($P < 0.01$; Figure 3b).

Relationship of chicken total Lpin1 expression levels in hepatic tissue with some traits

In the energy restriction population, some traits including AFR, and serum total CHO, HDL, LDL, TG, GLU and INS level were measured (Chen *et al*., 2012). Conducting the correlation analysis between chicken total *Lpin1* expression (absolute qPCR value) and these traits, it was seen that chicken total *Lpin1* mRNA expression level was significantly negatively correlated with AFR ($P < 0.01$), serum HDL ($P < 0.05$) and total CHO ($P < 0.05$), whereas chicken *Lpin1* gene mRNA expression level had no significant correlation with serum GLU, TG and INS level (Table 2).

Discussion

LPIN1 is a PAP1 enzyme, which is required in the *de novo* biosynthesis of TG, phosphatidylcholine and phosphatidylethanolamine (Donkor *et al*., 2007). Here chicken *Lpin1* ORF was cloned and its conservative elements were revealed. DXDXT motif located in CLIP conservative region is the characteristic of a superfamily of magnesium-dependent phosphatases (Peterfy *et al*., 2001; Han *et al*., 2006; Donkor *et al*., 2007), whereas the LXXIL motif mediates interaction with nuclear receptors (Finck *et al*., 2006).

Lpin1 gene could generate multiple alternatively spliced mRNA forms. Transcript variants *Lpin1-α*, *-β* and *-γ* have been identified in human and mouse (Peterfy *et al*., 2005; Han and Carman, 2010). It was found that the PAP of *Lpin1-α*, *-β* and *-γ* isoforms were dependent on Mg^{2+} or Mn^{2+} ions (Han and Carman, 2010). *Lpin1-α* and *Lpin1-β* had different and complementary function (Peterfy *et al*., 2005; van Harmelen *et al*., 2007; Reue and Zhang, 2008). Here we identified a novel isoform *Lpin1-δ*, which has an insertion between coding exons 12 and 13 of chicken *Lpin-α*, different from the reported transcript variants *Lpin1-β* and *Lpin1-γ* of human/mouse, which have an insertion between coding

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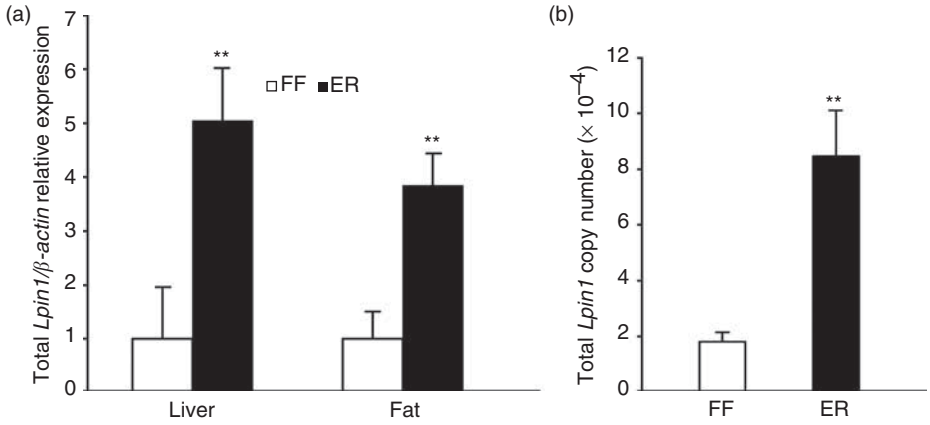


Figure 3 The differential expression of total *Lpin1* in liver and abdominal fat tissues between free feeding (FF) group and energy restriction (ER) group by real-time quantitative PCR. Fat, $n = 5$ for ER and FF group, respectively; liver, $n = 20$ for ER and FF group, respectively. $^{**}P < 0.01$. (a) Relative expression of chicken *Lpin1*/*β-actin* detected by SYBR GREEN qRT-PCR and (b) absolute expression of chicken *Lpin1* in hepatic tissue detected by TaqMan qRT-PCR.

Table 2 *The Pearson coefficients between the expression of chicken* Lpin1 *gene in hepatic tissue with physiological and biochemical indicators of broilers*

	GLU	CHO	TG	HDL	LDL	INS	AFR
Correlation	−0.1070	−0.3323	0.0126	−0.3550	−0.1730	0.1955	−0.5325
Significance[a]	0.5286	0.0445*	0.9410	0.0311*	0.3131	0.2532	0.0008**

GLU = glucose; CHO = total cholesterol; TG = triacylglycerols; HDL = high-density lipids; LDL = low-density lipids; INS = insulin; AFR = abdominal fat rate.
[a]Two-tailed test: $^{*}P < 0.05$; $^{**}P < 0.01$.

exons 4 and 5, and coding exon 10 and 11 of *Lpin1*-α sequence, respectively (Han and Carman, 2010). The *Lpin1*-*δ*-specific sequence consisting of 16 amino acids is located in a non-homologous region and does not alter the conserved NLIP and haloacid dehalogenase-like lipin domains (Han *et al.*, 2006). Elements controlling alternative splicing may be present in introns: the existence of intronic splicing enhancers and silencers has been documented (Hare and Palumbi, 2003). The identified conservative element from intron 12 of chicken *Lpin1-N* may have potential important function in the regulation of *Lpin1*-*δ* alternative splicing.

Previous studies revealed that *Lpin1* mRNA was abundant in adipose tissue and muscle tissue of humans and mice (Peterfy *et al.*, 2001; Donkor *et al.*, 2007). Chicken total *Lpin1*/*Lpin1*-*α* also presented high expression in pectoralis major tissue. The high expression levels of *Lpin1* mRNA in metabolically active tissues, such as skeletal muscle were in accordance with the roles of *Lpin1* in energy metabolism.

Previous studies *in vitro* and *in vivo* have linked *Lpin1* to the maintenance of metabolic homeostasis (Reue *et al.*, 2000; Phan and Reue, 2005). Ryu *et al.* (2009) found that fasting, high-fat diet and db/db mouse enhanced the expression of hepatic *Lpin1* transcript and protein. Similar to the fasting effect in mice, our study showed that 30% energy restriction increased the relative expression of chicken total *Lpin1* in both adipose tissue and hepatic tissues.

Phan *et al.* (2004) showed that *Lpin1*-deficient mice exhibit normal food intake but reduced fat deposition. Overexpressed *Lpin1* in either mouse adipose tissue or skeletal muscle promotes obesity (Phan and Reue, 2005). In contrast, in natural human population *Lpin1* levels has been revealed to be negatively correlated with obesity (Yao-Borengasser *et al.*, 2006; van Harmelen *et al.*, 2007; Tsuchiya *et al.*, 2009; Miranda *et al.*, 2010), which shows the complicacy of expression regulation for *Lpin1* gene. In accordance with the reports from natural human population, in our experiment chicken hepatic *Lpin1* gene mRNA expression level presented significantly negative correlation with abdominal adipose deposition (AFR; $P < 0.01$).

It was reported that *Lpin1* mRNA expression had a strong negative correlation with GLU and INS levels in mouse and human, TG levels in human (Suviolahti *et al.*, 2006). *Lpin1* RNAi in db/db mice had not showed significant effect on plasma TG and INS level (Ryu *et al.*, 2009). Our study showed that chicken *Lpin1* gene mRNA expression level was significantly negatively correlated with serum HDL and total CHO level ($P < 0.05$), whereas had no significant correlation with serum INS, TG and GLU level.

Overall, we identified two transcript isoforms (*Lpin1*-*α* and *Lpin1*-*δ*) of chicken *Lpin1* and conducted their tissue expression analysis by RT-PCR. The qRT-PCR analysis revealed that 30% energy restriction significantly elevated the total *Lpin1* mRNA expression level in hepatic and adipose tissues of birds. Chicken total *Lpin1* gene mRNA expression level had a significantly inverse correlation with AFR ($P < 0.01$), serum HDL ($P < 0.05$) and total CHO ($P < 0.05$), which shows that chicken *Lpin1* may play an important role in modulation of energy metabolism balance as human and mouse *Lpin1*.

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Supplementary materials

For supplementary materials referred to in this article, please visit http://dx.doi.org/doi:10.1017/S1751731112001358

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