Microarray analysis of adipose tissue gene expression profiles between two chicken breeds

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The chicken is an important model organism that bridges the evolutionary gap between mammals and other vertebrates and provides a major protein source from meat and eggs throughout the world. Excessive accumulation of lipids in the adipose tissue is one of the main problems faced by the broiler industry nowadays. In order to visualize the mechanisms involved in the gene expression and regulation of lipid metabolism in adipose tissue, cDNA microarray containing 9 024 cDNA was used to construct gene expression profile and screen differentially expressed genes in adipose tissue between broilers and layers of 10 wk of age. Sixty-seven differentially expressed sequences were screened out, and 42 genes were found when blasted with the GenBank database. These genes are mainly related to lipid metabolism, energy metabolism, transcription and splicing factor, protein synthesis and degradation. The remained 25 sequences had no annotation available in the GenBank database. Furthermore, Northern blot and semi-quantitative RT-PCR were developed to confirm 4 differentially expressed genes screened by cDNA microarray, and it showed great consistency between the microarray data and Northern blot results or semi-quantitative RT-PCR results. The present study will be helpful for clarifying the molecular mechanism of obesity in chickens.

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1. Introduction

The quantitative regulation of adipose in humans and animals is becoming more and more conspicuous. In human, obesity increases the risk of morbidity by type 2 diabetes, hypertension, and cardiovascular disease (Kopelman *et al* 2000). For agricultural animals, they are facing the similar problem. The over thick of swine backfat and excessive fat in chicken abdomen have already been serious problems. Excessive deposition of fat does not just reduce carcass yield and feed efficiency, but also cause rejection of the meat by the consumers (Kessler *et al* 2000) and difficulties in processing (Chambers 1990).

Accompanied with the rapid development of molecular biotechnology, researches on a number of complex biological responses are turning into molecular level. Via elucidating the molecular mechanism of lipid metabolism, people expect to investigate the mechanism of obesity occurrence, and find an effective method to solve it. Traditional research methods merely study the functions of genes one by one, and ignore the relationship among different genes. So they can not explain the biological responses comprehensively, especially for some complex biological responses, just like obesity, which are involved in multigenes, multipathways and multiphases.

DNA microarray analysis, proved as a fruitful strategy for the identification of functional genes in several model organisms (i.e. human, rodents, fruit fly, etc.) (Cogburn *et al* 2003), is considered as a powerful tool to visualize the molecular mechanism that govern complex biological

Keywords. Adipose tissue; cDNA microarray; chicken; gene expression profile

Abbreviations used: Apo-AI, Apolipoprotein AI gene; ARP8, actin-related protein 8 gene; GAPDH, glyseraldehyde-3-phosphate dehydrogenase gene; HDL, high-density lipoprotein; LPL, lipoprotein lipase gene; RT-PCR, reverse transcription polymerase chain reaction; SNP, single nucleotide polymorphism; TnI, troponin I gene; VLDL, very low density lipoprotein.

responses, and is used in gene expression analysis (Van Hal *et al* 2000). It is used in the analysis of aging and caloric restriction (Lee 1999), cancer classification (Golub *et al* 1999; Nadler *et al* 2000), and other diseases (Kaminski *et al* 2000).

The broilers used in present study were from modern commercial broilers – they are the product of intensive selection over many generations for rapid growth and enhanced muscle mass, and display strong capability of fat deposit. Oppositely, the Bai'er layer, which is a Chinese local breed, displays slow growth and weak capability for fat deposit. Obviously, the two breeds are excellent models to investigate the mechanism of lipid metabolism in chickens. The objectives of the present study were to construct and compare the adipose tissue gene expression profiles of the two chicken breeds, develop proper analysis method to find differentially expressed genes and elucidate the molecular mechanism of lipid metabolism in chickens.

2. Materials and methods

2.1 *Animals*

Fat line broilers, derived from a commercial Arbor Acres (AA) grandsire line, and Bai'er layers, a Chinese local breed were used in the current study. Birds had access to feed and water *ad libitum*. Birds were fed with commercial corn-soybean-based diet, which met all the NRC requirements (National Research Council 1994). From hatch to 3 wk of age, birds received a starter feed (3,000 kal ME/kg and 210 g/kg CP) and from 4 to 12 wk of age, birds were fed a grower diet (3,100 kal ME/kg and 190 g/kg CP).

2.2 *Sample preparation*

Birds were slaughtered at 10 wk of age, abdominal fat were isolated, frozen in liquid nitrogen immediately, and then conserved at -80°C. Total RNA was extracted from 300 to 800 mg of bulk abdominal adipose tissue using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's recommendations, and quantified in a spectrophotometer. Isolation of mRNA was carried out using Oligotex mRNA Mini Kit (QIAGEN, Valencia, CA, USA). cDNA was prepared by oligo-dT-primed reverse transcription reaction using Superscript II (Life Technologies, Inc., Grand Island, NY, USA). Labelled cDNA probes were prepared using CyScribe GFX Purification Kit (Amershan Biosciences, Buckinghamshire, UK) according to the manufacturer's protocol. The cDNA of broiler was labelled using fluorochrome Cy5, and layer's was labelled with Cy3.

2.3 *Microarrays and hybridization*

cDNA microarrays, containing 9024 chicken cDNA, 192 housekeeping genes and negative controls, were purchased from Beijing Genomics Institute. Every sequence was printed triply on the chip. Prior to hybridization, microarrays were prehybridized in hybridization buffer [5×SSC, 0.2% SDS, 5× Denhardt's solution, 50% (vol/vol) formamide, 0.2 mg/ml BSA] at 42°C for 1 h. After prehybridization, slides were rinsed twice in Millipore filtered water, once in isopropanol, and dried by centrifugation (2 min, 470 g). Cy3- and Cy5-labelled cDNA probes were purified using Microcon 30 (Millipore), and then mixed 1:1(vol/vol). The final volume was adjusted to 32 μl with hybridization buffer. After heating at 100°C for 2 min, the probes were added to the array and covered with a 24×60 mm cover slip. The array chip was placed in a sealed hybridization chamber, and the hybridization was performed overnight at 42°C. After hybridization, slides were washed at room temperature first in 1× SSC/0.1% SDS (5 min) and subsequently in 0.1× SSC/0.1% SDS (5 min) and 0.1 × SSC (1 min) and then dried by centrifugation (2 min, 470 g). The array was then immediately scanned using an Axon4100A scanner.

2.4 *cDNA microarray data analysis*

The scanned images were analysed using GenePix Pro3.0 software (Axon Instruments, Union City, CA, USA). The net fluorescent signal at each spot from Cy5 and Cy3 dyes was subsequently compared. Automatic and manual flagging were used to localize absent spots, which were then excluded from analysis. In addition, very weak spots, with a mean of Cy3 and Cy5 signals lower than twice of the mean background, were also excluded from data analysis. The signal from each spot was calculated as the average intensity of the spot minus the background. Average spot intensities were collected for each individual spot and stored for further data processing in Microsoft Excel. For further analysis, Cy5/Cy3 ratios were calculated using the mean of the Cy3 signals and of the Cy5 signals from triplicate spots. The ratios then were transformed Log2(ratio), that is genes of a Log2(ratio)>1 or < -1, will be considered as differentially expressed genes between two chicken breeds.

2.5 *Primer design*

Northern blots and semi-quantitative RT-PCR were used to confirm the four differentially expressed genes from the data obtained by cDNA microarrays. Gene-specific primers were designed according to the mRNA sequences in the GenBank. The primer sequences and GenBank accession No. were showed in table 1.

Table1 The primer sequences and GenBank accession No.

Gene	Accession No.	Primer sequence 5' to 3'
Apo-AI	M96012	F:AGACTGCTACCTGGCCTGACA
		R:CATCCTACTGGAATACGG
LPL	X60547	F:CCGTGCTCAGATGCCCTAC
		R:CTGACTCTCTGAATGGTGAATGC
TnI	U19926	F:GACTCGGGTTCACAACCATC
		R:ATGGCAAGAGTGGAGAGACC
ARP8	XM_414336	F:TATGCAGCGAGCAGGGTT
		R:TCTCACGTCTCCCTCAAAGG
GAPDH	K01458	F:TGACGTGCAGCAGGAACAC
		R:CAGTTGGTGGTGCACGATG

2.6 *Northern blots*

For Northern blots, target cDNA probes were labelled with ^{32}P-dCTP using Random Primers DNA Labeling Kit (Invitrogen, Rockville, MD, USA). The rRNA (18S and 28S) markers were used as standards for calculation of the transcript size. Total RNA (15 μg of each sample) isolated from the adipose tissue were electrophoresesd in a 1.2% agarose gel that contains formaldehyde and transfered to a nylon membrane (Hybond N, Amersham). The membranes were prehybridized at 42°C in UltraHyb (Ambion, Austin, TX, USA) for 60 min, and then denatured probe (at 95°C for 10 min) was added to the hybridization solution at a concentration of 1×10^6 cpm/ml. Hybridization reaction was performed at 42°C over night. Following hybridization, the membrane was washed twice with 2×SSC and 0.1% SDS for 5 min at 42°C, twice with 0.2× SSC and 0.1% SDS for 15 min at 42°C, dried in air, and then radioautographed. A phosphor-imager (UVP, Inc.,Upland, CA, USA) was used for analysis and quantitation of radiolabelled signals.

2.7 *Semi-quantitative RT-PCR*

Six samples of abdominal fat total RNA were isolated from three fat line broilers and three Bai'er layers of 10 wk of age. Reverse transcriptions were performed according to the directions of TaKaRa RNA PCR Kit ver. 3.0 (TaKaRa, Dalian, China). For the first strand cDNA synthesis, the 10 μl reaction mixture included 1 μl total RNA (1 μg/μl), 2 μl MgCl2 (25 mM), 1 μl 10×RT buffer, 1 μl dNTP mixture (10 mM each), 0.5 μl oligo-dT-adaptor primer (2.5 pmol/μl), 0.25 μl RNase inhibitor (40 U/μl), 0.5 μl avian myeloblastosis virus (AMV) reverse transcriptase (5 U/μl) and 3.75 μl RNase free H_2O. The reaction mixture was incubated for 30 min at 42°C, and then heated at 99°C for 5 min to extinguish reverse transcriptase activity. The expressions of *Actin-related protein 8* (*ARP8*) and *troponin I* (*TnI*) were detected by semi-quantitative RT-PCR. Reactions were carried out in the Gene Amp PCR system 9700. For *ARP8* gene, the reaction conditions were 94°C for 7 min followed by 28 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 45 s, and an extension at 72°C for 7 min. For *TnI* gene, the reaction conditions were 94°C for 7 min followed by 28 cycles of 94°C for 30 s, 63°C for 30 s, 72°C for 45 s, and an extension at 72°C for 7 min.

Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was considered to be a stably expressed house keeping gene, and was used as an inter-reference gene. Its PCR amplification conditions is 94°C for 10 min, followed by 24 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 45 s, extension at 72°C for 7 min. The PCR products of three reactions were electrophoresed on 1% agarose gel, and intensity of the bands was analysed with the Laboratory Imaging and Analysis System (UVP, Inc., CA, USA)

3. Results

3.1 *Characterizations of the two chicken breeds*

It is clear that these two breeds, fat line broilers and Bai'er layers, display great difference in growth and fat deposition. Differences in body weight became significantly distinguished from 2 wk of age. By investigating, we found that the body weight of the fat birds was 4.03, 5.41, 4.36, 4.92 and 4.48 folds higher than that of the Bai'er layers at 2, 4, 6, 8 and 10 wk of age, respectively (see figure 4), and the abdominal fat weight and percentage of abdominal fat of the fat birds were 17.5 and 4.2 folds higher respectively, than that of Bai'er layers at 12 wk of age (data were not shown). Obviously, these two breeds

Table 2. Part of differentially expressed genes from cDNA microarray[1,2].

Category	GenBank ID	Average Log_2 ratio
Fat metabolism and energy metabolism		
Apolipoprotein AI (Apo-AI)	M18746.1	-1.78
Lipoprotein lipase gene	X60547.1	1.17
Leptin receptor overlapping transcript	NM_001007958	1.13
Creatine kinase-M (CK-M)	X00954.1	-1.45
Troponin I (tni)	U19926.1	-1.57
SkTmod mRNA for skeletal muscle type tropomodulin	AB052717.1	-1.06
Actin-related protein 8	NM_022899.1	-1.64
Transcription and splicing factor		
Basic transcription factor 3	BC008062.1	-1.68
Similar to Homo sapiens U4/U6-associated RNA splicing factor (PRP3)(AF001947)	AJ720549	-1.35
Similar to protein phosphatase 1 regulatory (inhibitor)	AJ851823	-1.19
Similar to Homo sapiens protein phosphatase 2	AJ719537	-1.37
Similar to Homo sapiens poly(A)-specific ribonuclease(PARN)	AJ720562	-1.41
Similar to Homo sapiens tight junction protein 1 (zona occludens 1) (TJP1)	CV855732,BU306962	-1.58
Heat shock protein 70 (HSP70)	NM_001006685	1.15
Protein tyrosine phosphatase type IVA, member 1(PTP4A1)	NM_001008461	1.20
v-maf musculoaponeurotic fibrosarcoma oncogene	NM_001030852	1.24
Similar to Homo sapiens importin 9 (IPO9)	BU414284	-1.70
Similar to Homo sapiens SAPS domain family, member 3 (SAPS3)	AJ851429	-1.33
Cardiac and skeletal muscle-specific BOP1	AF410781	1.13
Protein synthesis and degradation		
Eukaryotic initiation factor 2 alpha kinase	AF330008.1	-1.15
Ring finger protein 30	NM_021447.1	-1.04
Branched chain keto acid dehydrogenase E1	NM_204657	1.02
Similar to Homo sapiens brefeldin A-inhibited guanine nucleotide-exchange	XM_037726.4	1.11
5'-nucleotidase, cytosolic III (NT5C3)	NM_204436	1.05
Histidine ammonia lyase	AY227348	-1.09
Similar to Homo sapiens WD and tetratricopeptide repeats 1 (WDTC1)	BX933307	-1.27
Brefeldin A-inhibited guanine nucleotide-exchange	XM_037726.4	1.11
Others		
MHC class II antigen alpha (B-LA)	NM_001001762	-1.07
Gallus gallus TOP AP mRNA	GGU17000	1.15
Similar to chromosome 20 open reading frame 72	XM_415017	1.42
G. gallus cDNA clone ChEST439g11	BU271424	-1.52
G. gallus finished cDNA, clone ChEST358k19	ABX932287	-1.07
G. gallus finished cDNA, clone ChEST830g7	CR389015	-1.49
G. gallus finished cDNA, clone ChEST124a24	CR524367	-1.18
G. gallus finished cDNA, clone ChEST158l24	BX932086	1.41
G. gallus finished cDNA, clone ChEST136j22	CR387244	1.19
G. gallus finished cDNA, clone ChEST320g10	CR387865	1.11

Table 1. (*continued*)

G. gallus mRNA for hypothetical protein, clone 12j16	AJ720206	1.12
G. gallus mRNA for hypothetical protein, clone 6l6	AJ719807	1.04
G. gallus mRNA for hypothetical protein, clone 17g4	AJ851681	1.01
G. gallus mRNA for hypothetical protein, clone 33p3	AJ721067	1.16
G. gallus mRNA for hypothetical protein, clone 2k20	CR352397	1.13

[1]*Ratio < -1 means the gene expresses higher in bai'er layers than in broilers,*
[2]*Ratio > 1 means the gene expresses higher in broilers than in Bai'er layers.*

are excellent models to investigate the mechanism of lipid metabolism in chickens.

3.2 *Differential gene expression*

Of the 9024 cDNA, 67 (about 0.7%) differentially expressed sequences were screened out. A summary of the most representative genes implicated in lipid metabolism, energy metabolism, cytoskeleton, transcription and splicing factor, protein synthesis and degradation (table 2).

3.3 *Verification of microarray results*

Northern blots and semi-quantitative RT-PCR were performed to verify four genes of our microarray results. *ApoAI* and *LPL* are important genes to lipid metabolism, and we have performed some studies on them. The *TnI* and *ARP8*, were involved in energy metabolism and cytoskeleton remodeling, and the two processes were reported to be related to lipid metabolism and adipocyte differentiation, and the two genes displayed greater difference in the microarray results. Great concordances between these two methods were observed. Northern blot showed that the expression of *LPL* in fat line broilers was higher than that in Bai'er layers, corresponding to three red dots on the microarray, and the expression of *Apo-AI* in fat line broilers was lower than that in Bai'er layers, corresponding to three green dots on the microarray. The results of Northern blots and relevant result of microarray are depicted in figure 1.

For two other genes, *ARP8* and *TnI*, semi-quantitative RT-PCR were performed to confirm microarray data. The results of semi-quantitative RT-PCR are in figure 2. The consistent trend between these two methods was found, and both of them showed that the expressions of *ARP8* and *TnI* in fat line broilers are lower than that of in Bai'er layers.

3.4 *Comparison of cDNA microarrays and Northern blot or semiquantitative RT-PCR*

A general trend of the expression in the four genes is given in figures 1 and 2. To compare and analyse the results among these methods, a phosphor-imager was used. The ratio of signal intensity of two breeds were calculated and turned into the Log_2 data. The results showed that the four genes that differentially expressed on the cDNA microarray also had a significant difference [$\text{Log}_2(\text{ratio}) < -1$ or >1] by two other methods (figure 3).

4. Discussion

The genetic mechanisms that govern differentiation, growth, and function of metabolic, somatic, and reproductive systems in the chicken are largely unknown (Cogburn *et al* 2003). With the gene-by-gene approach it would be difficult and time-consuming to obtain a global picture of gene expression patterns. cDNA array technology allows comparison of thousands of mRNAs from a given tissue simultaneously providing a comprehensive assessment of expression levels. This represents a powerful strategy for research in the obesity field (Gabrielsson *et al* 2000; Moreno *et al* 2001; Nadler and Attie 2001).

In our present study, cDNA microarrays were used to identify changes in gene expression between fat line broilers and Bai'er layers of 10 wk of age. Of the over 9,000 genes examined, 67 sequences, whose expression levels in adipose tissue showed significant difference between the two breeds, were identified. Four of the differentially expressed genes were verified by Northern blot or semi-quantitative RT-PCR. The results indicated that the results of the cDNA microarray were credible by two other methods as well, though small differences existed between the methods (see figure 3). It was reported Northern blot analysis is superior to microarrays for quantitative analysis (Taniguchi *et al* 2001). Nonetheless, microarrays offer a high-throughput method that generally captures changes of multigenes expression at the same time.

Cornelius *et al* (1994) organized genes in adipogenesis into five groups: hormone signalling and action, lipogenesis and lipolysis, cytoskeletal and extracellular secreted, and proteins of unknown function. The point of view has been validated by many researches in several model organisms (i.e. human, mouse, zebra fish, fruit fly, and yeast) (Samuel *et al* 2000; Lopez *et al* 2003). Our data from chicken showed the similar results.

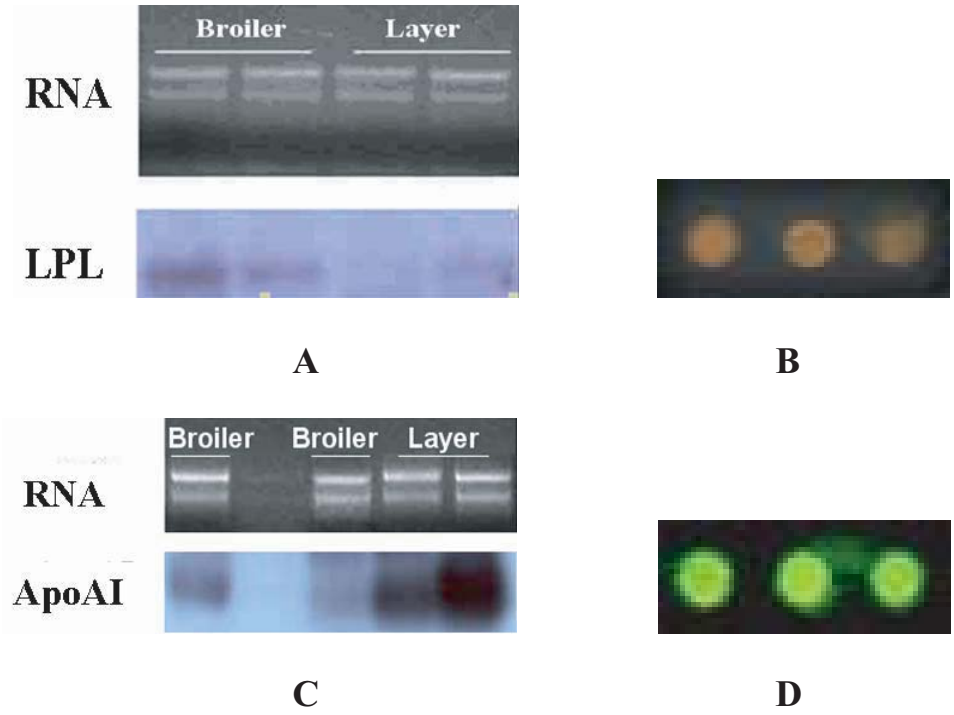

Figure 1. **(A)** The Northern blot results of LPL. **(B)** The result of *LPL* on chip. **(C)** The Northern blot results of *Apo-AI*. **(D)** The result of *Apo-AI* on chip. Two broilers and two layers were used in the validation. The red spots mean that the gene expressed higher in broilers' adipose tissue than in layers', and the green spots mean that the gene expressed higher in layers' adipose tissue than in broilers'.

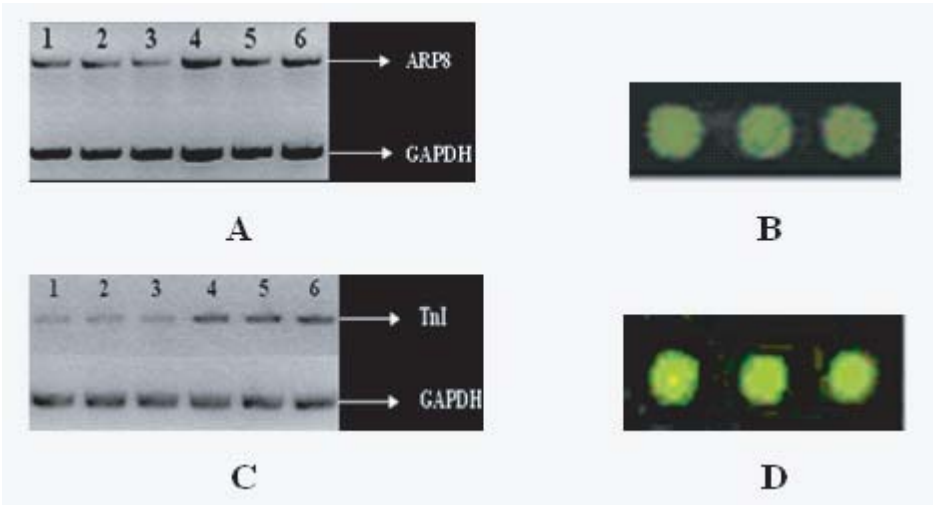

Figure 2. **(A)** The RT-PCR result of *ARP8*. **(B)** The result of *ARP8* on chip. **(C)** The RT-PCR result of *TnI*. **(D)** The result of *TnI* on chip. Lanes 1–3 are broilers; lanes 4–6 are layers. Three broilers and three layers were used in the validation. The red spots mean that the gene expressed higher in broilers' adipose tissue than in layers', and the green spots mean that the gene expressed higher in layers' adipose tissue than in broilers'.

Two genes that are important to lipid metabolism were screened out. Lipoprotein lipase (LPL) plays a crucial role in lipid metabolism and transport by catalyzing the hydrolysis of triglyceride-rich (TG-rich) lipoproteins such as chylomicrons and very-low-density lipoproteins (VLDLs) and causes a wide variety of alterations in lipoprotein metabolism (Goldberg 1996). Importantly, its activity is finely regulated to deliver fatty acids released by its action

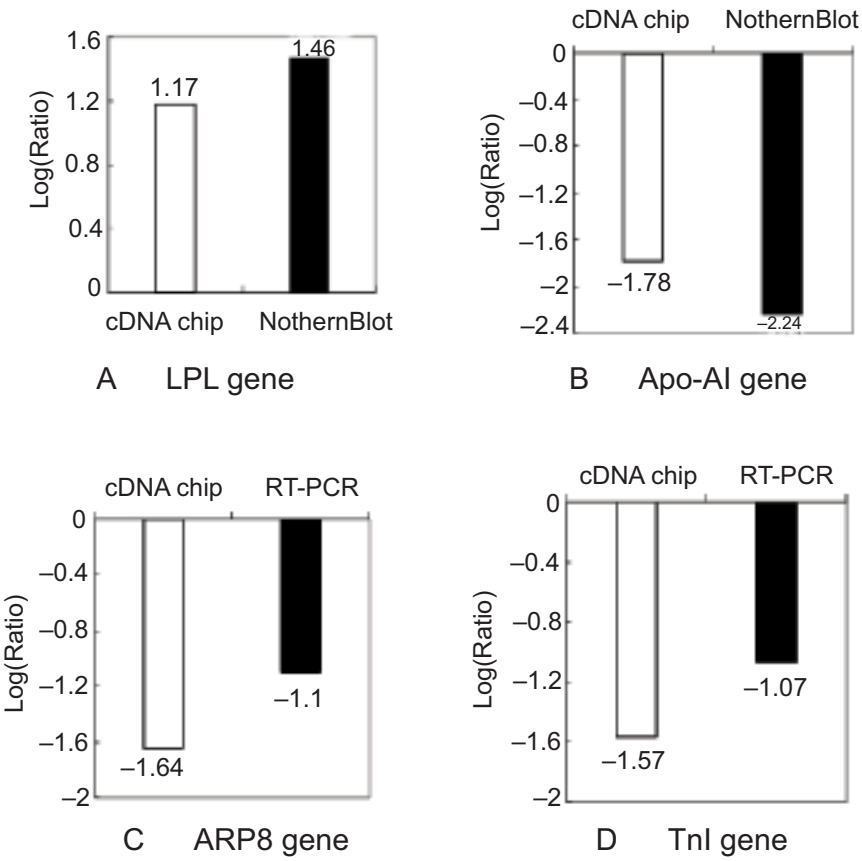

Figure 3. **(A, B)** Comparisons of gene expression levels measured by Northern blots and by cDNA microarrays. **(C, D)** Comparisons of gene expression levels measured by semiquantitative RT-PCR and by cDNA microarrays.

Note: Results are presented as the average Log_2 (ratio) of a given gene expression quantity in fat line broilers and the Bai'er layers. For Northern blots, analyses were performed for two broilers and two layers. Semiquantitative RT-PCR was performed for three broilers and three layers.

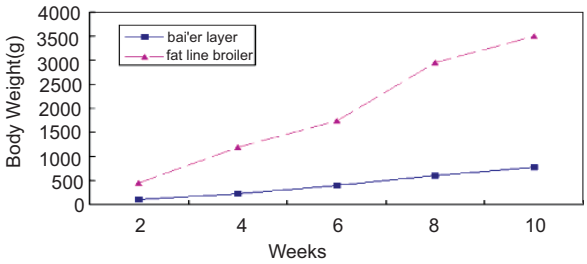

Figure 4. Change in weight during 10 weeks of fat line broiler and Bai'er layer.

into tissues that utilize them at a given moment (Fielding and Frayn 1998). The research performed in human showed that obese individuals have higher adipose tissue LPL activity than lean ones (Bosello *et al* 1984; Taskinen and Nikkila 1977), and LPL has been considered as a candidate gene for obesity (Kern 1997). In our previous research, three SNPs were found in chicken *LPL* 5'UTR for the first time, and the polymorphisms are significantly related to body weight and abdominal fat weight ($P<0.05$) (Wang 2004). In present research, we observed that the expression level of *LPL* in broilers was higher than that in layers. The two breeds have conspicuous differences in adipose sediment. It is presumed that due to the keener lipid metabolism in broilers than in layers, the triglyceride synthesized in liver will be transported to adipose tissue more quickly in broilers. LPL plays very important roles in this process, so it is necessary to express high level *LPL* and complete adipose sediment.

Apolipoprotein A-I (apoA-I) is a lipid-binding protein that participates in the transport of cholesterol and other lipids in the plasma. It is a major protein component of plasma high-density lipoprotein in all species, and plays an important role in cholesterol homeostasis (Bhattacharyya *et al* 1993). Cholesterol is removed from peripheral tissues and transported either directly or via other plasma

lipoproteins to the liver, where it is excreted. It is thought that, because of their involvement in cholesterol excretion, apoAI and HDL are important in protection against coronary heart disease. Genetic deficiencies in apoAI and HDL are associated with excessive intracellular cholesterol accumulation and premature atherosclerosis in human (Scriver and Charles 1989). In poultry, the studies performed in chicken *apoAI* had demonstrated that it was equivalent to human in its ability of depleting cholesterol (Kiss *et al* 2001). Furthermore, significant differences for *apoAI* mRNA levels were in the liver of broiler lines divergently selected for abdominal fat, and the total quantity of mRNA has significantly relation with abdominal fat (r = 0.58, P<0.05) (Douaire *et al* 1992). In our previous research, we had found two SNPs in 5' flanking region between broilers and layers, an A / T mutation at base positions 163 bp upstream of the ATG initiation codon lead to the change of a transcription initiation cap signal site, and the polymorphism was significant associated with abdominal fat weight and percentage of abdominal fat (P<0.05) (Wang *et al* 2003).

Talmud *et al* (2002) examined the *apolipoprotein AI* (*apoAI*) gene expression levels in 2773 healthy middle-aged men, and found that the S447X polymorphism in *LPL* was associated significantly with *apoAI* expression levels (P<0.01) (Talmud *et al* 2002). It seems that interaction of the *LPL* and *ApoAI* may be notable. From the results of our cDNA microarray data, it may be an adverse interaction between the two genes, but it remains to be proved.

The fact that energy expenditure is related to body weight and even more close to fat-free mass is well recognized (Ravussin *et al* 1986). Skeletal muscle is important in the regulation of energy expenditure and constitutes an important site for the utilization of both carbohydrates and lipids (Kelley *et al* 1990). In the genes screened, *Creatine kinase-M* (*CK-M*), *Troponin I* (*TnI*) and *Skeletal muscle type tropomodulin* (*SK-Tomd*) play prominent roles in energy metabolism and muscle contraction, the expression of these genes conspicuously decreased in fat line broilers, which just fit for theirs stronger ability of adipose sediment and less physiological activity controlled by muscle contraction.

In addition, *ARP8*, which is an important gene involved in cytoskeleton remodeling, was down-regulation in broilers. The previous researches have revealed that in obesity individuals, the expressions of genes mostly involved in adipocyte differentiation were decreased, suggesting that adipocytes are engaged in a dedifferentiation process that could be the result of their enlargement induced by obesity (Moraes *et al* 2003). The genes that refer to transcription and mRNA splicing (*Basic transcription factor 3*, *U4/U6-associated RNA splicing factor*) had low expression levels in fat line broilers, other genes (*Eukaryotic initiation factor 2 alpha kinase*, *Ring finger protein 30*) participatig in protein metabolism also decreased in the fat line broilers. These phenomena may suggest that the protein synthesis is not active in broilers, but further studies must be performed to demonstrate it.

In conclusion, our microarray expression profiling reveals specific genes that are differentially expressed in the adipose tissue between broilers and layers of 10 wk of age. These genes are mostly involved in lipid metabolism, energy metabolism, cytoskeleton, etc. If confirmed in future studies, these patterns of gene expression can contribute to our understanding of the pathogenesis of obesity and application in breeding lean line chickens.

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