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Gene Expression Patterns of Geese Expression Patterns of L-Fabp, Spot 14, Ob and Apo A1 Genes in Different Tissues of Overfed and Control Geese

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ABSTRACT

Ten-week-old Langde geese in similar body weight were randomly selected, four for overfeeding and four for routinly feeding. The abundance of *liver fatty acid-binding protein* (*L-FABP*), *thyroid hormone-responsive* (*THRSP* or *Spot 14*), *obese* (*OB*), and *apolipoprotein A1* (*Apo A1*) genes in goose were detected by quantitative RT-PCR. *L-FABP* was higher expressed in liver and intestine than other tissues, but no expression was detected in the pancreas or brain. The other three genes were widely expressed in different tissues, *OB* was higher expressed in pancreas and abdominal fat, *Spot 14* and *Apo A1* was higher expressed in sebum and abdominal fat. *Spot 14* and *Apo A1* genes were up-regulated in overfed goose livers compared with that in the control. Thus, *Spot 14* and *Apo A1* genes may play important roles in lipid metabolism in goose fat liver.

INTRODUCTION

Fatty acid-binding proteins (FABPs) play important roles in the transport of fatty acids from the plasma membrane to the sites of β oxidation or/and triacylglycerol or phospholipid synthesis (Veerkamp *et al*., 1995). FABPs are members of the intracellular lipid-binding protein family. Among the 12 known FABPs (Liu *et al*., 2008), the liver-type FABP (L-FABP) was the first to be cloned from recombinant resources. The *L-FABP* gene was highly expressed in the liver, and *L-FABP* gene-knock-down rats had significantly lower transportation of fatty acids compared with the control (Wang *et al*., 2006). The *thyroid hormone-responsive* (*THRSP* or *Spot 14*) gene was determined to be highly expressed in the liver of chicken and also expressed in fat tissues (Wang *et al*., 2002). *Spot 14* expression was associated with the expression of the thyroid hormone (Wang *et al*., 2002). In human lipid metabolism, *Spot 14* was proved to participate in the regulation of the transcription of six enzymes, namely, malic enzyme, ATP-citrate lyase, fatty acid synthase, pyruvate kinase, phosphoenolpyruvate carboxy kinase, and acetyl-CoA carboxylase (Brown *et al*., 1997). *Apolipoprotein A1* (*Apo A1*) is the main activating factor of lecithin cholesterol acyltransferase and plays the key role in reverse cholesterol transportation. *Apo A1* gene expression was detected and associated with the level of high-density lipoprotein cholesterol (HDLC) (Vega *et al*., 2001). Leptin is the product of *obese* (*OB*) gene. Leptin could suppress appetite, increase energy consumption, and reduce fat content in mice (Pelleymounter *et al*., 1995; Halaas *et al*., 1995). Lipid metabolisms in mammal and chicken have been reported clearly, but not in goose.

This study was performed to elucidate the lipid metabolism in goose. We selected several important genes related to lipid metabolism,

namely, *L-FABP*, *Spot 14*, *Apo A1*, and *OB* genes. We detected the expression patterns of these genes in different tissues and determined their differences between control and overfed (OF) geese.

MATERIALS AND METHODS

Sample collection

Ten-week-old Langde geese were randomly assigned into control group (C) and overfed group (OF), each group contain four geese. C geese were fed with basal diet and OF geese were fed with basal diet mixed with 0.4% goose fat and 0.1% salt. The overfeeding period lasted for 20 days after a period of 1 week of pre-overfeeding. Quantitative real-time PCR analysis was applied to detect mRNA abundance and differential expression patterns of the *L-FABP*, *Spot 14*, *OB,* and *Apo A1* genes in the different tissues of C and OF geese. Fourteen tissues (heart, liver, spleen, lung, kidney, brain, chest muscle, leg muscle, sebum, abdominal fat, intestine, proventriculus, gizzard, and pancreas) were collected from each individual. The tissues were immediately frozen in liquid nitrogen and stored at -70 °C for total RNA extraction. The geese were slaughtered following ethical standards.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All surgery was performed according to recommendations proposed by European Commission, and all efforts were made to minimize suffering of animals.

Total RNA extraction and reverse transcription

Trizol regent (Takara, Osaka, Japan) was used to isolate the total RNA, and M-MLV reverse transcriptase (Takara) was used to synthesize the complementary DNA. Aforementioned processes were performed according to the manufacturer´s protocols.

Quantitative RT-PCR

Primers used in this study are listed in Table 1. Quantitative RT-PCR analysis was performed in 20 μL of reaction mixture, which included 2 μL of cDNA template, 10 μL of 2 × SYBR qPCR mix, 0.4 μM forward/reverse primer, and 0.4 μL of ROX reference dye (Takara). The reaction was performed on ABI 7300 (Applied Biosystems, Foster City, CA) using the following protocol: 94 °C for 3 min, 40 cycles of denaturation at 94 °C for 10 s, and annealing and extension at 60 °C for 31 s. Each detection was performed thrice simultaneously.

Statistical analysis

Relative expression levels of goose *L-FABP*, *Spot 14*, *Apo A1*, and *OB* genes were indicated by $2^{-\Delta Ct}$, where $\Delta Ct = Ct_{target\ gene} - Ct_{GAPDH}$. Statistical significance was calculated using the Student's *t*-test with 2-tailed p-values (SPSS version 16.0). Differences were considered significant when $p<0.05$. Tests were performed among tissues and between treatments.

RESULTS

Expression patterns of L-FABP, Spot 14, OB, and Apo A1 genes in goose

The expression patterns of goose *L-FABP*, *Spot 14*, *OB*, and *Apo A1* genes in the different tissues are shown in Fig. 1. *L-FABP* gene was found to be highly expressed in the liver and intestine and also expressed in various tissues. No *L-FABP* expression was detected in the pancreas or brain. *Spot 14* gene was highly expressed in all tissues, especially in the fat tissues and intestine. *OB* gene was expressed in all tissues. *Apo A1* gene was highly expressed in the fat tissues, intestine, and liver, compared with those in the other tissues.

Table 1 – Sequences of primers used in this study.

	Nucleotide sequence (5′→3′)			
Primer	Sense	Antisense	Accession no.	Size
L-FABP[1]	actgcccccactgcgtt	cgtcaccacaaagtcgtctcct	HQ640427	184
Spot 14	tgcgtgtgtctaaaccacct	tcgaggctttgcgttttatt	GW713791	83
Apo A1[2]	aacttgcgcgagaagatgac	aagcgggtcttgaggttctc	NM_205525	83
OB[2]	gacttcattcctgggcttca	ccaggtcatcggctatctgt	AF082500	124
GAPDH	gtggtgctaagcgtgtca	aggctgggataatgttctgg	AY436595	290

[1]Referenced the sequences in duck

[2]Referenced the sequences in chicken

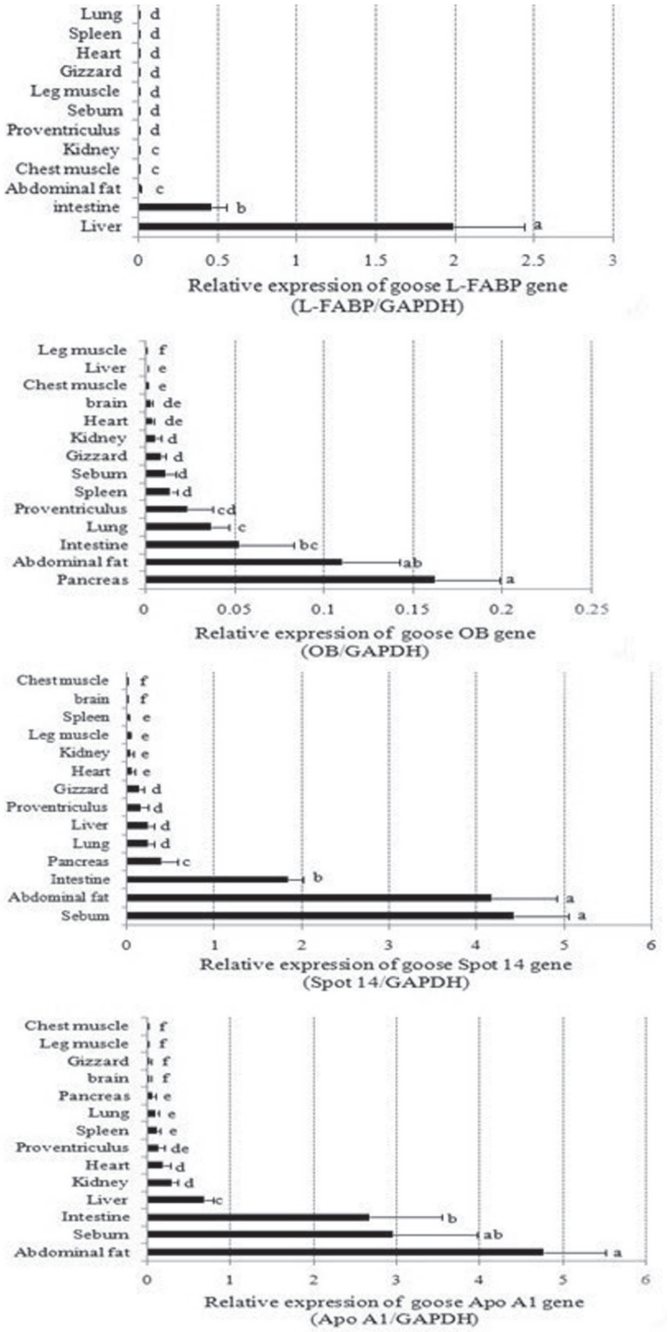

Figure 1 – Expression patterns of liver fatty acid-binding protein (L-FABP), thyroid hormone-responsive (THRSP or Spot 14), obese (OB), and apolipoprotein A1 (Apo A1) genes in goose.

Expression levels of these genes in the different tissues were determined by quantitative RT-PCR and normalized to that of GAPDH. No L-FABP gene was detected in the pancreas or brain. Each column in each diagram represents the mean ± SEM of four individuals. This condition applies to the following diagram. Different letters indicate significant difference at $p<0.05$.

Differential expression in control and OF geese

Significant higher expression of *Spot 14* and *Apo A1* genes was detected in OF goose livers, compared with those of the control ($p<0.05$) (Fig. 2). No significant difference was detected in other tissues ($P > 0.05$). No differential expression in the goose *L-FABP* or *OB* gene was detected ($p>0.05$)

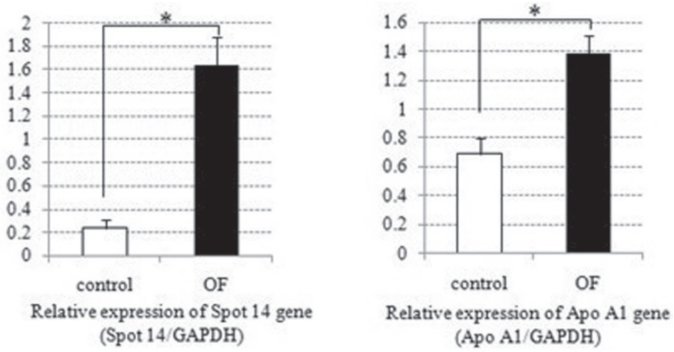

Figure 2 – Differential expression levels of *Spot 14* and *Apo A1* genes.

Expression levels of *Spot 14* and *Apo A1* genes in overfed (OF) geese were significantly higher than those in the control group ($p<0.05$).

DISCUSSION

Goose fat liver is one of the three most famous cuisines worldwide. This study was primarily performed to investigate several lipid metabolism-related genes in goose and their differential expression patterns between OF geese and control. Goose *L-FABP* gene was detected to be highly expressed in the liver and intestine, similar to the reports of Besnard *et al*. (2002) and Guilmeau *et al*. (2007). No *L-FABP* expression was detected in the pancreas or brain. Goose *Spot 14* gene was found to be highly expressed in the fat tissues, intestine, lung, and liver, which is consistent to those in human and rat (Jump *et al*., 1993). The OB gene was reported to be highly expressed in the fat and intestine, which is consistent with the results in Fig. 1. Goose *Apo A1* gene was also found to be highly expressed in the fat tissues, intestine, and liver, which are important organs for lipid metabolism. These lipid metabolism-related genes were all detected to widely exist in different tissues in goose.

Quantitative RT-PCR analysis showed that *Spot 14* and *Apo A1* genes were expressed higher in OF goose livers than those in the control (Fig. 2). The liver is a very important organ for lipid metabolism in vivo. These results indicated that lipid metabolism was more active in OF geese than control. *Spot 14* was proved to regulate the transcriptions of six key enzymes in lipid metabolism (Brown *et al*., 1997), and Spot 14 protein was indispensable in fat synthesis (Oppenheimer *et al*., 1987). *Apo A1* functions in reverse cholesterol transportation (Vega *et al*., 2001). Apo A1 is part of high-density lipoprotein (HDL), and HDLC was negatively associated with incidence of coronary atherosclerosis disease (Vega *et al*., 1996). The results

of *Apo A1* gene in this study might indicate that goose fat liver was healthier for human. Otherwise, the regulations of these genes in goose liver remain ambiguous.

In conclusion, the expression patterns of goose *L-FABP*, *Spot 14*, *OB*, and *Apo A1* genes in various tissues were detected. Differential expression analysis showed that the expression levels of *Spot 14* and *Apo A1* genes in OF goose livers were significantly higher than those in the control.

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