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

ABSTRACT

Ten-week-old Langde geese in similar body weight were randomly selected, four for overfeeding and four for routinely 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 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 (HDL-C) (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 (Pellegronymter 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 x 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 $2^{-\Delta \Delta Ct}$, where $\Delta Ct = Ct_{\text{target} \text{ gene}} - Ct_{\text{GAPDH}}$. 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.**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence (5’→3’)</th>
<th>Accession no.</th>
<th>Size</th>
</tr>
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<tbody>
<tr>
<td>L-FABP</td>
<td>actgcccccactgtcgg</td>
<td>HQ640427</td>
<td>184</td>
</tr>
<tr>
<td>Spot 14</td>
<td>tcgcgtgcctaaaccacct</td>
<td>GW713791</td>
<td>83</td>
</tr>
<tr>
<td>Apo A1</td>
<td>aacggtcggcggagatgac</td>
<td>NM_205525</td>
<td>83</td>
</tr>
<tr>
<td>OB</td>
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<td>AF082500</td>
<td>124</td>
</tr>
<tr>
<td>GAPDH</td>
<td>gttgtgtatagcgttgtc</td>
<td>AV436595</td>
<td>290</td>
</tr>
</tbody>
</table>

1Referenced the sequences in duck
2Referenced the sequences in chicken
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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)
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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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REFERENCES


