Cloning and Expression of the Genes Associated with Lipid Metabolism in Tsaiya Ducks[1]

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ABSTRACT Sterol regulatory element binding protein 1 (SREBP1) drives the expression of several lipogenic genes, whereas SREBP2 dictates the expression of every gene involved in cholesterolgenesis in mammals. In the current study, we cloned the cDNA fragments for SREBP1, SREBP2, fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), and very low density apolipoprotein-II (apoVLDL-II), the genes associated with lipid metabolism. Fifteen ducks immediately before the first egg was laid (18 wk old) and 15 ducks from the same population at an egg production rate of 80% were killed. Total RNA was extracted from liver and used to amplify the targeted genes by reverse transcription-PCR and screening of a cDNA library. The sequence data showed that Tsaiya duck SREBP1, SREBP2, FAS, and HMG-CoA reductase were highly homologous to that of chicken. Tsaiya duck SREBP1 mRNA was expressed in adipose tissue, cardiac muscle, skeletal muscle, liver, and ovary. The SREBP2 mRNA concentration was highest in liver and ovary. Concentrations of FAS and HMG-CoA reductase mRNA were high in liver and lower in other tissues. The apoVLDL-II mRNA was specifically expressed in the liver. The differences between mRNA concentrations of SREBP1, SREBP2, and FAS in the livers of laying and prelay ducks were not significant. However, the concentrations of hepatic HMG-CoA reductase and apoVLDL-II mRNA were higher in the laying ducks than in prelay ducks. Therefore, laying may affect particular aspects of lipid metabolism, especially biochemical pathways that involved apoVLDL-II and HMG-CoA reductase.

(*Key words*: duck, sterol regulatory element-binding protein 1, fatty acid synthase, 3-hydroxy-3-methylgutaryl-coenzyme A reductase, very low density apolipoprotein)

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INTRODUCTION

Tsaiya duck is the major egg-laying duck in Taiwan with a very high egg production rate (300 eggs/year). This high egg production rate requires a high lipid generation machinery to support egg yolk lipid accumulation. In mammals, the transcription factor sterol regulatory element-binding protein 1 (SREBP1) drives the expression of lipogenic genes such as fatty acid synthase (FAS) and acetyl CoA carboxylase (Brown and Goldstein, 1997). Sterol regulatory element-binding protein 2 (SREBP2) is a transcription factor that belongs to the same family but dictates the expression of every gene involved in cholesterolgenesis [for example, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) and HMG-CoA synthase] (Sakakura et al., 2001). The expression of SREBP1 and SREBP2 and their target genes is an indication of the capability of hepatic lipid synthesis.

Sterol regulatory element-binding protein 1 is expressed highly in the liver of rodents, humans, and chickens (Shimoura et al., 1997; Assaf et al., 2003) and in the adipose tissue of pigs (Ding et al., 1999; 2000; Hsu et al., 2004). It has been demonstrated that genes involved in de novo fatty acid (FAS and acetyl CoA carboxylase) and triacylglycerol synthesis (glycerol-3-phosphate acyltransferase) have SREBP1-responsive elements in their 5′-end promoter region (Magana and Osborne, 1996; Ericsson et al., 1997; Magana et al., 1997), indicating that these genes are regulated by SREBP1. Studies also show that inhibition of SREBP1 reduces FAS and acetyl CoA carboxylase expression (Horton et al., 1998; Shimano, 2001). With much of the emphasis on mammalian species, the expression of SREBP1 and SREBP2 and their relationship with avian lipid metabolism has not been well characterized.

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Abbreviation Key: ApoVLDL-II = very low density apolipoprotein-II; FAS = fatty acid synthase; HMG-CoA reductase = 3-hydroxy-3-methylglutaryl-CoA reductase; SREBP1 = sterol regulatory element-binding protein 1; SREBP2 = sterol regulatory element-binding protein 2.

Very low density apolipoprotein-II (apoVLDL-II), a protein only expressed in female birds, inhibits the activity of lipoprotein lipase, and plays an important role in lipid transportation from liver to ovary (Schneider et al., 1990; Zsigmond et al., 1995).

We hypothesize that the Tsaiya duck may express these genes in tissues with high lipid metabolism such as liver, ovary, and adipose tissues. Because the gene sequences of SREBP1, SREBP2, FAS, HMG-CoA reductase, and apoVLDL-II for Tsaiya duck are not known, one of our purposes was to clone these duck cDNA fragments. Another objective of this study was to determine the effect of egg laying on the mRNA concentrations of SREBP1, SREBP2, FAS, HMG-CoA reductase, and apoVLDL-II in the liver of Tsaiya ducks.

MATERIALS AND MATHODS

Birds

Tsaiya ducks, purchased from a commercial duck farm, were raised at the National Taiwan University. They were housed in individual cages and offered standard Tsaiya duck diets[3] ad libitum. The ducks were fed layer diets from 20 wk of age under a lighting program of 15L:9D. In experiment 1, 3 ducks with very high egg production records were chosen for cDNA fragment cloning. They were also used to study the tissue distribution of the mRNA of these genes. In experiment 2, the difference in gene expression in the livers of Tsaiya ducks before and after laying was studied. Fifteen ducks immediately before egg laying (18 wk old) and 15 ducks from the same population at an egg production rate of 80% were killed to study hepatic gene expression before and after egg laying. The National Taiwan University Animal Care and Use Committee approved the protocol for animal treatments. Adipose tissue, cardiac muscle, skeletal muscle, liver, and ovary were quickly dissected, frozen in liquid nitrogen, and stored at –80°C until total RNA extraction. Total RNA was extracted by the guanidinium-phenol-chloroform extraction method of Chomczynski and Sacchi (1987), as described previously (Hsu and Ding, 2003).

Cloning of the Tsaiya Duck cDNA Fragments

Two micrograms of Tsaiya duck liver total RNA were reverse transcribed at 42°C with a SuperScript II kit.[4] The transcribed cDNA was amplified by PCR for 36 cycles, using paired sense and antisense primers (5′-GCGCTACCGCTCATCCATCA-3′,5′-GGTCGGCATCTCCATCACCT-3′ for SREBP1; 5′-CAAGTCCTTCAGCCTCAAGT-3′, 5′-CATTGTGGTCAGAATGGTCC-3′ for SREBP2; 5′-ATTGACACAGCCTGCTCCTC-3′,5′-ACGGCTCTCTCTCACATTGG-3′ for FAS; 5′-CCAATGGCAACAACAGAAGG-3′, 5′-GGAATGACTGCTTCACAGAC-3′ for HMG-CoA reductase). The conditions for PCR were denaturation at 94°C for 30 s (5 min in the first cycle), annealing for 30 s, and extension at 72°C for 2 min (12 min in the last cycle). The annealing temperatures for SREBP1, SREBP2, FAS, and HMG-CoA reductase were 60, 60, 58, and 62°C, respectively. The PCR product for each gene was purified by gel electrophoresis and DNA extraction. The PCR products with correct cDNA fragment sizes were cloned into pGemTEasy[5] vectors and the cloned products were sequenced (forward and reverse) for verification. Sequence comparisons were performed with the Align Plus 2 software program.[6]

cDNA Library Construction

Five micrograms of egg-laying Tsaiya duck liver mRNA were purified with MicroPoly(A)Pure small scale mRNA purification kit[4] and the mRNA was used to construct a cDNA library. The mRNA was reverse transcribed with SuperScript Plasmid System with Gateway Technology for cDNA Synthesis and Cloning kit,[4] and the cDNA inserts were cloned into *Sal* I/*Not* I restriction enzyme-digested pCMV·SPORT 6 vectors. The ligation products were transformed into competent cells and cultured in Luria-Bertoni medium. The library was used for cloning duck apoVLDL-II full-length cDNA. One hundred colonies of the transformed *Escherichia coli* that contained Tsaiya duck liver cDNA were randomly selected and sequenced. About 10% of the cDNA sequences were complete coding sequences for apoVLDL-II.

Northern Analysis

The cloned duck cDNA fragments were used to synthesize DNA probes by PCR for Northern analysis. The protocol used by Hsu et al. (2004) was followed in this study. Briefly, total RNA (20 μg of each sample) was electrophoresed and transferred to nylon membranes. The membrane was prehybridized at 60°C in UltraHyb[7] for 30 min and then hybridized with the cDNA probe labeled with [α-^{32}P]dCTP[8] at 42°C overnight. Following hybridization, the membrane was washed twice in 2× SSC (300 m*M* NaCl, 30 m*M* sodium citrate, at pH 7.0) containing 0.1% SDS at 42°C for 5 min. The membrane was then washed twice in 0.2× SSC containing 0.1% SDS for 15 min at 42°C. All membranes were hybridized at the same time with a single probe, so comparisons could be made. The mRNA concentrations were quantified by a Fuji phosphoimager (BAS-1500)[9] and each transcript was normalized to the value for the glyceraldehyde-3-phosphate dehydrogenase mRNA in that sample. The same membranes were stripped according to the manufacturer's instruction and then hybridized with different probes to determine transcript concentrations.

[3]CP Feed Company, Taipei 104, Taiwan.
[4]Invitrogen Corporation, Carlsbad, CA.
[5]Promega, Madison, WI.
[6]Scientific and Educational Software, Durham, NC.
[7]Ambion, Inc., Austin, TX.
[8]Amersham Pharmacia, Piscataway, NJ.
[9]Fuji Photo Film Co., Ltd., Kanagawa, Japan.

TABLE 1. Amino acid sequence identity between Tsaiya duck and other species (GenBank accession number is indicated parenthetically)

Tsaiya duck	Chicken	Goose	Quail	Mouse	Human
SREBP1[1] (AY613441)	90%	—	—	76%	77%
SREBP2[2] (AY613442)	93%	—	—	89%	89%
FAS[3] (AY613443)	91%	96%	—	70%	71%
HMG-CoA reductase[4] (AY613444)	84%	—	—	71%	70%
ApoVLDL-II[5] (AY613440)	79%	—	67%	—	—

[1]The sequence of the duck sterol regulatory element-binding protein 1 (SREBP1) cDNA fragment was equivalent to nucleotides 232 to 511 in chicken (GGA310768), 806 to 1082 in mouse (AF374266), and 1162 to 1441 in human (XM008168).

[2]The sequence of the duck sterol regulatory element-binding protein 2 (SREBP2) cDNA fragment was equivalent to nucleotides 472 to 837 in chicken (GGA414379), 489 to 860 in mouse (AF374267), and 652 to 1023 in human (NM004599).

[3]The sequence of the duck fatty acid synthase (FAS) cDNA fragment was equivalent to nucleotides 555 to 1044 in chicken (J04485), 643 to 1132 in goose (M60622), 513 to 1002 in mouse (AF127033), and 596 to 1085 in human (NM004104).

[4]The sequence of the duck 3-hydroxy-3-methylgutaryl-CoA reductase (HMG-CoA reductase) cDNA fragment was equivalent to nucleotides 80 to 558 in chicken (BI066342), 1752 to 2230 in mouse (XM127496), and 1710 to 2188 in human (NM000859).

[5]The sequence of the duck very low density apolipoprotein-II (apoVLDL-II) cDNA fragment was equivalent to nucleotides 87 to 407 in chicken (M25774) and 102 to 419 in quail (S82591).

Statistical Analysis

The data for transcript concentrations in various tissues were analyzed by ANOVA (SAS Institute, 2001). The egg-laying effect on gene expression was analyzed by Student's *t*-test. ANOVA and Student's *t*-test were performed using SAS statistical software (SAS Institute, 2001). The means and SE for each transcript are presented.

RESULTS

The amino acid sequences for the Tsaiya duck genes were deduced from their cDNA sequences and compared with the sequences from other species. The sequence identity of SREBP1 amino acids between Tsaiya duck, chicken, mouse, and human was 90, 76, and 77%, respectively (Table 1). The sequence identity of SREBP2 between Tsaiya duck, chicken, mouse, and human was 93, 89, and 89%, respectively. The sequence identity of FAS between Tsaiya duck, chicken, goose, mouse, and human was 91, 96, 70, and 71%, respectively. The sequence identity of HMG-CoA reductase between Tsaiya duck and chicken, mouse, and human was 84, 71, and 70%, respectively. The data show that amino acid sequences of Tsaiya duck genes are very similar to that of chicken, confirming that, genetically, these species are more closely related than either is to the mammalian species.

Tsaiya duck apoVLDL-II complete coding sequence was obtained by sequencing a clone from a liver cDNA library. The sequence data showed that the cDNA fragment included a translation start (ATG) and a stop codon (TAG), suggesting that this cDNA fragment contains the complete open reading frame. The sequence identity of apoVLDL-II gene open reading frame between Tsaiya duck, chicken, and quail was 87 and 82%, respectively (Figure 1). The sequence of duck apoVLDL-II was equivalent to nucleotides 87 to 407 in chicken, and nucleotides 102 to 419 in quail. The amino acid sequence deduced from this cDNA showed that Tsaiya duck apoVLDL-II comprised 106 amino acids. The sequence identity of apoVLDL-II amino acids sequence between Tsaiya duck, chicken, and quail was 79 and 67%, respectively (Figure 2).

Tsaiya duck SREBP1 mRNA was expressed in all tissues studied (adipose tissue, cardiac muscle, skeletal muscle, liver, and ovary). The SREBP2 mRNA concentration was greater in the liver and ovary than in other tissues. The FAS and HMG-CoA reductase mRNA concentrations were high in the liver and low in the other tissues ($P < 0.05$). The duck apoVLDL-II mRNA, as in other avian species, was only expressed in the liver ($P < 0.05$; Figure 3).

The mRNA concentrations for hepatic genes associated with lipid metabolism are presented in Figure 4. There was no difference between prelay and laying duck mRNA concentrations for SREBP1, SREBP2, and FAS ($P > 0.05$; Figure 4). The concentration of HMG-CoA reductase mRNA in laying ducks was significantly higher than in prelay ducks ($P < 0.05$). The concentration of apoVLDL-II mRNA in the liver of prelay ducks was significantly lower than in laying ducks ($P < 0.05$).

DISCUSSION

The present study was the first to clone cDNA fragments for SREBP1, SREBP2, FAS, and HMG-CoA reductase from the liver of Tsaiya ducks. These Tsaiya duck cDNA sequences were highly identical to that of chicken, indicating the close genetic relationship between the 2 species.

In mammalian species, SREBP1 mRNA is detected in many tissues. It is expressed highly in tissues that have considerable lipid metabolism activity such as liver, adrenal grand, and adipose tissue (Shimomura et al., 1997; Ding et al., 2000). Chicken SREBP1 mRNA is detected in most tissues, and expressed highly in the liver and uropygial gland, both of which have high lipid synthetic

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Duck      (52) atggtgcaatacagggcattggtgatagctctgatcctgctccttagcac
Chicken   (87) ............................g....t.............
Quail    (102) .....a.........a.................t.......c.....

Duck     (102) caccgttcccgaagtgcactcaaagtccatctttgagagagaccgtcgtg
Chicken  (137) ...t..c..t.....................a....c.....a.......
Quail    (152) ...t..c..............g....c..ag...t....g........

Duck     (152) actggttggtcatccccgatgcaattgcagcttacatctatgaaactgtg
Chicken  (187) .....c.........t......gc..............c...g.....
Quail    (202) .....a....tta...t......c.....t.............g.....

Duck     (202) aacaagatgtcccctagagttggtcagttcttggcggatgctgcccagac
Chicken  (237) ......g...........c.............ct.....t.t.......
Quail    (252) ....c.g.t..a.......c..c..........ct.....t.t.......

Duck     (252) tccagtagttgttgggaccaggaccttcctcatcagagaaacgaccaaac
Chicken  (287) .aacagt...tc.....t.....a...........at......g.t.g..
Quail    (302) .a.g......tc....g......a.c...............a.....ga

Duck     (302) tcagtttactggctgaacagctgatggaaaaaataaagaacctgtggtat
Chicken  (337) ...c.aa......g.............................t..c
Quail    (352) ...c.a......tg......a..g.a.........g..g.a.t...a.c

Duck     (352) acaaaagtgctaggctactag
Chicken  (387) ....................
Quail    (402) gg....---............
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FIGURE 1. Full length open reading frame sequence for Tsaiya duck very low density apolipoprotein-II (apoVLDL-II; AY613440). The boldface type sequences are initiation and stop codons. The dots indicate identical nucleotides. The nucleotide sequence identities of open reading frames between Tsaiya duck and chicken (M25774) and quail (S82591) were 87 and 82%, respectively. The chicken sequence was from nucleotides 87 to 407 and the quail sequence was from nucleotides 102 to 419.

activity (Assaf et al., 2003). In the current study, laying Tsaiya duck SREBP1 mRNA was detected in adipose tissue, cardiac muscle, skeletal muscle, liver, and ovary. The Northern analysis results from the present study do not show whether there are 2 forms of the SREBP1 mRNA as in the mammalian species. Functions of SREBP1 in these tissues in the laying Tsaiya ducks await further investigation.

Mammalian SREBP2 mRNA can be found in most tissues with the highest concentration in the liver (Hua et al., 1993). Chicken SREBP2 mRNA is detected in most tissues (Assaf et al., 2003). We found that Tsaiya duck SREBP2 mRNA concentration was greater in liver and ovary when compared with other tissues (adipose tissue, skeletal muscle, and cardiac muscle). This observation is similar to that of chickens (Assaf et al., 2003). Liver is the major tissue with high cholesterol synthetic activity in avian species. The high hepatic SREBP2 mRNA concentration suggests this gene is involved in upregulation in expression of genes related to cholesterolgenesis, a

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Duck       (1) mvqyralvialilllsttvpevhsksiferdrrdwlvipdaiaayiyetv
Chicken    (1) ..........v..............id.e..........a......a.
Quail      (1) .....e.................r.lsd.g....m.y...l.s....a.

Duck      (51) nkmsprvgqfladaaqtpvvvgtrtflirettklsllaeqlmekiknlwy
Chicken   (51) ..v...a....l.vs..ns.s.i.n...n..ar.tk...........c.
Quail     (51) .tv...aa...l.vs..t..s.a.nl.......iti.v..mv...rsi.n

Duck     (101) tkvlgy
Chicken  (101) ......
Quail    (101) g.-...
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FIGURE 2. The deduced amino acid sequences for very low density apolipoprotein-II (apoVLDL-II) in Tsaiya duck and other species. The dots indicate the same amino acid. The amino acid sequence identities of apoVLDL-II amino acid sequences between Tsaiya duck, chicken, and quail was 79 and 67%, respectively.

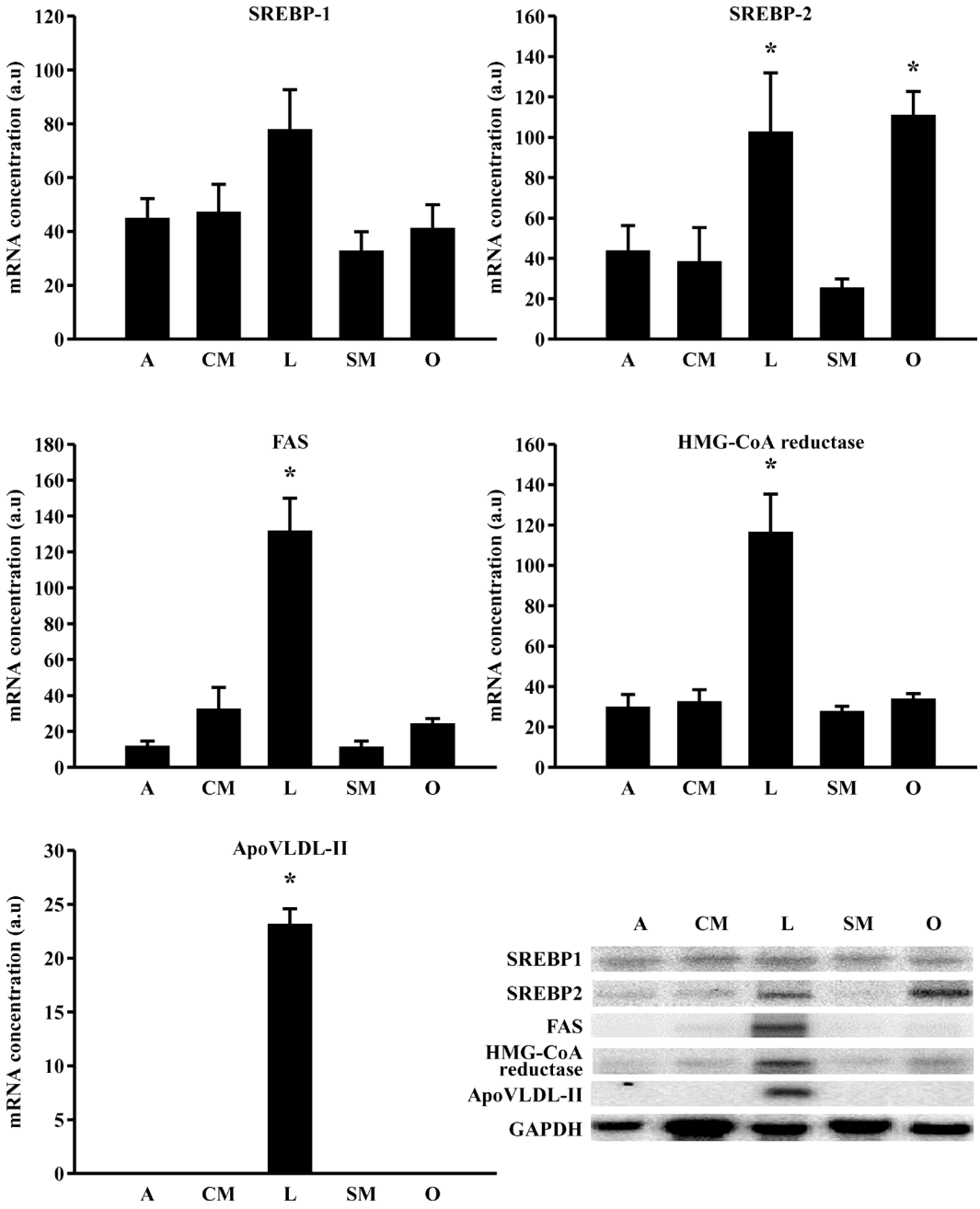

FIGURE 3. Tissue distribution of Tsaiya duck sterol regulatory element-binding protein 1 and 2 (SREBP1 and 2), fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), and very low density apolipoprotein-II (ApoVLDL-II) mRNA. Total RNA was isolated from adipose tissue (A), cardiac muscle (CM), liver (L), skeletal muscle (SM), and ovary (O), obtained from 30-wk-old female ducks. Twenty micrograms of total RNA was electrophoresed and transferred to nylon membranes. The membranes were hybridized with duck DNA probes for various gene fragments. The data are shown as arbitrary units using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a normalization value. Bars indicate SE of the means (n = 3). *Denotes significant difference among treatments ($P < 0.05$). A typical blot of each of the genes is presented in the figure.

function similar to that in mammals (Sakakura et al., 2001). We found that Tsaiya duck HMG-CoA reductase mRNA was expressed in all 5 tissues examined, similar to that of chickens, and that the mRNA concentration was greater in liver than in other tissues. Because HMG-CoA reductase is a rate-limiting enzyme for cholesterol synthesis, the data suggest greater cholesterol synthesis in the liver than in other tissues of Tsaiya ducks. In chickens, HMG-CoA reductase mRNA was detected in most tissues with greater concentrations in liver, brain, and ileum than in other tissues (Sato et al., 2003).

Fatty acid synthase is the major enzyme for de novo fatty acid synthesis. In humans, FAS mRNA is expressed in most tissues, and is highly expressed in the brain, lung, and liver (Jayakumar et al., 1995). In pigs, FAS mRNA is expressed in adipose tissue, liver, heart, lung, kidney, and small intestine, and is highly expressed in liver and adipose tissue (Mildner and Clarke, 1991; Ding

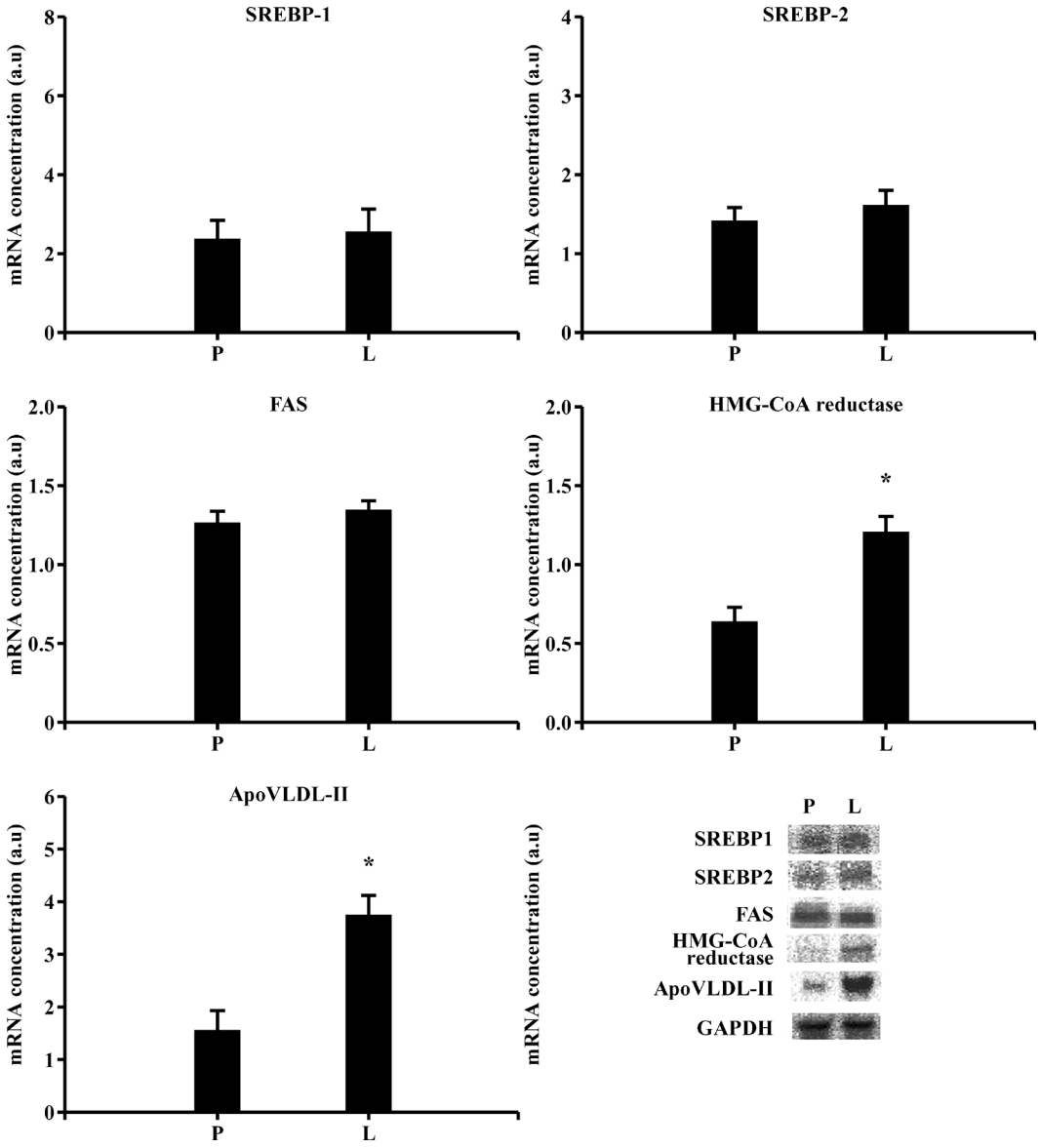

FIGURE 4. The mRNA concentrations of sterol regulatory element binding protein 1 and 2 (SREBP1 and 2), fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), and very low density apolipoprotein-II (ApoVLDL-II) in the liver of prelay and laying Tsaiya ducks. Total RNA was isolated from liver obtained from 18-wk-old (prelay) and 30-wk-old (laying) female ducks. Twenty micrograms of total RNA was electrophoresed and transferred to nylon membranes. The membranes were hybridized with duck DNA probes for various gene fragments. P = prelay; L = laying. The data are shown as arbitrary unit using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a normalization value. Bars indicate SE of the means. *Denotes significant difference between treatments ($P < 0.05$). A typical blot of each of the genes is presented in the figure.

et al., 2000). Liver is the major organ for fatty acid synthesis in avian species (Goodridge and Ball, 1967; Leveille, 1967; Yeh and Leveille, 1971; Evans, 1972). In the current study, we found that Tsaiya duck FAS mRNA was highly expressed in the liver and to a lesser extent in other tissues, suggesting that the liver is the major organ of fatty acid synthesis in Tsaiya ducks.

The Tsaiya Duck apoVLDL-II gene full-length cDNA sequence was similar to the sequences from the chicken and quail, suggesting that the function of duck apoVLDL-II is similar to that in other avian species. There are 2 forms of plasma apoVLDL-II, with molecular weights of 15.5 and 6 kDa in the laying poultry, but only 1 form (6 kDa) in the laying quail (MacLachlan et al., 1996). Chicken apoVLDL-II can form a secondary structure by a disulfide bond between the cysteine residues, but in quail, this cysteine (at amino acid 99) is mutated to tryptophan. Therefore, quail apoVLDL-II would not form the 15.5-kDa form. Tsaiya duck apoVLDL-II contained a tryptophan residue at position 99, suggesting that the Tsaiya duck apoVLDL-II protein would be similar to that of quail with a molecular weight of approximately 6 kDa. The finding that apoVLDL-II mRNA was only present in the liver of laying Tsaiya duck confirms that the laying Tsaiya ducks are similar to other mature female avian species with high apoVLDL-II in the liver (Schneider et al., 1990; Evans et al., 1994). The expression of apoVLDL-II protects apoVLDL-II containing very low

density lipoprotein (also called VLDLy) so that it can be transported to the ovary and not hydrolyzed by lipoprotein lipase in other peripheral tissues in laying ducks.

The expression of genes related to lipogenesis is elevated in laying poultry because the increase in circulating estrogen induces the expression of these genes (Speake et al., 1998). Lacasa et al. (2001) indicated that progesterone induced the expression of preadipocyte SREBP1c gene, which would then induce the expression of lipogenic genes in mammals. Therefore the expression of genes related to lipogenesis can be associated with hormonal status. We demonstrated that the gene expression of hepatic SREBP1, SREBP2, and FAS was not different in prelay and laying ducks. In chickens, the concentration of plasma estradiol increases from 7 wk of age, reaches a peak 2 to 3 wk before laying, and then drops to a stable level similar to that in birds 1 to 2 wk before laying (Senior, 1974). The plasma progesterone concentration of the duck increases steeply 7 d before laying and is maintained at a high concentration after laying (Wilson and Morris, 1982). Therefore the expression of genes (SREBP1, SREBP2, and FAS) regulated by these 2 hormones should not be different immediately before and after laying. We speculate that the prelay ducks used in the current experiment were close to laying their first eggs and therefore were equipped with egg-laying hormones similar to the layers at their laying peaks. Perhaps there is divergence in gene expression between juvenile and laying ducks. We did observe a greater concentration of HMG-CoA reductase mRNA in laying ducks compared with prelay ducks. In chickens, the expression of hepatic HMG-CoA reductase was greater in 30-wk-old chickens than that in 19-wk-old chickens (Sato et al., 2003). We found a similar pattern of expression for the hepatic HMG-CoA reductase gene expression in ducks.

The expression of apoVLDL-II is partially controlled by the presence of estrogen in laying poultry (Berkowitz and Evans, 1992). In the current study, we found that the hepatic apoVLDL-II mRNA concentration was significantly greater in laying ducks compared with prelay ducks, suggesting that the expression of hepatic apoVLDL-II is also associated with laying in the Tsaiya ducks. Because the estrogen concentrations in prelay and laying Tsaiya ducks have not been studied, the association of estrogen with the expression of apoVLDL-II is not known. Taken together, the data suggest that laying may affect particular aspects of lipid metabolism, especially biochemical pathways in which apoVLDL-II and HMG-CoA reductase are involved.

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