Apolipoprotein A-1 of Japanese Quail: cDNA Sequence and Modulation of Tissue Expression by Cholesterol Feeding

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Apolipoprotein (apo) A-1 cDNA was amplified by the reverse-transcriptase-polymerase chain reaction (RT-PCR). Primers were synthesized according to the nucleotide sequence of chicken apo A-1, and the identity of apo A-1 cDNA was confirmed by comparing with the N-terminal amino acid sequence. The open reading frame of apo A-1 cDNA consists of 795 nucleotides, and it is capable of coding a polypeptide of 264 amino acids. A comparison between quail and chicken apo A-1 revealed 94.5% homology in the nucleotide sequence and 91.7% homology in the amino acid sequence. There was a similar 11- or 22-amino acid repeat in quail apo A-1 as was the case for chicken apo A-1. Apo A-1 mRNA was evaluated to be 1.4k in length and was expressed in various tissues of Japanese quail: the liver, small intestine, lung, kidney, heart, and muscle. A quantitative evaluation, however, revealed that the liver and small intestine were the major organs for apo A-1 synthesis, accounting for more than 90% of the total expression of apo A-1 mRNA. Besides apo A-1 mRNA (1.4k in length), a transcript of 4.1k was detected in all the tissues examined, with a magnitude ranging from 5 to 10% of the apo A-1 mRNA level. The effect of cholesterol level on the expression of apo A-1 mRNA was studied to address the physiological significance of apo A-1 in the liver, small intestine, and muscle. The level of cholesterol in the liver and breast muscle was increased by feeding with cholesterol and reached a saturation level at day 7. There was also a temporal rise of cholesterol level at day 7 in the small intestine. Dietary cholesterol increased the expression of apo A-1 mRNA two fold in both the liver and small intestine. This was not the case for breast muscle, in which the expression of apo A-1 mRNA was not modulated by the cholesterol level.

Key words: apolipoprotein A-1; cDNA sequence; Japanese quail; tissue expression; cholesterol feeding

Apolipoprotein A-1 (apo A-1) is the major protein moiety of plasma HDL isolated from chicken and most animal sera.1-3 There is a reverse relationship between the propensity to develop atherosclerosis and the concentration of plasma HDL and apo A-1.4-9 For this reason, the protein structure and nucleotide sequence of apo A-1 has attracted many investigators’ attention.4-9

There has been increasing interest in the Japanese quail as an animal model for studying atherosclerosis.7,7 Our previous study delineated the lipoprotein and apolipoprotein profile of Japanese quail,10 apolipoprotein A-1 being detected in the entire lipoprotein subclasses from chylomicron to HDL.10 This result suggested that apo A-1 functions as a vehicle for plasma cholesterol transport in Japanese quail, and it has thus become of importance to investigate the molecular organization of quail apo A-1.

The present study describes the nucleotide sequence of apo A-1 mRNA and its expression in the various tissues of Japanese quail. In mammals, apo A-1 is only synthesized in the liver and intestine, the major sites of lipoprotein synthesis,11 while in the chicken, apo A-1 synthesis has been observed in various peripheral tissues as muscle and kidney. To address the physiological significance of local apo A-1 synthesis, the present study also describes the effect of cholesterol feeding on the expression of apo A-1 mRNA in the liver, small intestine, and muscle.

Materials and Methods

Reagents and enzymes. Restriction endonuclease, Klenow fragment, T4 DNA ligase, T4 polynucleotide kinase, and Taq polymerase were all purchased from Takara Shuzo Co. (Kyoto, Japan), while [α-32P]-dCTP was from Amersham International (Buckinghamshire, England).

Extraction of total RNA. Fresh quail tissues (0.5g) were homogenized in 4m guanidine thiocyanate, and the homogenate (1.6ml) was overlaid on 1.6ml of 5m cesium chloride and centrifuged at 93,000 × g for 16 hours. Pelleted total RNA was purified as described previously12 and used for the cDNA synthesis or Northern blotting analysis.

Amplification of apo A-1 cDNA. Total RNA isolated from quail liver was used as a template for the cDNA synthesis. First-strand cDNA was primed with oligo dT primers and synthesized by using reverse transcriptase from Rous associated virus 2 (Takara Shuzo). Our previous results showed that the N-terminal sequence of quail apo A-1 was very similar to that of chicken apo A-1 with 92% homology.10 This fact suggested that the nucleotide sequence of quail apo A-1 cDNA would also be similar to that of chicken. Thus, primers homologous (forward) and complementary (reverse) to chicken apo A-1 cDNA were used to amplify the quail apo A-1 cDNA, the amplification strategy by the polymerase chain reaction (PCR) being shown in Fig. 1. The primers used were 5’GCAAGGAGAGCAGCCTTCAAGC (forward) and 5’GCTCTCAAGTCTAAGCAGACAGCA (reverse) for the amplification of segment 1: 5’CTGG-
Fig. 1. Amplification Strategy for Quail Apo A-1 cDNA.

The hatched box denotes the sequence that encodes the N-terminus of the mature apo A-1 peptide.

CAGCAGATGACCCAGACGC (forward) and 5'CAGGGTGACAGCTTGGCTCGAAGCT (reverse) for segment 2, and 5'TCAGGAGCTGAAGGCATGCTC (forward) and 5'CAGCATGAGCTGGGCTCGAC (reverse) for segment 3. An mRNA-cDNA hybrid derived from 1 μg of total RNA was subjected to amplification with 30 pmol of primers and 2 units of Taq polymerase in a 50-μl reaction mixture. This reaction mixture was subjected to a repeated cycle of 45s at 94°C, 30s at 58°C, and 1 min at 72°C. The amplified DNA segment was purified in a minispin column (Pharmacia Biotech), and subsequently ligated into the pUC 119 plasmid.

DNA sequencing. The amplified DNA segment-blunt ended by the Klenow fragment was cloned into the pUC 119 plasmid at the Smal site. Two or three clones were used to confirm sequences, DNA sequencing being performed with a Sequenase 7-deaza-dGTP kit supplied by United States Biochemical. The reactions were separated in a 6% denaturing polyacrylamide gel.

Northern blotting analysis. Total RNA (4.5 μg) prepared as just mentioned was denatured in the presence of 35% formaldehyde and electrophoresed in a 1% agarose gel. The sample size was 4.5 μg unless otherwise stated. After completing the electrophoresis, RNAs were transferred to a nitrocellulose membrane, and cross-linked by baking at 80°C for 30 min. The membranes were then prehybridized at 68°C for 60 min in 6 × SSC, 2 × Denhardt's reagent, and 0.1% SDS. Hybridization was carried out for 16 hours in the same solution containing probe DNA labeled with 32P at 5 × 10^6 cpm/μg of DNA. The membranes were washed with 1 × SSC containing 0.1% SDS for 15 min at room temperature and subsequently washed 3 times with 0.5 × SSC containing 0.1% SDS for 15 min at 68°C. The blot was exposed to medical X-ray film (Fuji Film, Tokyo) at ~20°C, the radioactivity of the spots localized by autoradiography being determined with a scintillation counter (Hewlett Packard) and ACS-2 scintillation fluid (Amersham).

Labeling of the probes. The probes used for Northern blotting were of the [32P]-radiolabeled PCR products. Segment 1 (see Fig. 1) was amplified by PCR in the presence of [α-32P]-dCTP with the plasmid as a template. Unreacted [α-32P]-dCTP was removed with Centricon-30 (Amicon, MA, U.S.A.), and the concentrated products were used for probing.

Cholesterol-feeding experiment. Japanese quail (4 weeks old) were fed with a commercial diet containing 1% cholesterol and 10% corn oil for

CCGAAGATGACCCAGACGC (forward) and 5'CAGGGTGACAGCTTGGCTCGAAGCT (reverse) for segment 2, and 5'TCAGGAGCTGAAGGCATGCTC (forward) and 5'CAGCATGAGCTGGGCTCGAC (reverse) for segment 3. An mRNA-cDNA hybrid derived from 1 μg of total RNA was subjected to amplification with 30 pmol of primers and 2 units of Taq polymerase in a 50-μl reaction mixture. This reaction mixture was subjected to a repeated cycle of 45s at 94°C, 30s at 58°C, and 1 min at 72°C. The amplified DNA segment was purified in a minispin column (Pharmacia Biotech), and subsequently ligated into the pUC 119 plasmid.

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Cholesterol-feeding experiment. Japanese quail (4 weeks old) were fed with a commercial diet containing 1% cholesterol and 10% corn oil for
2 weeks. The indicated composition of the commercial diet was 22% protein, 2.5% fat, 5% fiber, 14% ash, and 2700 kcal/kg. The animals were killed on days 0, 3, 7, and 14. Total RNAs were isolated from the liver, small intestine, and muscle by the ultracentrifugal method as already described. The expression of apo A-1 mRNA was analyzed by Northern blotting as already described.

**Lipid analysis.** Total lipids were extracted from the liver, small intestine, and muscle by the method of Folch. The concentration of cholesterol was assayed as described elsewhere.

**Results**

Figure 1 shows the amplification strategy for apo A-1 cDNA of Japanese quail. Primers for segment 1 were synthesized to amplify a 312-bp length of segment from the initial signal, while segment 2 overlaps with segment 1 and segment 3 that contains the stop signal. Thus, it was possible to obtain the full length of the nucleotide sequence for quail apo A-1 cDNA. The authenticity of apo A-1 cDNA was supported by amino acid sequencing of the N-terminal, the amino acid sequence deduced from segment 1 being identical with that of the N-terminal of mature quail apo A-1 protein. This shows that primers synthesized on the basis of the sequence for chicken apo A-1 are useful for amplifying quail apo A-1 cDNA.

Figure 2 shows the nucleotide sequence and deduced amino acid sequence of quail apo A-1 cDNA obtained by overlapping segments 1, 2, and 3. The sequence comprises 802 bp and has a single open reading frame (ORF). The amino acid sequence flanking the N-terminal was tentatively designated as the signal and propeptide (boxed sequence) based on the known amino acid sequence of chicken apo A-1. A cDNA comparison between quail and chicken revealed 94.5% homology in the nucleotide sequence of ORF, while the deduced amino acid sequence of quail apo A-1 showed 91.3% homology to that of the mature chicken apo A-1 peptide. Chicken apo A-1 consists of a multiple repeat of 22 amino acids, each of which is a tandem array of two 11-mers (refer to refs. 4–6 for the detailed alignment of the amino acids). This structural feature was completely applicable to quail apo A-1.

In mammals, apo A-1 is synthesized in the liver and intestinal mucosa, while in chicken, apo A-1 synthesis takes place in peripheral tissues such as the kidney, brain, skeletal muscle, and heart. Figure 3 shows the Northern blotting analysis of apo A-1 mRNA from various tissues. A previous study proposed the widespread synthesis of apo A-1 by peripheral tissues of the chicken. Our data, however, do not support this view in that apo A-1 mRNA was detected almost exclusively in the liver and small intestine. An evaluation of the total expression of apo A-1 in the tissues revealed that the liver and small intestine accounted for more than 90% of total synthesized apo A-1.

We observed two transcripts on the Northern blotting analysis, whose nucleotide lengths were estimated to be 1.4 k and 4.1 k, respectively, based on the assumption that the

![Fig. 3. Northern Blot Analysis of the Apo A-1 mRNA Distribution in Various Tissues of Japanese Quail.](image)

Total RNA was analyzed as described elsewhere. Sample sizes for the liver, small intestine, breast muscle, heart, kidney, and lung were 9, 13, 7, 11, 11, and 6 µg, respectively.

![Fig. 4. Time-Course for the Cholesterol Concentration in the Liver (A), Small Intestine (B), and Breast Muscle (C).](image)

Data are mean ± SE values for 4 quail.

*Significantly different from day 0 at p < 0.05 by Student's t-test.
nucleotide lengths for 18 s and 28 s RNA would be identical to those for rabbit and comprises 6333 and 2366 bases, respectively. Chicken apo A-1 mRNA has been shown to be about 1 kbp in length, so RNA of 1.4 k was thus taken as quail apo A-1 mRNA in the present study. An inspection of the radioautogram revealed that the intensity of 4.1 k correlated well with that for 1.4 k throughout the tissues. Radioactivity probed to 4.1 k was usually in the 5–10% range of that for the 1.4 k transcript (apo A-1 mRNA).

Apolipoprotein A-1 is the major protein moiety of HDL, and has been implicated in the reverse transport of cholesterol from peripheral tissue to the liver for disposal. The effect of cholesterol feeding on the expression of apo A-1 mRNA was studied to delineate the function of this protein in the liver, small intestine and peripheral tissues. Animals were fed on an atherogenic diet containing 1% cholesterol and 10% corn oil, and were sacrificed on days 0, 3, 7, and 14.

Figure 4 illustrates the time-course for cholesterol concentration and the proportion of esterified cholesterol in the liver (A), small intestine (B), and muscle (C). The concentration of cholesterol in the liver increased with increasing feeding period of cholesterol, and appeared to reach the saturation level at day 7, while the percentage of esterified cholesterol remained unaffected throughout the experiment. The concentration of cholesterol in the small intestine had increased by day 7, but returned to the initial level by day 14. The rate of esterification in the small intestine was much lower than that in the liver, and was not affected by cholesterol feeding. The cholesterol concentration in the muscle was much lower than that in the liver and small intestine. Feeding cholesterol significantly increased the concentration of cholesterol in the muscle, with a concomitant increase in the proportion of esterified cholesterol, the highest concentration of cholesterol being seen on day 7.

The expression of apo A-1 mRNA in the liver was increased to its maximal level on day 3, and this level was sustained to day 14 (Fig. 5); a large change in the expression was noted on day 3. This pattern appeared to match the time-course for the apo A-1 mRNA level in the small intestine, but cholesterol feeding did not similarly affect the expression of apo A-1 mRNA in the muscle.

Consistent with the results shown in Fig. 3, the presence of the 4.1 k transcript was noted in this experiment. Feeding cholesterol significantly increased not only the level of apo A-1 mRNA (1.4K) but also that of the 4.1 k transcript in the liver and small intestine.

**Discussion**

We have described the cDNA sequence and tissue expression of quail apo A-1. The mRNA of chicken apo A-1 is expressed in a variety of peripheral tissues, and this widespread expression implies that the function of this protein may be related to reverse cholesterol transport to the liver from peripheral tissues. To examine this possibility, we studied the effect of feeding cholesterol on the expression of apo A-1 in the central tissues of cholesterol metabolism, the liver and small intestine, and in the peripheral tissue of the breast muscle. Feeding cholesterol increased the level of apo A-1 mRNA approx. two-fold in the liver and small intestine. Apolipoprotein A-1 is the major protein moiety of quail lipoproteins through chylomicron to the HDL subfractions. This observation was therefore interpreted as the increased flux of cholesterol in the liver and small intestine inducing the production of apo A-1 for the assembly of lipoproteins. The increase of apo A-1 mRNA in the liver may reflect an elevated synthesis of lipoproteins, VLDL or β-VLDL by this tissue. Analogous to this situation, the small intestine also may need to produce a large quantity of apo A-1 to process the absorbed cholesterol.

The cholesterol concentration in the muscle was much lower than that in the liver or small intestine. The level, however, remained with progressive cholesterol feeding. If one can assume that apo A-1 is involved in the transport of cholesterol, the level of apo A-1 may be increased with the concentration of cholesterol in this tissue. The expression of apo A-1 mRNA in this tissue was not influenced by the level of cholesterol. Thus, present findings may argue against the view that apo A-1 plays a role in the reverse transport of cholesterol from peripheral tissue to the liver. An alternative explanation for the absence of any increase in apo A-1 mRNA level is that a post-transcriptional mechanisms could be involved in regulating apo A-1 synthesis in the breast muscle.

A transcript of 4.1 k in nucleotide length was detected in all the tissues currently examined. The expression of this transcript correlated well with the expression of apo A-1 mRNA (1.4 k) throughout the tissues of the liver, small intestine, kidney, lung, heart, and breast muscle. This observation would rule out the possibility that the 4.1 k band was an artifact. Feeding cholesterol apparently increased the transcript of 4.1 k in both the liver and small intestine. Thus, the 4.1 k transcript might be a primary one, which thereafter was converted to apo A-1 mRNA by splicing.

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