Structure, Evolution, and Regulation of Chicken Apolipoprotein A-I*

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A full-length cDNA clone for the precursor form of chicken liver apolipoprotein A-I (apoA-I) was isolated by antibody screening of a chicken liver cDNA library in the expression vector λgt11. The complete nucleotide sequence and predicted amino acid sequence of this clone is presented. The identity of the clone was confirmed by comparison with partial amino acid sequences for chicken apolipoprotein A-I. Chicken preproapolipoprotein A-I consists of an 18-amino acid prepeptide, a 6-amino acid propeptide, and 240 amino acids of mature protein. The sequence of the protein is homologous to mammalian apoA-I and is highly internally repetitive, consisting largely of 11-amino acid repeats predicted to have an amphipathic α-helical structure. The sequence of the propeptide (Arg-Ser-Pro-Arg-Ser-Arg-Glu-Asp) differs in two positions from that of mammalian apoA-I. The mRNA for chicken apoA-I is about 1 kilobase in length and is expressed in a variety of tissues including liver, intestine, brain, adrenals, kidneys, heart, and muscle. This quantitative tissue distribution has been determined and is similar to that observed for mammalian apoE and different from that of mammalian apoA-I mRNA. This reinforces the concept that avian apoA-I performs functions analogous to those of mammalian apoE. Moreover, comparisons revealed sequences of chicken apoA-I similar to the region of mammalian apoE responsible for interaction with cellular receptors. Previous studies have demonstrated striking changes in the rates of synthesis of apoA-I in breast muscle during development and in optic nerve after retinal ablation. We now demonstrate that these changes are paralleled by changes in mRNA levels. ApoA-I mRNA levels increase approximately 50-fold in breast muscle between 14 days postconception and hatching and then decrease about 15-fold to adult levels. The levels of apoA-I mRNA increase about 3-fold in optic nerve following retinal ablation. ApoA-I mRNA is also found in the brain in the absence of nerve injury. This may indicate that locally synthesized apoA-I has a routine or housekeeping function in lipid metabolism in the central nervous system.

Apolipoprotein A-I (apoA-I) is the major protein of high density lipoproteins and is an activator of lecithin:cholesterol acyltransferase (1, 2). The sequences of human and rat apoA-I have been determined (3, 4). ApoA-I is a member of a dispersed gene family which also includes apolipoproteins A-II, A-IV, C-I, C-II, C-III, and E (5–7). The members of this family are internally repetitive, consisting in large part of 11-amino acid or higher order 22-amino acid repeats. A prominent structural feature of these apolipoproteins is an amphipathic α-helix in which the hydrophobic and hydrophilic faces are separated by positively charged residues. This structure is thought to mediate binding to lipid (8). In mammals apoA-I is synthesized only in liver and intestine, the major sites of lipoprotein synthesis (9), while in chickens, apoA-I synthesis has been observed in a number of peripheral tissues as well (10, 11). In this respect, chicken apoA-I resembles mammalian apoE, which is expressed at high levels in brain, kidney, lung, and other peripheral tissues (12, 13). The function of extrahepatic apoE in mammals may be related to "reverse cholesterol transport," a mechanism of cholesterol homeostasis involving the return of cholesterol to liver from peripheral tissues by a subtraction of high density lipoprotein (12). Since apoE has not been detected in avian lipoproteins, it has been suggested that, in birds, apoA-I may be the functional homologue of mammalian extrahepatic apoE (14). Synthesis of chicken apoA-I also undergoes profound developmental changes in breast muscle, exhibiting very high levels of synthesis in this tissue around the time of hatching followed by progressive decline to adult levels in the weeks after hatching (11). It has been suggested that the apoA-I produced at hatching may be involved in the mobilization of yolk lipids (11).

We now report the cloning and characterization of chicken apoA-I. We address questions relating to: (i) the structure and evolution of apolipoprotein A-I, (ii) the expression of apolipoprotein A-I in peripheral tissues, and (iii) the regulation of chicken apoA-I synthesis.

EXPERIMENTAL PROCEDURES

Animals and Tissues—White Leghorn roosters (SPAFAS, Norwich, CT; or Red Wing Hatchery, Los Angeles, CA) were maintained on a standard chicken diet. Tissues were removed, quick frozen in liquid nitrogen, and stored at ~70 °C. Chick embryo fibroblasts were dissociated from 11 day embryos with trypsin and cultured as described (15). Bone marrow-derived macrophages were provided by Dr. Paul Enrietta (Dept. of Microbiology, University of New York at...
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Stony Brook). The bone marrow macrophages were isolated from 10-day-old chick femurs and cultured for 5 days in growth media containing chicken myelomonocytic growth factor as described (16). Peritoneal macrophages were elicited with corn starch and isolated by adherence to plastic (17). For optic nerve samples, the right eye of 3-4-week-old chickens underwent retinal ablation under pentobarbital anesthesia; contralateral optic nerve of the same animal served as the control (14).

**Partial Amino Acid Sequencing**—Chicken apoA-I was purified from White Leghorn rooster plasma as described (10) and the amino-terminal amino acid sequence was determined using an Applied Biosystems Model 740A gas-phase sequencer. Phenylthiohydantoin amino acids released after each cycle of Edman degradation were identified by high performance liquid chromatography as described (18). To obtain sequence near the COOH-terminal end, apoA-I was cleaved with cyanogen bromide, and the fragments were isolated utilizing Sephadex G-50. The COOH-terminal fragment was identified by the absence of homoserine and subjected to sequential Edman degradation as described (19). The amino acid sequence of the cyanoan bromide fragment is: Met-Thr-Pro-Leu-Val-Gln-Glu-Phe-Arg-Agl-Leu-Pro-Tyr-Ala-Glu-Asn-Leu-Lys-Asn-Arg-Leu-Le-Ser-Phe-Leu.

**Isolation of Chicken ApoA-I cDNA**—A chicken liver cDNA library was prepared according to previously described methods (20). Briefly, double-stranded cDNA was prepared from poly(A) containing chicken liver RNA according to the method of Gubler and Hoffman (21), methylated at EcoRI sites, and EcoRI linkers were added. This was then ligated into λgll arms, packaged, and amplified on Escherichia coli. The library was screened. Phage DNA was isolated from plate lysates as described (20). Positive plaques were purified by multiple rounds of plating and screening. Phage DNA was isolated from plate lysates as described (20).

**DNA Sequencing**—cDNA inserts were subcloned in M13mp19 and single-stranded templates were prepared from the recombinant phage as described (23). Sequencing was carried out in both orientations of the template. A rapid chain termination method of Sanger et al. (24) using M13 universal and other synthetic primers. **RNA and DNA Blotting Analysis**—RNAs were prepared from chicken tissues as described (25). Poly(A)-containing RNA was electroblotted on 1.5% agarose gels in the presence of 2× MDE buffer and then transferred to a Hybond nylon from Amersham (U.K.) in 10× SSC, 0.15 M NaCl, 15 mM trisodium citrate, 0.1% sodium dodecyl sulfate. Hybrids were precipitated, collected on glass fiber filters, and radioactivity determined as described (28). ApoA-I mRNA values were determined by protein sequencing are underlined. The sites of cleavage of the pre- and propeptide regions are indicated by arrows. The prepropeptide is rich in hydrophobic amino acids and exhibits other features characteristic of a signal peptide (29). The 6-amino acid prepropeptide of chicken apoA-I (Arg-Ser-Phe-Try-Gln-His) differs from that of mammalian apoA-I (Arg-His-Phe-Try-Gln-Gln), in contrast to results reported from amino acids sequencing (30). Mature chicken apoA-I contains 240 amino acids with a calculated molecular weight of 27,360. The coding region is flanked by a 5' untranslated region of 23 nucleotides and a 3' untranslated region of 140 nucleotides. The polyadenylation signal AATAAA is located 13 bases upstream of the polyadenylation site (Fig. 1).

**Structure and Evolution**—A alignment of the amino acid sequence of chicken preproapolipoprotein A-I with the human protein is shown in Fig. 2. In order to maximally align the three sequences, gaps were introduced in the chicken sequence for the best alignment. The amino acid sequence of mature apoA-I is a 24-amino acid leader sequence which corresponds to an 18-amino acid prepropeptide and a 6-amino acid propeptide. The prepropeptide is rich in hydrophobic amino acids and exhibits other features characteristic of a signal peptide (29). The 6-amino acid prepropeptide of chicken apoA-I (Arg-Ser-Phe-Try-Gln-His) differs from that of mammalian apoA-I (Arg-His-Phe-Try-Gln-Gln), in contrast to results reported from amino acids sequencing (30). Mature chicken apoA-I contains 240 amino acids with a calculated molecular weight of 27,360. The coding region is flanked by a 5' untranslated region of 23 nucleotides and a 3' untranslated region of 140 nucleotides. The polyadenylation signal AATAAA is located 13 bases upstream of the polyadenylation site (Fig. 1).
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Table 1: Alignment of 11-amino acid repeats of chicken apoA-I. Conserved residues are indicated: hydrophobic (O), acidic (V), basic (K), and prolines (P).

| Sequence Position | Repeat | Human (H) | Chicken (C) | Rat (R) |
|-------------------|--------|-----------|-------------|---------|
| 1                 |        | MKAVLTAVLFLGQQA | RHPMQQ (Hu) |        |
|                   |        | MKAVLAVLFLGQQA | WEPMQQ (Ra) |        |

Fig. 2. Alignment of human (Hu), chicken (Ch), and rat (Ra) apoA-I protein sequences. The standard single letter code amino acid designation is used and the identities are indicated with dots. The sequences are divided into regions corresponding to the signal peptide and propeptide (residues 1–24) and the mature protein (residues 25–240).

Fig. 3. Alignment of 11-amino acid repeats of chicken apoA-I. Consered residues are indicated: hydrophobic (O), acidic (V), basic (K), and prolines (P).

As described below, the expression of chicken apoA-I in peripheral tissues is similar to that of mammalian apoE but not mammalian apoA-I. This raises the possibility that chicken apoA-I has functions analogous to those of mammalian apoB. Since mammalian apoB interacts with at least two...
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### Table I

An 11-nucleotide ancestral sequence for apolipoproteins

| Proposed ancestral sequence | G | C | G | A | C | G | A | G | G | C |
|-----------------------------|---|---|---|---|---|---|---|---|---|---|
| Consensus chicken apoA-I sequence (frequency of occurrence at each position) | (0.40) | (0.44) | (0.42) | (0.46) | (0.42) | (0.38) | (0.42) | (0.50) | (0.42) | (0.30) | (0.36) |
| T* | G* | A* | C* | A* | A* | C* | A* | G* | T* |
| (0.28) | (0.26) | (0.34) | (0.30) | (0.34) | (0.30) | (0.32) | (0.24) | (0.28) | (0.26) | (0.30) |
| C | T | C | C | T | C | G | A | G | T | G* |
| (0.28) | (0.20) | (0.16) | (0.18) | (0.20) | (0.28) | (0.22) | (0.20) | (0.16) | (0.24) | (0.22) |
| A | A | T | C | T | T | T | T | C | T | A |
| (0.04) | (0.10) | (0.08) | (0.06) | (0.04) | (0.04) | (0.04) | (0.06) | (0.14) | (0.20) | (0.12) |

**FIG. 4.** A region of chicken apoA-I homologous to the receptor-binding region of human apoE. Residues which are identical or have conservative substitutions at corresponding positions are indicated by boxes.

...peripheral tissues are summarized in Table II. The apoA-I mRNA values for small intestine, kidney, brain, and adrenal are substantial and range from 7 to 86% of the liver value. Values for the apoA-I mRNA content in thymus, heart, lung, and skeletal muscle fell in the range of 2–3% of the liver value.

Measurement of tissue RNA and DNA concentrations allow...
expression of the data on a per cell basis (Table II). This calculation does not consider possible differences in DNA ploidy but simply shows the apoA-I mRNA content normalized to the diploid DNA value (2.4 pg/cell; Ref. 45). On a cell basis, apoA-I is most abundant in the intestine at approximately 1640 molecules/cell. On a whole organ basis, the content of apoA-I mRNA is very similar in liver and intestine, suggesting that both organs contribute almost equally to the whole body apoA-I synthesis (Table II). These data are in agreement with earlier measurements of the relative rates of apoA-I protein synthesis; chicken apoA-I represented about 4%, 5%, and 2.5% of total protein synthesis in liver, duodenum, and jejunum/ileum, respectively (10). A similar distribution for apoA-I synthesis in liver and small intestine was found in the rat using in vivo amino acid labeling techniques (46). Based on the nine tissues assayed, liver and intestine account for approximately 45% and 52% of the total apoA-I mRNA present in adult chickens while peripheral tissues account for about 3% of the total apoA-I mRNA. However, the muscle contribution to the total apoA-I mRNA content may be significantly underestimated since only the breast muscles (musculus supracoracoideus + musculus pectoralis) were assayed. Northern analysis (Fig. 5) showed significant apoA-I mRNA in leg muscle, which constitutes a significant portion of the musculature of adult chickens.

Measurements of apoE synthesis in mammalian tissues as well as isolated macrophages suggested that peripheral tissue apoE synthesis is due to a wide variety of cell types in addition to tissue macrophages (13, 28, 47, 48). Similarly, the question arises as to which cells are responsible for apoA-I synthesis in peripheral chicken tissues. In contrast to mammalian macrophages which synthesize apoE but not apoA-I, avian macrophages appear to synthesize apoA-I. Measurements of apoA-I mRNA levels in avian bone marrow-derived macrophages and peritoneal macrophages indicate that apoA-I mRNA is present in very small quantities in these cells. For example, in chicken bone marrow-derived macrophages, apoA-I mRNA was present at only 0.4 ± 0.04 pg/μg RNA, which is in the range of a few molecules/cell and is lower than the apoA-I mRNA content of the peripheral tissues examined (Table II). Chicken peritoneal macrophage gave similar low values for apoA-I mRNA, and protein-labeling experiments with corn starch-elicited chicken peritoneal macrophages indicated that the relative rate of apoA-I protein synthesis was less than 0.01% of total protein synthesis in these cells. These data indicate that the apoA-I mRNA detected in chicken tissues is due largely to nonmyeloid cells. The low level of apoA-I mRNA in avian macrophages is in contrast to the high level of apoE mRNA in mammalian macrophages.

Developmental Regulation of ApoA-I mRNA in Breast Muscle—Previous studies have demonstrated that skeletal muscle shows a dramatic increase in the relative rate of apoA-I protein synthesis and mRNA content during the late stages of embryonic development (11, 49). Northern blotting analysis indicated that a single mRNA species of about 1 kilobase in size is present in muscle at different developmental stages (Fig. 7). Using solution hybridization to measure the absolute quantity of apoA-I mRNA, the developmental accumulation of apoA-I mRNA in breast muscle can be placed in perspective with respect to other apoA-I mRNA containing tissues. Fig. 8 shows the results of a solution hybridization assay with template DNA and RNA isolated from breast muscle taken at different stages in development. The low level of apoA-I mRNA measured in breast muscle of 14 day embryos is probably not due to contaminating cells such as fibroblasts, since fibroblast-free myotube cultures from 12 day embryos synthesize and secrete apoA-I (10). The absolute content of muscle apoA-I mRNA increases approximately 34-fold from the 14 day embryo stage to the time of hatching (day 0). The increase continues so that 3 days after hatching, breast muscle apoA-I mRNA content is 50-fold higher than the 14 day embryo levels. Then, apoA-I mRNA values quickly fall, so that by 40 days post-hatching the apoA-I mRNA content is 1.5 pg/μg RNA or only 6% of the 3 day level. These data, summarized in Table III, agree with earlier studies which measured apoA-I relative rates of protein synthesis (11) and translatable apoA-I mRNA (49). The quantitative measurements presented here establish that breast muscle accounts for a major portion of total apoA-I mRNA near the time of hatching. Thus a major fraction of plasma apoA-I and high density lipoprotein is likely a secretory product of muscle cells.

Regulation of ApoA-I mRNA during Wallerian Degeneration—Another system where an active mobilization of lipid occurs is Wallerian degeneration in the central nervous system (50). Using the optic nerve as a model system for Wallerian degeneration, 3 to 5-fold increases in rat apoE and chicken apoA-I synthesis were observed following retinal ablation (14). This increase in protein synthesis was accompanied by an increase in the respective mRNAs for apoE and apoA-I. However, in these earlier studies, the relative concentrations
ApoA-I mRNA was measured in total RNA extracted from the indicated tissues as described under "Experimental Procedures." Each value is the mean ± S.D. of at least 8 determinations on tissues isolated from 3-4 adult White Leghorn roosters. Total RNA (column B) and total DNA contents (column F) were measured by colorimetric procedures (39-43). Organ weights (column D), expressed as grams/kg of body weight, were obtained from Ref. 44. Total apoA-I mRNA (column E) is expressed as micrograms/organ/kg of body weight. Calculations for column G are based on a diploid DNA content of 2.4 pg/cell (45).

### Table II

| Tissue            | A PoA-I mRNA | B Total RNA | C ApoA-I mRNA | D Organ weight | E ApoA-I mRNA | F Total DNA | G ApoA-I mRNA |
|-------------------|--------------|-------------|---------------|----------------|---------------|-------------|---------------|
| Liver             | 80.7 ± 9.0   | 8.1         | 653.7         | 25             | 16.34         | 2.0         | 1488          |
| Small intestine   | 69.5 ± 9.1   | 12.2        | 847.9         | 22             | 18.65         | 2.3         | 1642          |
| Kidneys           | 22.0 ± 2.5   | 4.1         | 90.2          | 6.2            | 0.56          | 4.5         | 90            |
| Brain             | 6.7 ± 2.7    | 5.1         | 34.2          | 4.0            | 0.14          | 1.0         | 153           |
| Adrenals          | 5.9 ± 1.0    | 12.5        | 73.8          | 0.09           | 0.007         | 2.8         | 117           |
| Thymus            | 2.7 ± 1.0    | 8.3         | 22.4          | 4.2            | 0.09          | 20.9        | 5             |
| Heart             | 2.2 ± 1.0    | 4.6         | 10.1          | 6.7            | 0.07          | 2.0         | 22            |
| Lungs             | 1.8 ± 0.4    | ND*         | ND            | ND             | ND            | ND          | ND            |
| Breast muscle     | 1.6 ± 0.4    | 1.0         | 1.6           | 28.0           | 0.03          | 0.4         | 17            |

* ND, not determined.

![Fig. 7. Northern blotting analysis of chicken apoA-I mRNA from breast muscle at different times of development.](image)

![Fig. 8. Quantitative solution hybridization assay for chicken apoA-I mRNA in breast muscle at various times of development.](image)

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of apoA-I mRNA were determined using an in vitro translation system (14). Here, we use solution hybridization to measure the absolute content of apoA-I mRNA in control and degenerating optic nerve. The results (Table IV) show that apoA-I mRNA is more abundant in control optic nerve than whole brain (Table I) on a per microgram RNA basis. ApoA-

I mRNA is present in control optic nerve and brain at levels of 13 and 8%, respectively, as compared to chicken liver. Following retinal ablation, apoA-I mRNA increases approximately 3-fold compared to the contralateral nerve. This in-
TABLE III  
ApoA-I mRNA in chicken breast muscle at different developmental stages

| Age      | ApoA-I mRNA | ApoA-I mRNA |
|----------|-------------|-------------|
|          | ps/pg RNA   | molecules/pg RNA |
| 14 day embryo | 0.5 ± 0.2 | 0.9 |
| 0 day     | 16.8 ± 3.0 | 31.3 |
| 3 day     | 24.8 ± 3.4 | 46.3 |
| 13 day    | 2.3 ± 0.7  | 4.3 |
| 40 day    | 1.5 ± 0.4  | 2.8 |

TABLE IV  
ApoA-I mRNA in chicken optic nerve

|          | ApoA-I mRNA | ApoA-I mRNA |
|----------|-------------|-------------|
|          | ps/pg RNA   | molecules/pg RNA |
| Control  | 10.8 ± 2.5  | 20.1 |
| Retinal ablation | 32.0 ± 10.0 | 59.8 |

The decrease closely parallels the increase in apoA-I protein synthesis in degenerating optic nerve.

DISCUSSION

In this report, we have presented the complete nucleotide and deduced amino acid sequence for a full-length cDNA clone of chicken apoA-I. The clone was identified by antibody screening of a chicken cDNA expression library and its identity was established by comparisons with partial amino acid sequences of plasma apoA-I. The clone corresponds to 23 amino acid signal peptide, a 6-amino acid propeptide, a 240-amino acid sequence, and a poly(A) tail. The sequence provides information about the evolution, structure, and function of chicken apoA-I. The cDNA was used as a probe to examine the expression of apoA-I mRNA in various chicken tissues, during development in breast muscle, and during Wallerian degeneration of optic nerve. These results indicate that the regulation of apoA-I synthesis occurs at the level of mRNA abundance, and they support the concept that chicken apoA-I may perform some functions analogous to those of apoE in mammals.

While the 18-amino acid signal peptide of chicken apoA-I is similar to the signal peptides of rat and human apoA-I (3, 4), there are important differences in the propeptide sequence. In contrast to previous results obtained by protein sequencing (30), we find that the chicken apoA-I propeptide (Arg-Ser-Phe-Trp-Gln-His) differs at two positions from the human propeptide (Arg-His-Phe-Trp-Gln-Gln). These differences may explain differences previously observed between avian and mammalian species in the sites of processing of proapoA-I to the mature form. Chicken hepatocytes, when cultured under serum-free conditions, have been reported to secrete largely mature apoA-I, while the mammalian hepatoma cell line, Hep G2, secretes only proapoA-I (51). Since the amino-terminal sequence of mature apoA-I (Asp-Glu-Pro) is identical in chickens, rats, and humans, processing of proapoA-I in mammals involves cleavage of a Gln-Asp peptide bond, while in chickens it involves cleavage of a His-Asp peptide bond. This difference could well affect recognition and cleavage by intracellular proteases involved in pro-protein processing. This possibility could be tested by expression of chicken apoA-I cDNA in mammalian hepatoma cell lines. Banerjee et al. (51) have recently shown that the extent of intracellular processing is regulated in part by the cells hormonal environment: the presence of serum inhibits intracellular processing in chick hepatocytes but stimulates intracellular processing in Hep G2 cells. Moreover, both chicken and mammalian proapoA-I are capable of being processed in the presence of plasma (51). Thus, as suggested by Banerjee et al. (51), both intracellular and extracellular sites of pro- to mature apoA-I conversion exist.

The overall homology of chicken apoA-I to human and rat apoA-I is 49 and 42%, respectively. Most substitutions are conservative but some deletions or insertions have occurred. It has been proposed that rodent genes may undergo more rapid mutation on the basis of the greater homology observed between canines and human apoA-I (85%) as compared to rat and human apoA-I (64%) (4, 52, 53). Our results with chicken apoA-I are consistent with this hypothesis. The overall structure of chicken apoA-I is similar to mammalian apoA-I, consisting largely of tandemly repeated 11-amino acid segments with higher order 22-amino acid repeats. These repeats have the consensus sequence (Pro-X-hydrophobic-acid-acid-hydrophobic-base-acid-base-hydrophobic-X) and are predicted to exhibit largely an amphipathic a-helical structure. Thus, both the repeat unit organization and the amphipathic a-helical structure are present in this nonmammalian apoA-I. However, chicken apoA-I has significantly higher a-helical structure (about 90%) than mammalian apoA-I (60-70%) as predicted from the rules of Chou and Fasman or calculated from circular dichroism spectra (36, 37). The functional significance of this difference in structure of mammalian and avian apoA-I is unknown. We have previously hypothesized that the 33-nucleotide repeats corresponding to the 11-amino acid repeats of mammalian apolipoproteins were derived by repeated duplications of a more primitive 11-nucleotide ancestral unit (5). This proposal was based on theoretical considerations, homology alignments, and overall base composition of mammalian apolipoproteins. Our present results indicating that traces of the primitive repeats are retained in apoA-I from a distantly related species (birds diverged from mammals about 300 million years ago (54)) provides additional evidence for the hypothesis.

Quantitative measurements by solution hybridization showed that apoA-I was present in substantial amounts in numerous peripheral tissues. Since the mature macrophages contained very small amounts of apoA-I mRNA, most of the apoA-I mRNA in peripheral tissues is likely present in non-myeloid cells. Comparison of the total apoA-I mRNA in peripheral tissues versus the liver and small intestine suggests that peripheral tissues do not contribute a major portion of plasma apoA-I. It seems more likely that peripheral apoA-I functions in cellular cholesterol metabolism or local lipid transport. This is most likely the case with brain apoA-I since lipoproteins are not expected to readily cross the blood-brain barrier. Interestingly, the absolute amounts of apoA-I mRNA in peripheral chicken tissues are similar to the absolute amounts of apoE mRNA in monkey tissues, particularly in brain, adrenal, and kidney (28). This comparison suggests that the quantitative capacity for peripheral apoA-I synthesis in the chicken is similar to the capacity for peripheral apoE synthesis in mammals. This supports the idea that these two different proteins may perform some homologous functions with respect to peripheral tissue expression. It is noteworthy
that chicken apoA-I contains a region which resembles the receptor binding region of mammalian apoE, raising the possibility that chicken apoA-I may interact with receptors which are homologous to the apoE receptors. Although purely speculative, it would be of interest to examine the possibility in ligand competition experiments utilizing lipoproteins and receptors from mammalian or chicken tissues.

Northern gel analysis showed that the apoA-I mRNA transcript was the same size in all tissues and all situations studied. This result and the previous analyses of peripheral apoA-I by two-dimensional gel analysis (10) argue that peripheral apoA-I and liver apoA-I are the same protein and are encoded by a single apoA-I gene. More recently, Karathanasis' have used our cDNA to isolate overlapping apoA-I clones from a chicken genomic library. His analysis indicates a single apoA-I gene per haploid genome. Thus, it appears that tissue specific and developmental regulation of apoA-I synthesis is due to alterations in the cellular abundance of apoA-I mRNA transcripts produced from a single gene.

Results of the present study suggest that apoA-I mRNA levels in peripheral tissues are responsive to changes in lipid metabolism. In degenerating optic nerve, accumulation of apoA-I mRNA is induced at a time when myelin breakdown is occurring (28) suggesting that locally newly synthesized apoA-I participates in the transport of cholesterol released in this process. Similarly, apoA-I mRNA levels dramatically increase in skeletal muscle around the time of hatching. This accumulation of apoA-I mRNA and the increase in apoA-I protein synthesis occurs during and immediately following the time of massive absorption of yolk lipids associated with hatching (11). These associations between lipid levels and apoA-I mRNA suggest that cholesterol or other lipids may be involved in regulating the expression of this gene. The availability of cDNA and genomic clones for chicken apoA-I will facilitate the testing of this hypothesis.

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