Synthesis of Apolipoprotein AI by Peripheral Tissues of the Rooster

A POSSIBLE MECHANISM OF CELLULAR CHOLESTEROL EFFLUX*

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After in vitro incubation with radiolabeled amino acids, extracts of various chicken tissues were reacted with antiseraum against apolipoprotein AI (apo-AI) of plasma high density lipoprotein. Radiolabeled apo-AI was found in liver and intestine as well as in the kidney and a variety of peripheral tissues including aorta, peripheral arteries, veins, and skeletal muscle. The immunoreactive apo-AI synthesized by peripheral tissues had the same mobility as plasma apo-AI and newly synthesized liver apo-AI upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis. High resolution two-dimensional gel analysis showed that newly synthesized apo-AI exists in at least four isoforms in each of the tissues examined. Comparisons of isoform patterns as well as mixing experiments suggest that newly synthesized apo-AI isoforms have identical charge properties in each tissue examined. Plasma apo-AI also shows four isoforms with the same isoelectric points, but the quantitative distribution among the isoforms is different. The two basic isoforms predominate in newly synthesized apo-AI, while plasma apo-AI consists primarily of the two acidic isoforms.

Messenger RNA from liver, intestine, kidney, and skeletal muscle directed the synthesis of apo-AI in a wheat germ extract. The apo-AI cell-free product directed by each tissue RNA had the same molecular weight on sodium dodecyl sulfate-polyacrylamide gels. These products are 2000 daltons larger than mature apo-AI synthesized by liver tissue. Liver apo-AI is a secretory protein, and rat liver mRNA encodes an apo-AI precursor that can be processed in vitro to the size of mature apo-AI (Lin-Su, M.-H., Lin-Lee, Y.-C., Bradley, W. A., and Chan, L. (1981) Biochemistry 20, 2470-2475). Since the apo-AI synthesized in response to skeletal muscle and kidney RNA is larger than mature apo-AI but the same size as the cell-free product directed by liver RNA, it is likely that the apo-AI synthesized in peripheral chicken tissues is destined for secretion. Newly synthesized apo-AI was secreted from cultures of differentiated chick embryo muscle cells. Analysis of secreted apo-AI by isopycnic density gradient ultracentrifugation showed a substantial portion of the apo-AI in the density range <1.22 g/ml. This result implies an association of muscle apo-AI with lipid. These results are discussed with respect to the potential role of peripheral apo-AI in cellular cholesterol efflux and/or the movement of cholesterol from peripheral tissues to the liver.

The inverse correlation between plasma HDL levels and the incidence of atherosclerosis (1-4) suggests that some feature of HDL metabolism is closely related to the atherogenic process. The precise roles of HDL and its subfractions in lipid transport and metabolism, however, are not well understood. In 1968, Glomset (5) postulated that HDL participates in the removal of cholesterol from peripheral tissues and the transport of cholesterol to the liver for metabolism and excretion. This role of HDL could be of fundamental importance in preventing the accumulation of cholesterol and cholesteryl esters in the intima of the aorta and arteries as occurs in atherosclerosis. One line of evidence in support of the Glomset hypothesis derives from studies in which HDL and HDL apolipoproteins act as acceptors of cholesterol released from cultured cells (6-10). In vitro studies have indicated a negative correlation between the plasma HDL-cholesterol concentration and body cholesterol pool sizes (11). These results support the in vitro evidence for a role of HDL in cholesterol clearance from peripheral tissues.

The mechanism of cholesterol transfer to an acceptor and the exact nature of the acceptor in vivo are not known. The HDL which reaches peripheral tissues is believed to arise mainly from two sources. Discoidal HDL believed to be precursors to spherical plasma HDL are secreted directly by liver and small intestine (12, 13). In addition, surface components of chylomicrons and very low density lipoproteins may give rise to HDL via metabolism in plasma (14). Evidence from a variety of studies in experimental animals (15-17) and man (18-20) indicates that apo-AI, the major apolipoprotein of plasma HDL, is synthesized in liver and small intestine. Apo-AI appears to play a major role in cholesterol metabolism through its stimulation of lecithin:cholesterol acyltransferase (21, 22). In addition, apo-AI spontaneously binds phospholipid-cholesterol complexes (23) and in mixtures of phospholipids will promote cholesterol efflux from cultured cells (24-28). Thus, an understanding of the regulation of apo-AI synthesis is central to the further analysis of how HDL participates in cholesterol metabolism. Chicken and human apo-AI are very similar in molecular weight and amino acid composition (24, 25) and show partial immunological identity (25), suggesting that the chicken is a suitable model for studying apo-AI synthesis. In the course of studies on apo-AI synthesis in rooster liver and small intestine, it was found that intestinal smooth muscle synthesized apo-AI to an extent that could not be explained by contamination with mucosa. Subsequent analysis of vascular and other peripheral tissues showed apo-AI synthesis in all tissues and cell types examined with the exception of buffy coat leukocytes. In addition, newly synthesized apo-AI was secreted by two types of cultured chick

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1 The abbreviations used are: HDL, high density lipoprotein; apo, apolipoprotein; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride.
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embryo cells, and the secreted apo-AI had flotation properties indicative of association with lipid. Based on these findings and the known properties of apo-AI, we propose that apo-AI serves as a ligand in peripheral tissues of the rooster may play a role in cellular cholesterol efflux and/or the movement of cholesterol from peripheral tissues to the liver.

EXPERIMENTAL PROCEDURES

Preparation of Apo-AI and Anti-apo-AI—White Leghorn roosters (SPAFA, Norwich, CT) (0.9-1.2 kg) had access to a standard chicken diet. Blood was drawn from the heart, and plasma was prepared as described (26). HDL, isolated from plasma by ultracentrifugal flotation (27) between densities 1.063 and 1.210 g/ml as described (25) and dialyzed against 0.15 M NaCl, 0.01 M sodium phosphate (pH 7.3) at 4°C. After adjustment of HDL to 1% SDS, apo-AI was purified by chromatography on Sepharose 6B equilibrated with 1% SDS, 0.01 M sodium phosphate (pH 7.3), 100 μg/ml PMSF (26, 28), and column fractions were monitored by electrophoresis on SDS-10% polyacrylamide slab gels. Fractions containing apo-AI were pooled and the apo-AI was concentrated by precipitation from 80% acetone at -20°C overnight. The apo-AI was chromatographed a second time as described above. Antiserum against apo-AI was raised in a New Zealand White rabbit by subcutaneous administration of antigen (0.5-1 mg) in Freund’s complete adjuvant. Booster injections were given at 3-week intervals and the rabbit was bled 9 days after the third injection. Antiserum was adjusted to 0.02% NaN₃ and stored at -70°C. Prior to use, antisera was treated with 100 μg/ml PMSF and centrifuged at 10,000 x g for 5 min.

Labeling of Tissue Slices and Cell Cultures—Tissue slices from adult tissues (20 mg) were incubated for 1 h at 41°C in 0.1 ml of Krebs-Ringer bicarbonate solution containing 5 units of penicillin and 5 μg of streptomycin under an atmosphere of 95% O₂/5% CO₂ (28, 29). Each sample contained 100-250 μCi of [³H]leucine (1.45 μCi/μl), 60 Ci/mmol, or [³S]methionine (1.14 μCi/μl), 60 Ci/mmol, or [³P]orthophosphate (20 Ci/mmol). Radioisotopes were obtained from New England Nuclear. After incubation, tissue samples were washed, homogenized, and centrifuged to prepare a high speed supernatant as described (29).

Skeletal myoblasts were obtained by mechanical dissociation (31) of leg tissue from 12-day chick embryos and grown in vitro using the technique of Konigsberg (32). When myotube formation was complete after 4 days, cultures were labeled with [³H]leucine or [³S]methionine for 1 h (200 μCi/ml) or 4 h (50 μCi/ml). The labeling medium was minimal essential medium (Grand Island Biologicals, Grand Island, NY) containing 15% dialyzed horse serum, 5% dialyzed chick embryo serum, 50 μg/ml of streptomycin under an atmosphere of 95% O₂/5% CO₂ (29, 30). Each sample contained 100-250 μCi of [³H]leucine (1.45 μCi/μl), 60 Ci/mmol, or [³S]methionine (1.14 μCi/μl), 60 Ci/mmol, or [³P]orthophosphate (20 Ci/mmol). Isoelectric focusing gels were obtained from New England Nuclear. After incubation, tissue samples were washed, homogenized, and centrifuged to prepare a high speed supernatant as described (29).

Molecular weights of apo-AI were determined by polyacrylamide slab gels as described below. Various dilutions of the liver extract prepared according to Konigsberg (32), but lacking leucine, lysine, and methionine. Incorporation of radiolabeled amino acids into cell extracts prepared according to Scanu (40). Gradients extended from 1.02 to 1.36 g/ml. After centrifugation at 12°C for 72 h at 35,000 rpm in a Beckman SW 41 rotor, about 20 fractions were collected from the top. Densities were determined from refractive index measurements, and protein was monitored by absorbance at 280 nm. Radioactivity was monitored by scintillation spectrometry after the addition of a 25-μl sample to 1 ml of Scinti-Gel (Radiometric Instruments and Chemical Co., Addison, IL). Selected fractions were pooled and dialyzed against 0.15 M NaCl, 0.01 M sodium phosphate (pH 7.3), 100 μg/ml PMSF prior to analysis by immunoprecipitation, and electrophoresis in SDS-10% polyacrylamide gels as described above.

RNA Extraction and Cell-free Translation—Total cellular RNA was prepared from liver, skeletal muscle, kidney, and small intestine by the guanidine HCl technique (41). The RNA was subsequently precipitated four times from 0.2 M NaCl, 0.01 M Tris-HCl (pH 7), 0.01 M EDTA with 2 volumes of ethanol at -20°C. For cell-free translation, RNA was precipitated from 0.2 M ammonium acetate (pH 7), 0.01 M sodium phosphate (pH 7.3), 100 μg/ml PMSF, and centrifuged at 2°C for 60 min at 150,000 x g. The supernatant was assayed for radioactivity in total protein (36) and analyzed for apo-AI by immunoprecipitation as described above.

RESULTS

Characterization of Anti-apo-AI Serum—HDL was isolated from rooster plasma between densities 1.063 and 1.210 g/ml as described (25). When examined by CoCl isoponic
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density gradient ultracentrifugation, rooster HDL banded at 1.11 g/ml. Analysys by SDS-10% polyacrylamide gel electrophoresis showed that the major HDL protein was an apo-AI band of $M_r = 27,000$ which co-migrated with human apo-AI and represented about 90% of chicken HDL protein as reported previously (24, 25). Trace amounts of low molecular weight protein and plasma albumin were also present, while significant amounts of a protein with the mobility of apo-AI were not detected. These properties of chicken HDL are in agreement with previous work (24, 25). Apo-AI was purified by two cycles of chromatography on Sephacryl 6B in SDS as described under “Experimental Procedures.” Both the low molecular weight protein and plasma albumin were effectively removed. The final apo-AI preparation showed a single band upon electrophoresis in SDS-10% polyacrylamide gels with sample loads of 8 μg (Fig. 1A, lane 2) or 4 μg (Fig. 1A, lane 1).

Characterization by Ouchterlony double diffusion and precipitin analyses showed that rabbit antiserum against rooster apo-AI was a nonprecipitating antiserum. Consequently, radioligand competition procedures were used to test antiserum specificity. Since chicken liver is known to synthesize apo-AI (17), liver slices were labeled in vitro for 1 h with [3H]leucine, and an extract containing newly synthesized proteins was prepared. The radiolabeled extract was reacted with anti-apo-AI or normal rabbit serum in an indirect immunoprecipitation assay using goat anti-rabbit γ-globulin as second antibody. Analysis of the immunoprecipitates by electrophoresis on an SDS-10% polyacrylamide gel showed that anti-apo-AI, but not normal rabbit serum, precipitated a single radioactive band that co-migrated with purified apo-AI at $M_r = 27,000$. These results are shown in lanes 7 and 8 of Fig. 2. Several faint bands representing nonspecific precipitation are also present in both immunoprecipitates but are only seen with longer fluorographic exposure of the gel. Comparison of the anti-apo-AI immunoprecipitate (Fig. 2, lane 7) with the profile of total radioactive liver proteins (Fig. 2, lane 1) demonstrates that the antiserum is highly specific. The specificity of anti-apo-AI was further tested via competition as described under “Experimental Procedures” and analyzed by electrophoresis on an SDS-10% polyacrylamide gel followed by fluorography. Lanes 1-6 show newly synthesized proteins from the indicated tissues. Newly synthesized apo-AI was precipitated with anti-apo-AI (A1) using the double antibody method from liver (lane 7), kidney (lane 9), and brachial vein (lane 11). Control immunoprecipitates from these tissue supernatants formed with normal rabbit serum (N) are shown in lanes 8, 10, and 12. The arrow indicates the mobility of plasma apo-AI.

**Fig. 2.** Apo-AI synthesis by liver, kidney, and vein. Tissue extracts labeled with [35S]methionine were prepared as described under “Experimental Procedures” and analyzed by electrophoresis on an SDS-10% polyacrylamide gel followed by fluorography. Lanes 1-6 show newly synthesized proteins from the indicated tissues. Newly synthesized apo-AI was precipitated with anti-apo-AI (A1) using the double antibody method from liver (lane 7), kidney (lane 9), and brachial vein (lane 11). Control immunoprecipitates from these tissue supernatants formed with normal rabbit serum (N) are shown in lanes 8, 10, and 12. The arrow indicates the mobility of plasma apo-AI.

**Fig. 1.** Characterization of anti-apo-AI serum. A, purified apo-AI (lane 1, 24 μg; lane 2, 8 μg) was analyzed by SDS-10% polyacrylamide gel electrophoresis. The gel was stained with Coomassie blue. B, liver extract (5 μl) containing 1.2 × 10^4 cpm of [3H]leucine was immunoprecipitated with the indicated volumes of anti-apo-AI serum using the double antibody technique. Data points represent immunoprecipitate radioactivity after correction for nonspecific precipitation with normal rabbit serum. C, liver extract (10 μl) was reacted with 8 μl of anti-apo-AI serum in the presence of the indicated amounts of the following proteins: O, purified apo-AI; A, HDL; Δ, chicken immunoglobulin; O, purified apo-B; O, chicken serum albumin. D, immunoprecipitates containing [3H]apo-AI formed in the presence of competitors as in C were analyzed by SDS-10% polyacrylamide gel electrophoresis followed by fluorography. The competitors were (lanes 1-6, respectively): none, 15 μg of purified apo-AI, 15 μg of HDL, 2.5 μg of chicken serum albumin, 2.5 μg of chicken immunoglobulin, and 15 μg of chicken immunoglobulin. The arrow indicates the mobility of plasma apo-AI.
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Apo-AI Synthesis in Peripheral Tissues—In the course of experiments with small intestine, it was noted that the smooth muscle layer scraped free of mucosa synthesized apo-AI at a substantial rate. Histological analysis of the smooth muscle layer indicated that it was highly unlikely that the observed apo-AI synthesis was due to residual mucosa. Consequently, a variety of tissues were analyzed for apo-AI synthesis. Fig. 2 (lanes 1-6) shows the SDS-10% polyacrylamide gel profiles of newly synthesized proteins from liver, intestine, kidney, iliac artery, and brachial vein after labeling tissue slices in vitro for 1 h with [35S]methionine. Immunoprecipitation of the tissue extracts with anti-apo-AI yields a newly synthesized apo-AI band in liver (lane 7), kidney (lane 9), and brachial vein (lane 11). The apo-AI band is not present in immunoprecipitates with normal rabbit serum (lanes 8, 10, and 12). Results with a variety of other tissues (Fig. 3) show a newly synthesized apo-AI band in adrenal, adipose tissue, testis, iliac artery, thoracic aorta, and duodenum (A-F, respectively). In each case, the anti-apo-AI immunoprecipitate shows an apo-AI band that runs with authentic apo-AI at Mr = 27,000, while the normal serum precipitate does not. Other tissues which also showed a newly synthesized apo-AI band were lung and skeletal muscle as well as oviduct and uterus from laying hens (data not shown). Apo-AI synthesis was detectable in these tissues using [3H]leucine or [35S]methionine. Incorporation of radiolabeled amino acid into total protein and apo-AI was not detectable when tissue slices were incubated with 250 μM cycloheximide. Newly synthesized apo-AI was not detected in buffy coat leukocytes.

Relative Rates of Apo-AI Synthesis—The relative rates of apo-AI synthesis in liver, intestine, and kidney were measured by quantitative immunoprecipitation of [3H]leucine-labeled tissue extracts as illustrated in Fig. 1B. Haptic apo-AI synthesis represents about 4% of total protein synthesis, whereas kidney apo-AI synthesis is 2.5% of total protein synthesis (Table I). The relative rate of intestinal apo-AI is greatest in the jejunum at 5% and about one-half of this value in the duodenum and ileum. Relative synthetic rates are considerably less in the cecum and colon where apo-AI synthesis is 1% or less. Apo-AI synthesis in most peripheral tissues is too low to estimate by direct measurement of immunoprecipitate radioactivity. In these cases, apo-AI synthesis was estimated by densitometry of the apo-AI band on the fluorograph subsequent to electrophoresis of the immunoprecipitates in SDS-10% polyacrylamide gels (see “Experimental Procedures”). Relative synthetic rates between 0.1 and 0.5% were found for thoracic aorta, iliac artery, adrenal, and lung. Relative synthetic rates between 0.5 and 1.3% were found for brachial vein, testis, adipose tissue, skeletal muscle, and duodenal smooth muscle.

Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Supernatant protein</th>
<th>Supernatant protein</th>
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<tbody>
<tr>
<td></td>
<td>Precipitated by anti-apo-AI</td>
<td>Precipitated by anti-apo-AI</td>
</tr>
<tr>
<td></td>
<td>cpm/mg %</td>
<td>cpm/mg %</td>
</tr>
<tr>
<td>Liver</td>
<td>42,000 4.2</td>
<td>61,000 4.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>55,000 2.6</td>
<td>142,000 2.5</td>
</tr>
<tr>
<td>Duodenum</td>
<td>52,000 2.4</td>
<td>94,000 2.1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>17,000 5.4</td>
<td>91,000 4.8</td>
</tr>
<tr>
<td>Ileum</td>
<td>50,000 2.1</td>
<td>80,000 1.3</td>
</tr>
<tr>
<td>Cecum</td>
<td>47,000 0.2</td>
<td>31,000 1.6</td>
</tr>
<tr>
<td>Colon</td>
<td>45,000 0.9</td>
<td>27,000 0.7</td>
</tr>
</tbody>
</table>

Secretion of Apo-AI by Cultured Cells—Secretion of newly synthesized apo-AI was studied with primary cultures of chick embryo muscle cells. Fig. 4 shows the electrophoretic profiles of newly synthesized intracellular (lanes 1 and 2) and secreted (lanes 3 and 4) muscle proteins after labeling for 1 h (lanes 1 and 3) or 4 h (lanes 2 and 4) with [35S]methionine. Each sample in lanes 1-4 represents 15,000 cpm of protein radioactivity. Secreted proteins show a prominent band at the position of apo-AI which is precipitated by anti-apo-AI (lanes 9 and 11) but not by normal rabbit serum (lanes 10 and 12). The profiles of intracellular proteins (lanes 1 and 2) show that the apo-AI band is far less prominent. In addition, the intracellular apo-AI band is decreased in its relative intensity after labeling for 4 h (lane 2) as compared to 1 h (lane 1). This point is confirmed by the relative intensities of immunoreactive apo-AI after 4 h (lane 7) and 1 h (lane 5) of labeling. These data suggest that newly synthesized apo-AI does not accumulate intracellularly to a major extent but is rapidly secreted from the muscle cells. Since muscle cultures may also contain low amounts of fibroblast-like cells, pure myotube cultures were prepared as described under “Experimental
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**TABLE II**
Distribution of secreted apo-AI in CsCl gradients

The distribution of [35S]apo-AI secreted by muscle cell cultures was determined in two different gradients. Gradient I is the gradient illustrated in Fig. 5. Fractions containing labeled apo-AI were pooled and dialyzed. Distribution of apo-AI was calculated as a per cent of apo-AI radioactivity in combined pooled fractions based on immunoprecipitable labeled apo-AI per fraction. Density ranges were estimated from gradient profiles based on determinations of average density/fraction (density profile for gradient I is shown in Fig. 5).

Total protein radioactivity was measured by trichloroacetic acid precipitation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Density (g/ml)</th>
<th>% of total apo-AI</th>
<th>% of total protein radioactivity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gradient I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.10-1.13</td>
<td>7.7</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>1.13-1.19</td>
<td>18.4</td>
<td>7.8</td>
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<td>3</td>
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<td>4</td>
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<td>11.7</td>
<td>38.3</td>
</tr>
<tr>
<td>Gradient II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.08-1.14</td>
<td>4.4</td>
<td>4.8</td>
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<tr>
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</table>

**Fig. 4.** Secretion of apo-AI by muscle cell cultures. Muscle cell cultures were grown and labeled with [35S]methionine as described under “Experimental Procedures.” Newly synthesized intracellular and medium proteins as well as the corresponding immunoprecipitates were analyzed on an SDS-10% polyacrylamide slab gel followed by fluorography. Lanes 1-4 each represents 75,000 cpm of protein radioactivity of intracellular protein (lanes 1 and 2) and secreted protein (lanes 3 and 4) after labeling for 1 h (lanes 1 and 3) or 4 h (lanes 2 and 4). Lanes 5 and 7 represent anti-apo-AI immunoprecipitates of the samples in lanes 1 and 2, respectively, except that 10 times the volume of tissue extract was used. Lanes 6 and 8 are the normal serum immunoprecipitate controls for lanes 5 and 7, respectively. Lanes 9 and 11 are anti-apo-AI immunoprecipitates of the secreted proteins in lanes 3 and 4, respectively, except that twice the volume of culture medium was used. Lanes 10 and 12 are the normal serum immunoprecipitate controls for lanes 9 and 11, respectively. The arrow indicates the mobility of plasma apo-AI.

**Fig. 5.** Flotation properties of secreted muscle cell apo-AI. Dialyzed muscle cell medium containing [35S]-protein was analyzed by ultracentrifugation in a CsCl density gradient. Fractions were monitored for radioactivity (O-O) and density (--.--.) as indicated. Fractions 9 and 10, 11 and 12, 13 and 14, and 15 and 16 were pooled and dialyzed. Samples from these four fractions containing 10,000 cpm of protein radioactivity were immunoprecipitated with anti-apo-AI serum and analyzed by SDS-10% polyacrylamide gel electrophoresis followed by fluorography. The gel region showing the apo-AI band from fractions 9 and 10 (lane 1), 11 and 12 (lane 2), 13 and 14 (lane 3), and 15 and 16 (lane 4) is shown above the gradient profile.

**Fig. 6.** Two-dimensional gel analysis of peripheral apo-AI. [35S]Methionine-labeled tissue extracts were reacted with anti-apo-AI serum followed by goat anti-rabbit γ-globulin. Immunoprecipitates were prepared for isoelectric focusing as described under “Experimental Procedures.” The isoelectric focusing dimension is oriented with the basic end to the left. The second dimension SDS-10% polyacrylamide gel is run from top to bottom. Immunoprecipitates containing newly synthesized apo-AI of two different liver samples are shown in A and B. Other panels show anti-apo-AI immunoprecipitates of kidney (C), muscle cell medium (D), adipose tissue (E), and the mixture of adipose and liver (F).
Procedures.” Apo-AI was also synthesized and secreted by myotube cultures (data not shown). In addition, primary cultures of chick embryo fibroblasts were found to synthesize and secrete apo-AI (data not shown).

Dialyzed muscle cell medium was analyzed by isopycnic density gradient ultracentrifugation to determine the flotation characteristics of newly secreted apo-AI. As shown in Fig. 5, the bulk of the radiolabeled protein banded at a density of 1.25 g/ml, while purified HDL banded at 1.11 g/ml. Appropriate gradient fractions were pooled and dialyzed, and equal amounts of protein radioactivity from each sample were immunoprecipitated with anti-apo-AI and analyzed by SDS-10% polyacrylamide gel electrophoresis. As shown by the gel profile in Fig. 5, newly secreted apo-AI is significantly enriched in fractions of lower density as compared to the distribution of total protein radioactivity. The distribution of total radiolabeled apo-AI and total protein radioactivity in the gradient fractions is tabulated in Table II (Gradient I). Approximately 25% of the newly secreted apo-AI was in fractions of density <1.19 g/ml. The same muscle cell medium was centrifuged in another CsCl gradient, and fractions of slightly different densities were pooled and analyzed. In this case (Table II, Gradient II), 58% of the apo-AI was at densities <1.22 g/ml. Comparison of the distributions of apo-AI and total protein radioactivity shows that the bulk of the newly synthesized apo-AI is in a density range intermediate between HDL and total protein.

**Apo-AI Isoforms**—High resolution two-dimensional gel analysis was carried out to analyze apo-AI isoforms. Fig. 6 shows the isofrom patterns of newly synthesized apo-AI from liver (A and B), kidney (C), muscle cell medium (D), and adipose tissue (E). Apo-AI from each tissue yielded two major and two minor isoforms with isoelectric points of 5.76, 5.64, 5.42, and 5.30. The isofrom patterns of newly synthesized apo-AI from intestine and vein (data not shown) as well as the tissues shown in Fig. 6 were completely superimposable on the isoform pattern of liver apo-AI. Mixing experiments with liver and adipose apo-AI (Panel F), liver and vein apo-AI (data not shown), and liver and kidney apo-AI (data not shown) demonstrate apparent identity in the isofrom pattern. Two-dimensional analysis of plasma apo-AI (Fig. 7A) also

![Apo AI](image)

**Fig. 7.** Comparison of plasma and tissue apo-AI by two-dimensional gel analysis. A, purified plasma apo-AI was acetone precipitated, lyophilized, and analyzed by two-dimensional electrophoresis as described under “Experimental Procedures.” Four isoforms are distinguishable by Coomassie blue staining (Arrows 1–4). B–D, plasma apo-AI and an immunoprecipitate containing [35S]methionine-labeled apo-AI from liver were mixed and analyzed. Subsequently, the gel was stained with Coomassie blue, destained, soaked for 1 h in 5% glycerol, dried, and exposed by autoradiography instead of fluorography to avoid artifacts due to shrinkage. B shows the Coomassie blue-stained dried gel. C, the autoradiograph of the gel in B, and D is a duplicate of C except that the isoform pattern of the stained gel has been traced onto the X-ray film (dotted outlines) to illustrate superimposition of the isoforms. The orientations of the two dimensions are as in Fig. 6.
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Fig. 9. Two-dimensional analysis of apo-AI made in the cell-free system. Total liver (A) and kidney (B) RNA was translated in the wheat germ system, the cell-free products were immunoprecipitated with anti-apo-AI, and the immunoprecipitates were examined by two-dimensional gel analysis as described under “Experimental Procedures.” The isoelectric focusing dimension is oriented with the basic end to the left. The second dimension SDS-10% polyacrylamide gel is run from top to bottom. shows two major and two minor isomers, but in this case, the two acidic species are the major isomers. Analysis of a mixture of plasma apo-AI and newly synthesized liver apo-AI (Fig. 7B, C, and D) indicates that the two major liver isomers (1 and 2) correspond exactly to the two minor plasma apo-AI isomers and the two minor liver apo-AI isomers (3 and 4) correspond to the two major plasma isomers. This result suggests that newly synthesized apo-AI is subsequently modified to yield the major acidic shifted plasma apo-AI isomers. Analysis of apo-AI subsequent to incubation of liver slices with 32P showed no radiophosphate incorporation into any of the isomers, although many other liver proteins were labeled.

Cell-free Translations of Apo-AI mRNA—Since hepatic apo-AI is a secretory protein, its mRNA would most likely encode a larger precursor as has been shown for many secretory proteins (44, 45). Rat liver apo-AI mRNA, in fact, has been shown to encode such a precursor (46). Cell-free translations were carried out in the wheat germ system to compare the sizes of the apo-AI species directed by RNAs from various tissues. The SDS-polyacrylamide gel profiles of Fig. 8 show that the apo-AI species directed by RNAs from skeletal muscle, kidney, intestine, and liver are all the same size (lanes 1–4, respectively). Comparison to apo-AI made by liver tissue indicates that the cell-free products are approximately 2000 daltons larger than mature apo-AI. None of the apo-AI species directed by these RNAs is precipitated by normal rabbit serum (lanes 6–10). Two-dimensional gel analysis of the apo-AI species made in response to liver RNA (Fig. 9A) and kidney RNA (Fig. 9B) shows the cell-free products to consist of two major and one minor isoform. Intestinal RNA gave a similar result (data not shown).

Discussion

The results of these studies indicate that many peripheral chicken tissues and two types of cultured chick embryo cells synthesize apolipoprotein AI. Newly synthesized apo-AI was identified by reaction with antibody specific to apo-AI of plasma high density lipoprotein. The immunoreactive apo-AI made by peripheral tissues had the same mobility as plasma apo-AI or newly synthesized liver or intestinal apo-AI when examined by SDS-polyacrylamide gel electrophoresis (Figs. 2 and 3). Analysis by high resolution two-dimensional gel electrophoresis showed that newly synthesized liver apo-AI exists in four isoforms with the two most basic isoforms predominating. The isoform patterns of newly synthesized apo-AI from peripheral tissues were superimposable on the liver isoform patterns (Fig. 6). Mixing experiments indicated apparent identity in the isoform patterns of apo-AI made in liver, vein, kidney, and adipose tissue (Fig. 6). Apo-AI synthesis was detected in peripheral tissues using either [3H]leucine or [35S]methionine as precursor amino acid, and no apo-AI synthesis was detected in the presence of cycloheximide. These results provide strong evidence for the de novo synthesis of apo-AI by peripheral tissues.

Peripheral apo-AI synthesis was further confirmed by showing that the mRNA for apo-AI could be isolated from skeletal muscle and kidney as well as from liver and small intestine (Fig. 8). Translation of liver or intestinal mRNA in the wheat germ system yielded a cell-free product that was reactive with antibody against plasma apo-AI. As previously shown (46, 47), liver mRNA encodes an apo-AI precursor that is approximately 2000 daltons larger than mature apo-AI when examined by SDS-polyacrylamide gel electrophoresis (Fig. 8). This precursor form is believed to contain the signal peptide for the secretion of hepatic apo-AI (46, 47). Both skeletal muscle mRNA and kidney mRNA were shown to encode a product that was reactive with antibody against plasma apo-AI (Fig. 8), and these immunoreactive products had the same electrophoretic mobility as the apo-AI precursor directed by liver and intestinal mRNA. These results established the presence of apo-AI mRNA in adult skeletal muscle and kidney and suggest that these mRNAs encode a secretory precursor form of apo-AI.

The studies with differentiated chick embryo muscle cultures provide further evidence for the synthesis and secretion of apo-AI by peripheral tissues. These studies showed that chick embryo myotubes and fibroblasts synthesize apo-AI. Newly synthesized apo-AI did not accumulate intracellularly to a major extent but did accumulate extensively in the culture medium. This result suggests that apo-AI is made primarily for secretion from these cells. Analysis of newly secreted muscle cell apo-AI by high resolution two-dimensional gel electrophoresis showed the same isoform pattern as seen with newly synthesized apo-AI from adult liver or adult peripheral tissues. This result, as well as the reactivity of muscle cell apo-AI with antibody against adult plasma apo-AI, suggests that the apo-AI species made by adult and embryonic cells are very similar if not identical. The finding that apo-AI is made by tissues from arteries and veins (Figs. 2 and 3) also raised the possibility that the synthesis of apo-AI in peripheral tissues was due solely to vascular endothelium or other cells in the vascular network. The synthesis of apo-AI by cultured cells and, in particular, by pure myotube cultures suggests that vascular cells alone do not account for apo-AI synthesis.

Flotation studies indicate that at least some of the secreted apo-AI can associate with lipid. Analysis of muscle cell culture medium by density gradient ultracentrifugation revealed a substantial portion of newly synthesized apo-AI in fractions of density <1.21 g/ml, while the bulk of the labeled proteins was in the density range 1.21–1.22 g/ml, as expected for proteins not bound to lipid (Fig. 5 and Table II). Interestingly, the apo-AI is not found as a discrete band at 1.11 g/ml, indicating that newly secreted apo-AI is not a component of normal HDL particles. Further studies will be required to evaluate the lipoprotein character of newly secreted muscle cell apo-AI. It should be noted that these results do not establish whether the newly synthesized apo-AI in the density range <1.21 g/ml was secreted in association with lipid or became associated with exogenous lipids in the culture medium subsequent to secretion.

It is generally believed that HDL plays an important role in the movement of cholesterol from peripheral cells to the liver for metabolism and elimination. The mechanisms by which
Peripheral Apolipoprotein AI Synthesis

choleren leaves cells and the regulation of cellular cholos-
sterol eff lux, however, have remained elusive. Similarly, little
is known about the means by which extracellular cholesterol
is transported from interstitial fluid surrounding peripheral
cells to the vascular compartment for eventual delivery to the
liver. The present finding that many peripheral tissues syn-
thetize apo-AI suggests that peripheral apo-AI may play a
role in these processes. Several potential roles may be sug-
gested. First, apo-AI made in peripheral cells may serve to
transport cholesterol from the cell in the form of an apo-AI-
containing lipoprotein. Second, peripheral apo-AI may be
secreted as an apolipoprotein to serve as an extracellular
acceptor of cholesterol released from cell membranes. Pre-
vious studies have shown that human HDL and apolipo-
protein-lipid complexes can promote cholesterol efflux from
cultured cells (6-10). Fielding and Fielding (49) recently de-
scribed an apo-AI-containing lipoprotein in human plasma
that is largely responsible for promoting cholesterol efflux from
cultured cells. Interestingly, this lipoprotein appears to
contain only apo-AI and is a very minor component among all
apo-AI-containing lipoproteins. The possibility that the apo-
AI synthesized by peripheral chicken tissues corresponds to
this minor human apo-AI-containing lipoprotein is of clear
interest. A third potential role for peripheral apo-AI is as
an activator of lecithin:cholesterol acyltransferase. Since lec-
thin:cholesterol acyltransferase appears to be required for net
transfer of cholesterol from cultured cells to plasma lipopro-
tein acceptors (49), high local concentrations of apo-AI might
be essential to maintain maximal transferase activity in vivo.
Each of these possibilities might be advantageous to a periph-
eral cell in that the cell could regulate its cholesterol efflux
independently of the supply of HDL which reaches the cell
from the central vascular compartment. Similarly, local cell
to cell variation in the need to eliminate cholesterol could be
accommodated via intracellular regulation of apo-AI synthe-
sis. Further studies will be required to determine the precise
role of peripheral apo-AI in cholesterol metabolism.

Two-dimensional gel analysis showed that newly synthe-
sized apo-AI exists in at least four isoforms in each of the
tissues examined (Fig. 6). The four isoforms have similar
cationic properties in all tissues, with the two major isoforms
being the more basic of the four species. Plasma apo-AI also
shows four isoforms with charge properties identical with
tissue apo-AI isoforms (Fig. 7). In the case of plasma apo-AI,
however, the two major spots correspond to the more acidic
of the four species, suggesting that an acidic modification
occurs after synthesis or during circulation in the blood.
Similar differences between human plasma apo-AI and newly
synthesized intestinal apo-AI were observed by Zannis et al.
(50). Since the isoform pattern of secreted muscle apo-AI (Fig.
6D) is the same as newly synthesized liver apo-AI (Fig. 6B),
the acidic modification may be an extracellular event. It is in
not known whether the apo-AI isoforms have different functions
or to what extent this heterogeneity is due to post-transla-
tional modifications. Two-dimensional gel analyses of the apo-
AI made in cell-free translations, however, suggest that the
apo-AI isoprotein species are not generated solely by post-
translational events. Two major and one minor isoform were
synthesized in response to liver (Fig. 8A) and kidney (Fig. 8B)
RNA. Since post-translational modifications apparently do
not occur in the wheat germ cell-free system, the apo-AI
isoforms may differ in primary sequence. These isoform
charge differences could reflect very minor differences in
translation termination from a single apo-AI mRNA, multiple
mRNA species generated via RNA splicing events, or multiple
genomes for apo-AI. Analyses of cDNA clones to chicken apo-AI
are being carried out to distinguish among these possibilities.

REFERENCES

4. Gordon, T., Castelli, W. P., Hortland, M. C. Kannel, W. B., and
Biochim. Biophys. Acta 386, 106-118
Chem. 250, 7204-7209
9. Daniels, R. J., Guertler, L. S., Parker, T. S., and Steinberg, D.
sclerosis 23, 535-547
Invest. 61, 526-534
Sci. U. S. A. 74, 2569-2573
18. Glickman, R. M., Green, P. H. R., Lees, R. S., Lux, S. E., and
Kigore, A. (1979) Gastroenterology 75, 298-292
(1978) Gastroenterology 75, 677-682
Biophys. Res. Commun. 46, 1493-1498
22. Soutar, A. K., Garner, C. W., Baker, H. N., Sparrow, J. T.,
Biophys. Acta 400, 256-265
23. Fownell, H. R., Massey, J. B., Kusserow, S. K., and Gotto, A. M.
(1979) Biochemistry 18, 574-579
Biochim. Biophys. Acta 420, 342-349
Invest. 44, 1345-1353
Chem. 235, 10044-10051
34. Rubin, H. (1973) in Chicken Embryo Cells in Culture: Methods
and Applications (Krase, P., Jr., and Patterson, M. K., Jr., eds)
Acad. Sci. U. S. A. 75, 5974-5978
Operation of the ISO-DALT System, Argonne National Labo-
ratory Publication ANL-BIM-79-2, Argonne National Labo-
ratory, Argonne, IL
41. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter,
W. J. (1979) Biochemistry 18, 5294-5299
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