Hepatic apolipoprotein J is secreted as a lipoprotein

Bryan F. Burkey,* William D. Stuart,† and Judith A. K. Harmony[1,*,†]

Developmental Biology Program* and the Department of Pharmacology and Cell Biophysics,† University of Cincinnati, College of Medicine, Cincinnati, OH 45267-0575

Abstract Apolipoprotein J (apoJ) is a unique glycoprotein thought to be involved in a variety of physiological processes, including lipid transport, regulation of complement function, sperm maturation, programmed cell death, and membrane recycling. In the plasma, apoJ is associated with apoA-I in high and very high density lipoproteins. In this report we demonstrate that HepG2 human heptocellular carcinoma cells secrete apoJ in association with a significant amount of lipid, providing unequivocal evidence that apoJ can transport lipids. The HepG2 cell line has provided important clues about the structural organization of nascent lipoprotein particles. HepG2 cell apoJ-containing lipoproteins are dense and heterogenous in size, ranging from 100 to 910 kDa. Plasma and HepG2 cell apoJ-lipoproteins differ in size distribution. Both have α2 electrophoretic mobility, although their average mobilities differ within the α2 region. In contrast to plasma apoJ-HDL which contain little triglyceride and which can associate with apoA-I, HepG2 cell apoJ-lipoproteins are rich in triglyceride and lack apoA-I. By implication, nascent apoJ-lipoproteins undergo plasma remodeling that results in triglyceride depletion and apoA-I association. We propose that the metabolic consequences of this remodeling play an important role in lipid homeostasis in localized tissue environments, particularly where organs are isolated from the blood by cellular barriers such as in testis and brain. In such tissues, apoJ is expressed constitutively in high level compared to other lipid transport proteins.—**Burkey, B. F., W. D. Stuart, and J. A. K. Harmony.** Hepatic apolipoprotein J is secreted as a lipoprotein. *J. Lipid Res.* 1992. **33:** 1517–1526.

Supplementary key words nascent lipoproteins • HDL • HepG2 cells

Apolipoprotein J (apoJ), recently discovered (1–3) in human plasma in association with discrete but minor subclasses of high and very high density lipoproteins (HDL, VHDL), may link lipid metabolism and homeostasis with the immune response. The presence of apoJ in HDL implies a role of apoJ in lipoprotein metabolism. In addition to its association with lipoproteins, apoJ has been isolated from human plasma in association with soluble complexes of the terminal complement cascade components, C5b-9 (4). Its association with C5b-9 complexes implies a role in complement function and, in fact, apoJ is a potent inhibitor of complement-mediated cell lysis in vitro by interacting with C5b-7 to prevent activation of C8 and C9 (5, 6).

Potential roles in both lipid metabolism and complement function are substantiated by structural considerations. ApoJ and known apolipoproteins have in common the predicted amphipathic helices that are important in protein-lipid interactions (7, 8). Human apoJ has three such domains, one in Jα and two in Jβ (7). These domains are not, however, homologous with the 22-mer repeat that constitutes the amphipathic helices of members of the apolipoprotein gene family (9). Unlike other apolipoproteins but similar to certain coagulation and complement proteins, apoJ circulates as a disulfide-linked heterodimer for which the subunits, Jα and Jβ, are produced by proteolytic cleavage of the apoJ precursor (7, 8, 10). Each apoJ subunit is glycosylated via Asn, and carbohydrate accounts for 30% of the molecular mass. Moreover, apoJ shares some homology with complement components C7, C8, and C9, specifically within a Cys-rich motif in Jα (residues 75–98) (11).

Plasma apoJ-lipoproteins are spherical particles (1, 2) that have a bimodal distribution within HDL_2 and HDL_3+VHDL classes in the density range 1.16–1.25 g/ml (1). The apoJ-containing species are relatively poor in lipid: protein makes up 78–89%, and lipid 11–22% of the

Abbreviations: ACT, α1-antichymotrypsin; apo, apolipoprotein; BSA, bovine serum albumin; CETP, cholesteryl ester transfer protein; CBB, Coomassie brilliant blue; CLI, complement lysis inhibitor; DAG, dimeric acidic glycoprotein; EM, electron microscopy; FBS, fetal bovine serum; GGE, gradient gel electrophoresis; gp80, glycoprotein 80; HDL, high density lipoprotein; HRP, horseradish peroxidase; HTGL, hepatic triglyceride lipase; LDL, low density lipoprotein; LPL, lipoprotein lipase; MDCK, Madin Darby canine kidney; MEM, minimum essential medium; PAGE, polyacrylamide gel electrophoresis; PDB, plasma density buffer; PMSF, phenylmethylsulfonyl fluoride; SP-40,40, serum protein 40,40; SDS, sodium dodecyl sulfate; SGP-2, sulfated glycoprotein 2; TLC, thin-layer chromatography; TRPM-2, testosterone repressed protein message 2; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed at: Department of Pharmacology and Cell Biophysics, College of Medicine, University of Cincinnati, 231 Bethesda Avenue, Cincinnati, OH 45267-0575.

lipoprotein mass (1, 2). Of the lipids, phospholipid and cholesterol predominate; triglyceride accounts for about 1% of apoJ-HDL lipid (1, 2). The major proteins are apoJ and apoA-I; the mole ratio of apoJ:apoA-I present in affinity-purified apoJ-HDL is 5:1 (12). The apoA-I associated with apoJ represents only 2–4% of the total apoA-I in plasma (1, 2) and is tightly associated with apoJ, requiring nonionic detergents for dissociation (2).

The origin of blood apoJ-HDL, present at ~10 mg/dl (11, 13), is not known. The relatively high abundance of apoJ mRNA in hepatocytes (7) combined with the large size of the liver predict that the liver, rather than other organs that also express apoJ mRNA (7), is the source of the circulating pool of apoJ. Given apoJ's homology to both apolipoproteins and complement components, it is important to determine if the protein is secreted in association with lipid, as other apolipoproteins, or if it binds lipids after secretion, as other complement components. To address these alternatives, we used human hepatocellular carcinoma HepG2 cells (10) to compare the newly secreted apoJ species with apoJ-HDL isolated from human plasma. Our results demonstrate that apoJ is secreted by HepG2 cells as a lipoprotein. The secreted apoJ-lipoproteins are heterogeneous particles that have an average lipid composition significantly different from that of plasma apoJ-HDL. Moreover, apoA-I is not associated with the secreted apoJ-lipoproteins, implying independent pathways of apoJ and apoA-I secretion. Taken together, the data suggest that nascent apoJ-lipoproteins undergo remodeling to apoJ-HDL upon entering the circulation.

EXPERIMENTAL PROCEDURES

Cell culture

The human hepatoblastoma-derived cell line, HepG2, was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were grown in Eagle's minimal essential medium (MEM, Whittiker Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum (FBS, Hazelton, Lenexa, KS) and 4 mM L-glutamine (Sigma Chemical Co., St. Louis, MO) at 37°C in a humidified incubator with 5% CO_2–95% air in 75-cm^2 tissue culture flasks (Costar, Cambridge, MA). For passage, cells were harvested with 0.25% trypsin and reseeded at a density of 3×10^6 per flask. Experiments were initiated when cell monolayers became 90% confluent.

Metabolic labeling of cell protein and lipid

Newly synthesized HepG2 cell proteins were metabolically labeled with 500 μCi of [^{35}S]methionine (<1,200 Ci/mmol, Amersham, Arlington Heights, IL) in 4.5 ml of medium as previously described (10). Culture medium, maintained at 4°C, was centrifuged at 2000 *g* for 15 min to remove cellular debris. HepG2 cell lipids were labeled by the incorporation of [^{14}C]acetate (56 mCi/mmol, Amersham) into hydrocarbon chains and sterol groups. Cell monolayers were washed twice with serum-free MEM, then incubated for 18 h in 6 ml of serum-free MEM containing 80 mM bovine serum albumin (BSA). The medium was replaced with fresh medium containing 7.5 μCi of [^{14}C]acetate per ml. After 6 h the culture medium was collected, cell debris was removed by centrifugation at 2000 *g* for 15 min, and the labeled lipids were analyzed. For analysis of lipid and protein mass, parallel cultures were established in the absence of radioisotope.

Analysis of secreted proteins

Secreted HepG2 cell proteins were analyzed by molecular exclusion chromatography, agarose electrophoresis, and gradient gel electrophoresis (GGE). For molecular exclusion chromatography, culture medium (2 ml) from [^{35}S]methionine-labeled (6 h) cells was immediately fractionated on a Bio-Gel A5-m column (110 × 1 cm, Bio-Rad, Rockville Centre, NY), equilibrated, and eluted at 0.28 ml/min with plasma density buffer (PDB, 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM Na_2EDTA, and 0.02% NaN_2). The column was calibrated with very low density lipoproteins (VLDL, d 1.006–1.019 g/ml), dextran blue 2,000 (Pharmacia, Piscataway, NJ), thyroglobulin (Sigma), BSA (Sigma), and phenol red (Sigma). Fractions of 1.8 ml were collected and analyzed for total protein, apoJ, and apoA-I. Total protein was precipitated from 50 μl of sample adjusted to 10% trichloroacetic acid (TCA, Sigma). Protein pellets were washed at 4°C in acetone, then resuspended in 200 μl of sample buffer (20 mM Tris-HCl, pH 6.8, 2% SDS, 10% sucrose, 4 M urea, and 2 mM Na_2EDTA) by heating to 60°C for 2 h with occasional vortexing. ApoJ and apoA-I were immunoprecipitated from 2.5 ml and 0.5 ml of pooled column fractions, respectively, and analyzed as previously described (10).

For agarose electrophoresis, HepG2 cell-conditioned medium was concentrated 50-fold to a protein concentration of 20–30 mg/ml by ultrafiltration (Amicon, Beverly, MA). To obtain human plasma, blood (1 ml) from healthy fasted male donors was collected into 2 mM EDTA and immediately transferred to a microfuge tube containing preservatives and protease inhibitors (10 μg/ml of α2-macroglobulin, 0.01% NaN_2, 1 mM benzamidine, and 25 KU/ml aprotinin). After centrifugation at 4°C to remove red blood cells, phenylmethanesulfonyl fluoride (PMSF, final concentration, 1 mM) was added. HepG2 cell-conditioned medium or plasma was loaded onto a 1% agarose gel (Ciba Corning Diagnostics Corp., Palo Alto,

CA). Electrophoresis was performed at 10°C in 50 mM barbital buffer, pH 8.6, containing 1 mM Na_2EDTA in a Bio-Rad flatbed electrophoresis apparatus operated at 200 v until the marker dye, bromophenol blue, had migrated 4 cm (about 30 min). Proteins were transferred to PVDF paper (Bio-Rad) by press-blotting for 1 h. The blot was incubated overnight with primary antibodies in BLOTTO (14). Protein was stained with 0.2% Coomassie brilliant blue (CBB). ApoJ was visualized with rabbit antiapoJβ (10), diluted 1/500, followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (HRP), diluted 1/4000; apoA-I, with sheep antiapoA-I-HRP (The Binding Site, San Diego, CA), diluted 1/3000.

For GGE (15), plasma or concentrated HepG2 cell-conditioned medium was applied to a 4–30% precast polyacrylamide gel (Pharmacia LKB), and the gradient gels were electrophoresed in Tris-borate buffer at 6°C for 1800 v-h. Gels were soaked for 2 h in blotting buffer containing 0.5% SDS, rinsed for 5 min in blotting buffer, and transblotted at 200 mA constant current onto PDVF paper (Bio-Rad) for 21 h at 4°C. The blots were temporarily stained with Ponceau S and native standards were marked. ApoJ and apoA-I were visualized as described above. Apparent molecular weights were determined, based on a standard curve generated by plotting log (molecular weight) versus migration distance of the native standards. Vézina et al. (16) have reported that GGE conditions similar to these underestimate the sizes of lipoprotein particles.

Affinity isolation of apoJ-lipoproteins

Monoclonal antibody mAb11, specific for apoJ, was covalently attached to AffiGel (1) and the resulting 2-ml column was used to purify apoJ from plasma and HepG2 cell-conditioned medium. Freshly drawn human plasma (5 ml, diluted 1:3 with phosphate-buffered saline) containing protease inhibitors (25 kallikrein units/ml of aprotinin, 1 mM benzamidine, 1 mM PMSF, and 0.02% NaN_2) or HepG2 cell-conditioned medium (10 ml obtained from cells labeled for 6 h with [^{35}S]methionine) was applied to the immunoaffinity column. Unbound proteins were washed from the column with 300 bed-volumes of PDB. Bound proteins were eluted with 10 bed-volumes of 1 M acetic acid, dialyzed into 10 mM NH_4HCO_3, and concentrated by ultrafiltration (Amicon). Proteins were resolved on a 10% acrylamide gel containing SDS, and visualized with CBB or radioautography, as appropriate.

Immunoprecipitation of nascent HepG2 cell lipoproteins

Apolipoproteins secreted by HepG2 cells, cultured with [^{35}S]methionine or [^{14}C]acetate, were isolated by nondenaturing immunoprecipitation. One ml of medium was incubated at 4°C overnight (gentle rocking) with protein A-AffiGel (15 μl) and primary antibody: antiapoA-I, antiapoE, antiapoJβ, or antiα-1-antichymotrypsin (ACT, Calbiochem, San Francisco, CA). The protein A-Affigel-antibody complexes were pelleted by centrifugation for 2 min at low speed, and the pellets were washed twice with PDB, transferred to new microfuge tubes, and washed three more times with PDB. Proteins were resolved by SDS-PAGE, visualized by autoradiography, and quantitated. Lipids were analyzed as described below.

Analysis of lipoprotein lipids

The [^{14}C]acetate-labeled lipids associated with immunoprecipitated proteins were extracted and separated by thin-layer chromatography (TLC). The protein A-AffiGel immunoprecipitates were transferred to 13 × 100 mm screw-cap glass tubes (Teflon caps) and resuspended in 400 μl of PDB. Lipids were extracted with 2 ml of chloroform–methanol 2:1 (v/v), and the aqueous phases were extracted with 2 ml of chloroform–methanol 2:1 (v/v). The solvent phases were combined, washed once with 1 ml of H_2O, then dried at 37°C under a stream of nitrogen and resuspended in 200 μl of chloroform–methanol 9:1 (v/v). Lipids were resolved on silica gel G plastic-backed TLC plates in a solvent system of heptane–diethyl ether–acetic acid 85:15:1 (v/v) and visualized by iodine vapor. Bands corresponding to authentic lipid standards (Sigma) were excised and their radioactivity was determined by scintillation counting in 10 ml of scintillation fluid (4a20, Research Products International, Mt. Prospect, IL).

Cholesterol and triglycerides were determined chemically by enzymatic methods on an Hitachi 737 (17), using serum calibrators provided by the CDC. Phospholipid was quantitated by the method of Bartlett (18).

Density ultracentrifugation

HepG2 cells were labeled for 6 h with [^{35}S]methionine, and the culture medium was collected and centrifuged at 2,000 *g* for 15 min to remove cellular debris. Two ml of medium, raised to a density of 1.25 g/ml with solid KBr, was layered under 2.5 ml of 1.25 g/ml PDB-KBr in a polypropylene centrifuge tube, and centrifuged for 24 h at 45,000 rpm in a Beckman 50.3Ti rotor. The tube was sliced, and fractions corresponding to the upper one third and the lower two thirds were collected. Each fraction was dialyzed against PDB and stored at 4°C for immunoprecipitation of apoJ.

RESULTS

HepG2 apoJ is secreted as high molecular weight species

To determine the sizes of apoJ and/or apoJ-containing macromolecular complexes secreted by HepG2 cells, culture medium from [^{35}S]methionine-labeled HepG2 cells was analyzed by molecular exclusion chromatography.

The elution profile of total secreted HepG2 cell protein, precipitated from column fractions by TCA, is shown in **Fig. 1** (square symbols). Most of the radiolabeled proteins eluted in fractions 35–45, corresponding to 20–167 kDa; the remainder eluted in fractions 24–34, corresponding to 167–2100 kDa. The elution profiles of apoJ (△) and apoA-I (○), determined by immunoprecipitation, were compared. ApoA-I eluted with two distinct size distributions: large species in fractions 32–38 (110–390 kDa) and smaller species in fractions 38–44 (31–110 kDa). These results are consistent with findings of Cheung and Albers (19) who defined two similar size ranges for HepG2 cell HDL by using nondenaturing GGE and electron microscopy (EM). In contrast to apoA-I, apoJ eluted as a single broad peak in fractions 28–38 (100–910 kDa), with the majority in fractions 32–34 (250–400 kDa). Although there was significant overlap in the elution profiles of apoA-I and apoJ (Fig. 1, insert), much of the apoJ was associated with particles larger than those containing the majority of the apoA-I.

HepG2 cell apoJ- and apoA-I-containing species were also evaluated by nondenaturing GGE followed by immunostaining (**Fig. 2**). The predominant apoA-I-containing species secreted by HepG2 cells were < 70 kDa, with less abundant species of 100–400 kDa. The apparent sizes of HepG2 cell apoA-I-containing species determined by GGE agreed reasonably well with those determined by molecular exclusion chromatography. GGE has been reported to underestimate the sizes of lipoprotein particles (16). Secreted HepG2 cell apoA-I-containing species had a size distribution by GGE that was different from that of plasma apoA-I-containing species which were predominantly 100–300 kDa. HepG2 cell apoJ was distributed between 100–1000 kDa, with the majority concentrated between 240–300 kDa and 450–650 kDa, also in agreement with the results obtained by molecular exclusion chromatography. The size distribution of HepG2 cell and human plasma apoJ contrasted markedly, however. The majority of plasma apoJ was 70–90 kDa with very minor higher molecular weight species of 250 and 500 kDa. This range of molecular weights of plasma apoJ-lipoproteins is similar to that reported previously (1), except that the predominant species are smaller. ApoJ-lipoproteins in plasma increase in apparent

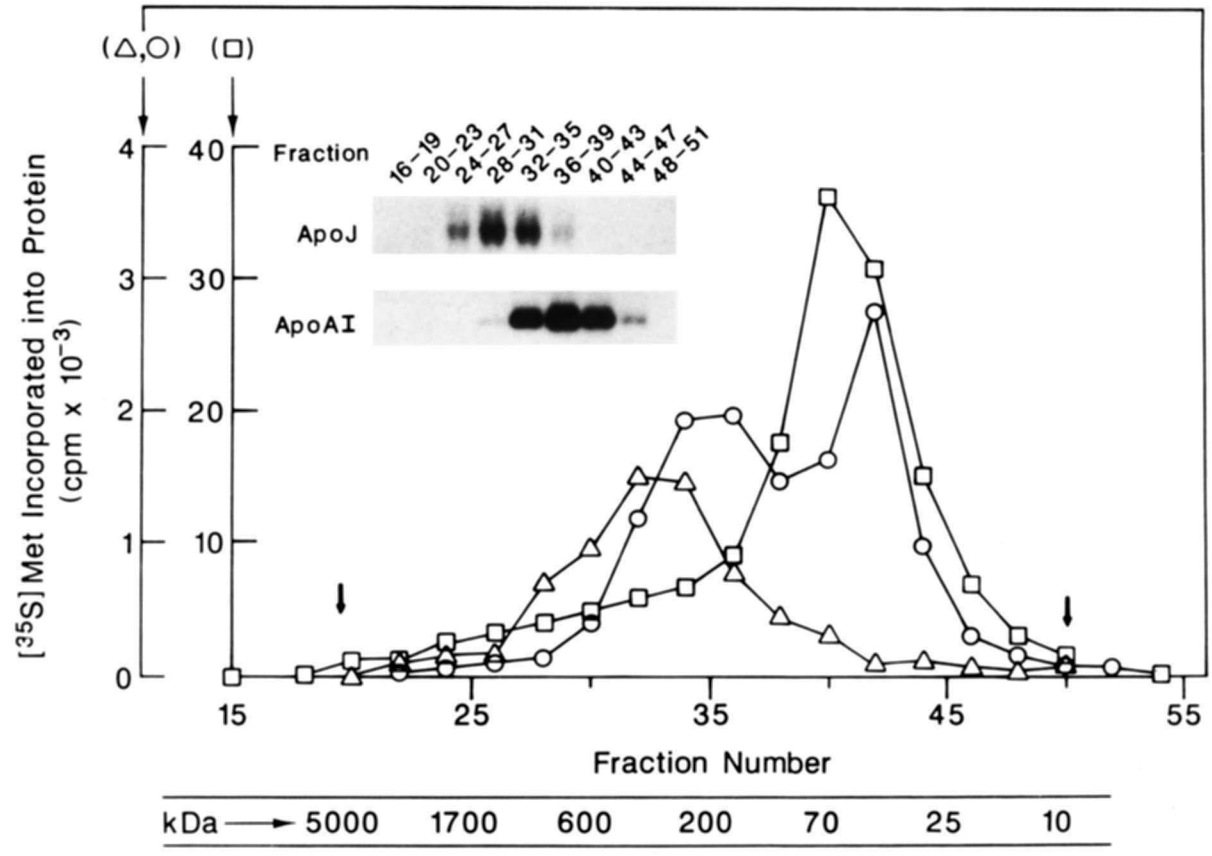

Fig. 1. ApoJ-containing species secreted by HepG2 cells are 100–900 kDa, based on molecular exclusion chromatography. Cell-conditioned medium was chromatographed on a Bio-Gel A5-m column, and 1.8-ml fractions were collected. The elution profile for total [^{35}S]methionine-labeled protein (-□-) was determined by TCA precipitation. ApoJ (-△-) and apoA-I (-○-) were immunoprecipitated and analyzed by SDS-PAGE followed by autoradiography (insert). The column was calibrated as indicated.

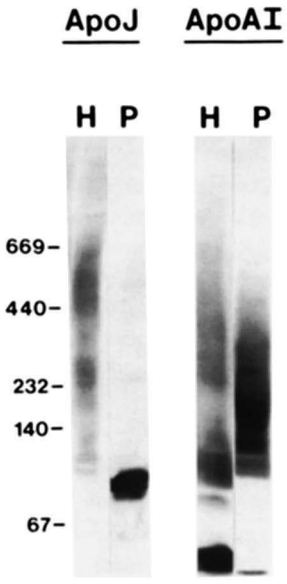

Fig. 2. HepG2 cell apoJ-containing species are larger and more heterogeneous than plasma apoJ-containing species. Proteins from HepG2 cell medium (24 h) (H) and from human plasma (P), 200 μg per lane, were resolved on a 4–30% nondenaturing gel. Albumin, lactate dehydrogenase, catalase, ferritin, and thyroglobulin were the molecular mass standards; their kDa values are shown at the right. The proteins were electroblotted onto nitrocellulose and incubated with antiapoJ or antiapoA-I antibodies as described in Experimental Procedures.

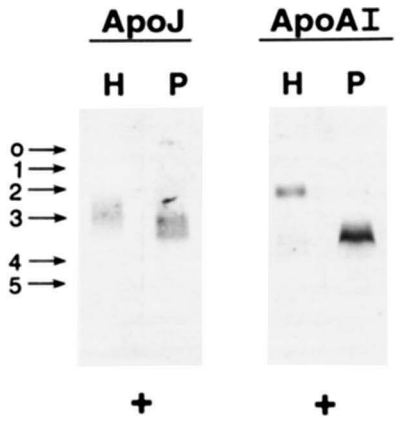

Fig. 3. HepG2 cell apoJ and human plasma apoJ have similar electrophoretic properties. Plasma (P, 2 μl–120 μg) or concentrated HepG2 cell-conditioned medium (H, 2 μl–46 μg) was analyzed by agarose electrophoresis as described in Experimental Procedures. Protein was transferred to PVDF paper by press blotting. ApoJ and apoA-I were visualized by immunostaining, as outlined in Experimental Procedures. Major plasma proteins were identified by CBB staining (48): 0 = margin, 1 = fibrinogen, 2 = transferrin (β_1 mobility), 3 = α2-macroglobulins, 4 = α1-antitrypsin, 5 = albumin.

size with even brief (e.g., 24 h) plasma storage (12). HepG2 cell apoJ and apoA-I overlapped, particularly in the 100–400 kDa range, in agreement with molecular exclusion chromatographic results.

The electrophoretic mobilities of HepG2 cell and plasma apoJ and apoA-I were compared by agarose electrophoresis, and the results are shown in **Fig. 3.** Although a minor fraction of HepG2 cell apoA-I migrated to the α position, typical of plasma apoA-I, the majority was preβ. Both HepG2 cell and plasma apoJ had electrophoretic mobilities in the α2 region, although HepG2 cell apoJ migrated slightly behind the α2 position of plasma apoJ but ahead of the preβ position of HepG2 cell apoA-I. There was overlap between the leading edge of the HepG2 cell apoA-I and the trailing edge of the apoJ.

Immunoaffinity-purified HepG2 apoJ is not associated with apoA-I

The overlap of HepG2 cell apoJ and apoA-I that occurred after three different separation procedures supports the possibility that HepG2 cells can secrete particles comprised of both apoJ and apoA-I that may be comparable in macromolecular structure to that of plasma apoJ/apoA-I-lipoproteins (1–3). To examine this possibility further, we asked if apoA-I were associated with apoJ isolated from HepG2 cell medium by immunoaffinity chromatography, using an apoJ-specific antibody (1). The protein composition (**Fig. 4**) of the eluate fraction (lane 1) was compared by SDS-PAGE, under reducing conditions, to that obtained by immunoaffinity chromatography of human plasma (lane 2). The small molecular mass difference between HepG2 cell and human plasma apoJ α and

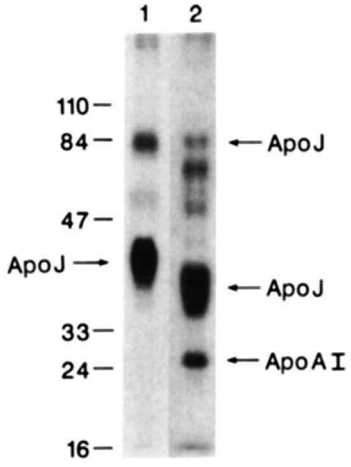

Fig. 4. ApoA-I is associated with apoJ immunoaffinity-purified from human plasma but not from HepG2 cell-conditioned medium. ApoJ was isolated by immunoaffinity chromatography from [^{35}S]methionine-labeled HepG2 cell culture medium (lane 1) or from human plasma (lane 2) as described in Experimental Procedures. Proteins were resolved by SDS-PAGE (11% acrylamide), the gel was cut and proteins were visualized by either autoradiography (lane 1) or CBB staining (lane 2). The positions of apoJ and apoAI are indicated at the right; the molecular mass markers in kDa, at the left.

β subunits, present in the 35–45 kDa region (lane 1 vs. 2), is likely the result of a difference in carbohydrate content (10). ApoJ immunoaffinity-purified from human plasma was associated with apoA-I, as reported previously (1). In contrast, eluate obtained from HepG2 cell medium contained only apoJ with no detectable apoA-I present. Even after long exposure of the gel to X-ray film, there was no detectable apoA-I.

The absence of apoA-I from apoJ preparations immunoaffinity-purified from HepG2 cell-conditioned medium was unexpected. To evaluate the possibility of an apoJ–apoA-I interaction by an alternative method, a nondenaturing immunoprecipitation protocol was developed. The protein compositions of macromolecular complexes secreted by HepG2 cells, isolated on the basis of their apoE, apoA-I, or apoJ, were compared by SDS-PAGE under reducing conditions (**Fig. 5**). To validate the system, we confirmed that the HepG2 cells secreted apoA-I-lipoproteins that also contain apoE. Antiserum specific for apoA-I (lane 5) immunoprecipitated both apoA-I and apoE; moreover, antiserum specific for apoE (lane 4) immunoprecipitated apoA-I in addition to apoE. The mole ratio of apoA-I:apoE in the HepG2 cell apoA-I-containing lipoproteins isolated by nondenaturing immunoprecipitation was 10:1, a result that agreed well with the 7:1 ratio reported by Cheung et al. (20). In contrast to the association of apoA-I with apoE in HepG2 cell HDL, immunoprecipitated apoJ-containing species contained an insignificant amount of apoA-I (Fig. 5, lane 3), although HepG2 cells secreted apoA-I in 25-fold molar excess of apoJ. The minor amount of apoA-I in apoJ immunoprecipitates was attributed to nonspecific binding of apoA-I to the secondary antibody. Similar amounts of [^{35}S]apoA-I were evident in the secondary antibody control (lane 1) and in the immunoprecipitate of α-1-antichymotrypsin (ACT) (lane 6), a protein that is not associated with lipoproteins. The length of time that HepG2 cells were incubated with [^{35}S]methionine did not influence the results: no apoA-I was specifically immunoprecipitated with apoJ isolated from 2-, 6-, or 24-h culture medium.

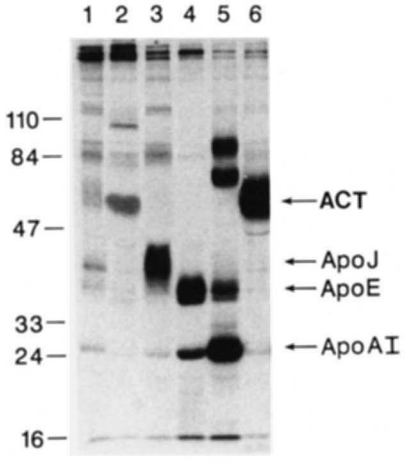

Fig. 5. Secreted HepG2 cell apoJ is not associated with apoA-I or apoE. [^{35}S]Methionine-labeled proteins were isolated from HepG2 cell-conditioned medium by nondenaturing immunoprecipitation, and analyzed by SDS-PAGE under reducing conditions. The antisera used to isolate the proteins were: lane 1, secondary control protein A-AffiGel alone; lane 2, preimmune control; lane 3, antiapoJ; lane 4, antiapoE; lane 5, antiapoA-I; and lane 6, antiACT. The resolved proteins, visualized by autoradiography, were isolated from 1 ml of medium, except for ACT which was isolated from 0.5 ml. Positions corresponding to apoJ, apoA-I, apoE, and ACT are indicated at the right; molecular mass markers in kDa, at the left.

Lipids are associated with HepG2 cell apoJ

To determine whether apoJ secreted by HepG2 cells is complexed with lipid, HepG2 cells were cultured with [^{14}C]acetate (21) to label cellular lipids prior to isolation of apoJ. Lipids associated with apoJ were evaluated relative to those associated with apoE and apoA-I, and to those associated with the control protein, ACT. Lipids, extracted from immunoprecipitates, were separated by TLC and values of each major lipid class were determined as dpm of lipid per fmol of immunoprecipitated protein.

The negative control, ACT, had very little associated lipid, and the values were considered to represent background. ApoA-I and apoE were both associated with lipid. ApoE was complexed with the highest amount of lipid, with triglyceride (7,600 dpm/fmol) and phospholipid (5,000 dpm/fmol) predominating over cholesterol (3,100 dpm/fmol) and cholesteryl ester (1,400 dpm/fmol). ApoA-I was complexed with only minor amounts of lipid: 500 dpm triglyceride, 800 dpm phospholipid, 300 dpm cholesterol, and 150 dpm cholesteryl ester per fmol of protein. ApoJ was associated with significant triglyceride (2,800 dpm), unesterified cholesterol (1,900 dpm), and phospholipid (1,300 dpm), and a minor amount of cholesteryl ester (600 dpm) per fmol. Based on radioactivity, the quantities of lipids associated per mol of apoJ were approximately one-third of those associated with apoE and 2–6 times greater than those associated with apoA-I. Based on the specific activity of secreted phospholipid, total cholesterol, and triglyceride, the approximate molar lipid composition of the immunoprecipitated lipoproteins was calculated (**Table 1**). The lipid composition of HepG2 cell apoA-I-lipoproteins (54 mol of phospholipid, 29 of cholesterol, and 17 of triglyceride per mol of apoA-I) was in reasonable agreement with that reported by Cheung et al. (20).

The significant amount of lipid associated with apoJ secreted by HepG2 cells and the size heterogeneity of the secreted product predict that the apoJ-containing particle would have a range of buoyant densities, a prediction tested by density ultracentrifugation. Cell-conditioned medium was centrifuged at density 1.25 g/ml, the density

TABLE 1. Lipid composition of HepG2 cell lipoproteins isolated by immunoprecipitation

Protein Precipitated	Phospholipid	Cholesterol	Triglyceride
		mol lipid/mol protein	
ACT	24	13	16
ApoE	1391	368	464
ApoA-I	223	34	29
ApoJ	367	233	179

The lipid composition of medium conditioned by HepG2 cells and the radioactivity of each lipid class was determined, as outlined in Experimental Procedures. Based on the specific activity of secreted lipid, the molar composition of lipids associated with each immunoprecipitated protein was calculated.

that separates typical plasma lipoproteins from poorly lipidated and nonlipidated proteins. The d < 1.25 and d > 1.25 g/ml fractions were analyzed for apoJ by immunoprecipitation under denaturing conditions. As is evident in **Fig. 6,** only the d > 1.25 g/ml fraction contained HepG2 cell apoJ.

ApoJ-lipoproteins were substantially enriched in triglyceride, relative to that associated with apoA-I-lipoproteins. In an attempt to determine whether the triglyceride content of HepG2 cell apoJ-HDL is responsive to cellular triglyceride content, HepG2 cells were fed oleic acid (0.8 mM, associated with 0.16 mM BSA) in the presence of tritiated glycerol. There was a 6.3-fold increase in the amount and a 36-fold increase in the radioactivity of intracellular triglyceride which was not accompanied by a significant change in the content or radioactivity of cholesterol. There was, however, a modest 40% increase in phospholipid content. In spite of the large increase in triglyceride, there was no consequent effect on the total amount of triglyceride secreted over a 6-h period: 24% and 4.6% increase in mass and radioactivity, respectively. Nor was there an increase in phospholipid or cholesterol secretion. The amount of triglyceride associated with immunoprecipitated apoJ, apoE, apoA-I, and apoB was also unchanged. Thus, HepG2 cells did not provide information about the possibility that the amount of apoJ-associated triglyceride is responsive to the size of the cellular triglyceride pool. Although the usefulness of HepG2 cells in studies of triglyceride-loading and lipoprotein secretion is indicated (21–24), our results are consistent with those of other investigators (25, 26).

DISCUSSION

ApoJ mRNA is present in moderate-high abundance in liver as well as in most other tissues, notably brain, stomach, testis, ovary, kidney, heart, spleen, and thymus (7, 8). However, the gene does not appear to be expressed in the adult intestine or lung (7). The broad tissue distribution of apoJ expression, coupled to the low levels of lipids associated with apoJ in the circulation (1–3), raise the possibility that, in spite of its amphipathic domains (7, 27), apoJ's primary function may not be lipid transport. ApoJ and its homologs are thought to participate in biological functions other than lipid transport and complement regulation (**Table 2**). ApoJ is particularly abundant in the male reproductive tract where it is secreted by testicular Sertoli cells and epididymal epithelium (28). In rodents, apoJ becomes bound to the heads and distal tails of spermatozoa, suggesting its participation in sperm maturation (28, 29). ApoJ can also bind to membranes of other cells, notably adrenal chromaffin cells (30) and erythrocytes (31), where it has been implicated in the processes of membrane retrieval (32) and cell–cell association, respectively. In addition, apoJ is up-regulated in tissues undergoing programmed cell death and degeneration (33).

In light of the diversity of functions proposed for apoJ, the results of our work reported here are significant. They establish the lipoprotein nature of apoJ secreted by HepG2 cells, and provide support for a role of apoJ in lipid transport. Although a long-term line of hepatocellular carcinoma origin, HepG2 cells have served as an important model in delineating the processes responsible for the production and post-translational processing of liver-specific proteins, and for the regulation of these processes (10, 34, 35). This is particularly true of apolipoprotein secretion (22–26, 36). Based on an analysis of HepG2 cell

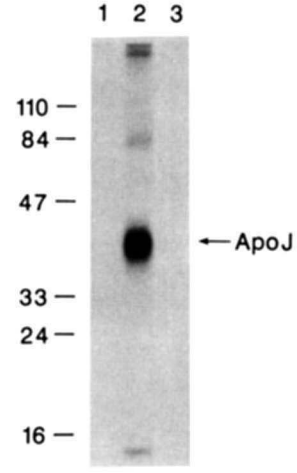

Fig. 6. HepG2 cell apoJ-lipoproteins have hydrated densities >1.25 g/ml. HepG2 cells were labeled with [^{35}S]methionine for 6 h and lipoproteins were isolated from cell-conditioned medium by continuous centrifugation at d 1.25 g/ml. ApoJ was immunoprecipitated from the d <1.25 g/ml top fraction (lane 1) and the d >1.25 g/ml bottom fraction (lane 2). Preimmune serum was used as a control with the d > 1.25 g/ml bottom fraction (lane 3).

TABLE 2. ApoJ homologs

Name	Species	Comments	References
Apolipoprotein J	human	HDLassociated protein	1
NA1/NA2	human	HDL-associated protein	3
SP-40,40,CLI	human	complement cell lysis inhibitor	4, 11, 41
TRPM-2	rat	programmed cell death	33, 42
SGP-2, DAG	rat	reproductive tract; Sertoli cell secretory protein	28, 43
S45-S35	rat	sperm binding protein	44
Clusterin	ram	cellular aggregation	31, 45
gp80	dog	MDCK apical secretory protein	46
Glycoprotein III	cow	adrenal medullary chromaffin granule secretory protein	30, 32, 47

apoJ, we conclude that apoJ is a secreted lipid transport protein associated with all of the major lipid classes that characterize other plasma lipoproteins: phospholipid, unesterified and esterified cholesterol, and triglyceride. The amount of lipid associated with HepG2 cell apoJ-lipoproteins is greater than that of apoA-I-lipoproteins but less than that of apoE-lipoproteins.

ApoJ-lipoproteins secreted by HepG2 cells share properties with those circulating in the blood, although there are notable differences. Both HepG2 cell and plasma apoJ-lipoproteins are dense (>1.25 g/ml) lipoproteins when analyzed by density ultracentrifugation. The low lipid content of plasma apoJ-HDL is consistent with its high hydrated density; however, the high density of HepG2 cell apoJ-lipoproteins was unexpected. It is possible that nascent apoJ-lipoproteins are unstable when exposed to concentrated salt solution and/or subjected to ultracentrifugation. While heterogeneity is typical of plasma apoJ-lipoproteins (1), HepG2 cell apoJ-lipoproteins show even greater size diversity. When subjected to molecular exclusion chromatography, both plasma (2, 3) and HepG2 cell apoJ-containing species elute in a broad peak at the leading edge of plasma HDL. However, within this 100–910 kDa range the majority of HepG2 cell apoJ-lipoproteins are considerably larger than plasma apoJ-HDL. In addition, while HepG2 cell apoJ-containing species and plasma apoJ-HDL both have $\alpha 2$ electrophoretic mobility, the average HepG2 cell species have reduced electrophoretic mobility. Plasma and HepG2 cell apoJ-lipoproteins also differ in apolipoprotein association. A fraction of plasma apoJ-HDL have apoA-I associated (1–3, 12) (1–3), whereas HepG2 cell apoJ-lipoproteins do not.

ApoJ-lipoproteins secreted into the blood may circulate without further modification. Alternatively, they may be secreted as species quite different in macromolecular structure and composition from those in plasma, and be converted to the plasma species by plasma enzymes and/or lipid transfer proteins. The differences in overall size and in lipid: protein ratios between HepG2 cell and plasma apoJ-lipoproteins suggest that HepG2 cells secrete "nascent" apoJ-lipoproteins that lose lipid, particularly triglyceride, and accumulate apoA-I as they circulate. Alternatively, circulating lipoproteins comprised of both apoJ and apoA-I may be derived from tissues other than the liver. Consistent with the concept of apoJ-lipoprotein remodeling is the finding that purified apoJ, added to apoJ-deficient plasma, binds apoA-I (12). We therefore propose that metabolic remodeling of nascent apoJ-lipoproteins occurs and requires plasma components for apoJ/apoA-I-HDL to be formed. This hypothesis is based on the absence of an apoJ-apoA-I interaction in HepG2 cell medium, in spite of the opportunity for apoJ to bind apoA-I. ApoA-I and apoJ have similar rates of production and secretion by HepG2 cells (10). Moreover, apoA-I is present in the cell-conditioned medium at a level 25 times that of apoJ. There is precedent for the model of post-secretion interaction of apoA-I with apoJ. For example, little apoA-IV and lecithin:cholesterol acyltransferase (LCAT) are associated with nascent apoA-I-containing lipoproteins secreted by HepG2 cells (20), yet both proteins are associated with apoA-I-containing HDL in plasma (37, 38).

The role of apoJ-lipoproteins in whole body lipid metabolism and homeostasis is unknown at present. The possibility that apoJ may bind apoA-I after entering the circulation leads to speculation about the circumstances of an interaction between them. The apoA-I that binds to apoJ-lipoproteins may be lipid-deficient apoA-I derived from several sources: secreted from hepatocytes or shed from triglyceride-rich lipoproteins as a consequence of lipoprotein lipase (LPL)- or hepatic triglyceride lipase (HTGL)-mediated triglyceride metabolism (39, 40). This latter potential source is intriguing in light of the positive correlation between plasma triglyceride and apoJ levels (13). Since high levels of triglyceride-rich lipoproteins and increased lipolysis can result in a rise in the level of lipid-deficient apoA-I, apoJ may serve as a "sink" for apoA-I shed from these lipoproteins. The associated apoA-I has the potential to alter the half-life of apoJ-lipoproteins in

the circulation by stabilizing them or by blocking their uptake and clearance, with a consequent increase in apoJ levels.

Although the lipid compositions of plasma apoJ-HDL and nascent HepG2 apoJ-lipoproteins are similar in that both contain significant phospholipid and cholesterol, HepG2 cell apoJ-lipoproteins contain significantly more triglyceride. HepG2 cell apoJ-lipoproteins secreted over a period of 6 h have a phospholipid:triglyceride ratio (2:1), similar to that of apoE-lipoproteins (3:1) but significantly greater than that of apoA-I-containing lipoproteins (7–8:1). Taken together, these findings suggest that apoJ is secreted by the liver as a triglyceride-rich lipoprotein that is transformed during circulation to a triglyceride-poor species. The corollary of this suggestion is the prediction that the triglyceride in nascent apoJ-lipoproteins is a substrate for LPL, HTGL, and/or cholesteryl ester transfer protein (CETP).

HDL are a heterogenous class of lipoproteins whose roles in lipid and lipoprotein metabolism are not fully understood. Different HDL subclasses can have distinct physiologic functions, e.g., acceptors of unesterified cholesterol from extrahepatic tissue, modulators of LCAT activity, donors and acceptors of neutral lipids transferred by CETP, or sources of HDL-cholesterol for liver or steroidogenic tissues. Even less well understood are the origins and metabolic pathways of the various HDL subclasses. ApoJ defines a new subclass of HDL that may be involved in such diverse processes as immunoregulation, programmed cell death, spermatogenesis, and neurodegeneration. ApoJ's basic lipid transport function may underlie its capacity to participate in these processes. The integration of potential apoJ functions with its wide tissue distribution and the complete elucidation of its macromolecular structure will aid in developing an understanding of the role of apoJ within and external to the plasma compartment. Clearly, the liver is an important source of circulating apoJ-HDL. A comparison of HepG2 cell apoJ-lipoproteins with apoJ secreted by tissues other than the liver should provide evidence in support of or contradictory to the importance of a basic localized lipid transport function of apoJ in all tissues in which it is expressed.

We appreciate the expert technical assistance of Ms. Terri Berning who prepared the manuscript and Mr. Gene Fellows who prepared the figures. We wish to thank Mr. Thomas Brown for providing the rabbit antiapoE and Dr. Sarah Jenkins for quantitating lipid mass. This project was supported by NIH grant HL22619. BFB was a Predoctoral Trainee sponsored by NIH training grant HL07527.

Manuscript received 2 April 1992 and in revised form 15 May 1992.

REFERENCES

1. de Silva, H. V., W. D. Stuart, C. R. Duvic, J. R. Wetterau, M. J. Ray, D. G. Ferguson, H. W. Albers, W. R. Smith, and J. A. K. Harmony. 1990. A 70-kDa apolipoprotein designated apoJ is a marker for subclasses of human plasma high density lipoproteins. *J. Biol. Chem.* **265:** 13240–13247.
2. Jenne, D. E., B. Lowin, M. C. Peitsch, A. Bottcher, G. Schmitz, and J. Tschopp. 1991. Clusterin (complement lysis inhibitor) forms a high density lipoprotein complex with apolipoprotein A-I in human plasma. *J. Biol. Chem.* **266:** 11030–11036.
3. James, R. W., A-C. Hochstrasser, I. Borghini, B. Martin, D. Pometta, and D. Hochstrasser. 1991. Characterization of a human high density lipoprotein-associated protein, NA1/NA2. *Arterioscler. Thromb.* **11:** 645–652.
4. Murphy, B. F., L. Kirszbaum, I. D. Walker, and A. J. F. d'Apice. 1988. SP-40,40 a newly identified normal human serum protein found in the SC5b-9 complex of complement and in the immune deposits in glomerulonephritis. *J. Clin. Invest.* **81:** 1858–1864.
5. Murphy, B. F., J. R. Saunders, M. K. O'Bryan, L. Kirszbaum, I. D. Walker, and A. J. F. d'Apice. 1989. SP-40,40 is an inhibitor of C5b-6-initiated haemolysis. *Int. Immunol.* **1:** 551–554.
6. Choi, N-H., T. Mazda, and M. Tomita. 1989. A serum protein SP-40,40 modulates the formation of membrane attack complex of complement on erythrocytes. *Mol. Immunol.* **26:** 835–840.
7. de Silva, H. V., J. A. K. Harmony, W. D. Stuart, C. M. Gil, and J. Robbins. 1990. Apolipoprotein J: structure and tissue distribution. *Biochemistry.* **29:** 5380–5389.
8. Collard, M. W., and M. D. Griswold. 1987. Biosynthesis and molecular cloning of sulfated glycoprotein 2 secreted by rat Sertoli cells. *Biochemistry.* **26:** 3297–3303.
9. Li, W-H., M. Tanimura, C-C. Luo, S. Datta, and L. Chan. 1988. The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. *J. Lipid Res.* **29:** 245–271.
10. Burkey, B. F., H. V. de Silva, and J. A. K. Harmony. 1991. Intracellular processing of apolipoprotein J precursor to the mature heterodimer. *J. Lipid Res.* **32:** 1039–1048.
11. Kirszbaum, L., J. A. Sharpe, B. Murphy, A. J. F. d'Apice, B. Classon, P. Hudson, and I. D. Walker. 1989. Molecular cloning and characterization of the novel, human complement-associated protein, SP-40,40: a link between the complement and reproductive systems. *EMBO J.* **8:** 711–718.
12. Stuart, W. D., B. Krol, S. H. Jenkins, and J. A. K. Harmony. 1992. Structure and stability of apolipoprotein J-containing high density lipoproteins. *Biochemistry.* **31:** In press.
13. Jenkins, S. H., W. D. Stuart, J. A. K. Harmony, and L. A. Kaplan. 1990. Development of competitive enzyme-linked immunosorbent assay (ELISA) for a new apoprotein (J). *Clin. Chem.* **36:** 963.
14. Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder. 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transfered to nitrocellulose. *Gene Anal. Tech.* **1:** 3–8.
15. Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* **128:** 417–431.
16. Vézina, C. A., R. W. Milne, P. K. Weech, and Y. L. Marcel. 1988. Apolipoprotein distribution in human lipoproteins separated by polyacrylamide gradient gel electrophoresis. *J. Lipid Res.* **29:** 573–585.
17. Steiner, P. M., J. Freidel, W. F. Bremner, and E. A. Stein. 1981. Standardization of micro-methods for plasma cholesterol, triglyceride and HDL-cholesterol with Lipid

Research Clinics' methodology. *J. Clin. Chem. Biochem.* **19:** 850.
18. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
19. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
20. Cheung, M. C., K. D. Lum, C. G. Brouillette, and C. L. Bisgaier. 1989. Characterization of apoA-I-containing lipoprotein subpopulations secreted by HepG2 cells. *J. Lipid Res.* **30:** 1429–1436.
21. Davis, R. A., S. C. Engelhorn, D. B. Weinstein, and D. Steinberg. 1980. Very low density lipoprotein secretion by cultured rat hepatocytes: inhibition by albumin and other macromolecules. *J. Biol. Chem.* **255:** 2039–2045.
22. Boström, K., J. Borén, M. Wettesten, A. Sjöberg, G. Bondjers, O. Wiklund, P. Carlsson, and S-O. Olofsson. 1988. Studies on the assembly of apoB-100-containing lipoproteins in HepG2 cells. *J. Biol. Chem.* **263:** 4434–4442.
23. Dashti, N., D. L. Williams, and P. Alaupovic. 1989. Effects of oleate and insulin on the production rates and cellular mRNA concentrations of apolipoproteins in HepG2 cells. *J. Lipid Res.* **30:** 1365–1373.
24. Pullinger, C. R., J. D. North, B-B. Teng, V. A. Rifici, A. E. Ronhild de Brito, and J. Scott. 1989. The apolipoprotein B gene is constitutively expressed in HepG2 cells: regulation of secretion by oleic acid, albumin, and insulin, and measurement of the mRNA half-life. *J. Lipid Res.* **30:** 1065–1077.
25. Craig, W. Y., R. Nutik, and A. D. Cooper. 1988. Regulation of apoprotein synthesis and secretion in the human hepatoma HepG2. *J. Biol. Chem.* **263:** 13880–13890.
26. Homan, R., J. E. Grossman, and H. J. Pownall. 1991. Differential effects of eicosapentaenoic acid and oleic acid on lipid synthesis and secretion by HepG2 cells. *J. Lipid Res.* **32:** 231–241.
27. Tsuruta, J. K., K. Wong, I. B. Fritz, and M. D. Griswold. 1990. Structural analysis of sulfated glycoprotein 2 from amino acid sequence. *Biochem. J.* **268:** 571–578.
28. Sylvester, S. R., M. K. Skinner, and M. D. Griswold. 1984. A sulfated glycoprotein synthesized by Sertoli cells and by epididymal cells is a component of the sperm membrane. *Biol. Reprod.* **31:** 1087–1101.
29. Sylvester, S. R., C. Morales, R. Oko, and M. D. Griswold. 1991. Localization of sulfated glycoprotein-2 (clusterin) on spermatozoa and in the reproductive tract of the male rat. *Biol. Reprod.* **45:** 195–207.
30. Fischer-Colbrie, R., R. Zangerle, I. Frischenschlager, A. Weber, and H. Winkler. 1984. Isolation and immunological characterization of a glycoprotein from adrenal chromaffin granules. *J. Neurochem.* **42:** 1008–1016.
31. Fritz, I. B., K. Burdzy, B. Setchell, and O. Blaschuk. 1983. Ram rete testis fluid contains a protein (clusterin) which influences cell-cell interaction in vitro. *Biol. Reprod.* **28:** 1173–1188.
32. Patzak, A., and H. Winkler. 1986. Exocytotic exposure and recycling of membrane antigens of chromaffin granules: ultrastructural evaluation after immunolabeling. *J. Cell Biol.* **102:** 510–515.
33. Buttyan, R., C. A. Olsson, J. Pintar, C. Chang, M. Bandyk, P-Y. Ng, and I. S. Sawczuk. 1989. Induction of the TRPM-2 gene in cells undergoing programmed death. *Mol. Cell Biol.* **9:** 3473–3481.
34. Morris, K. M., D. P. Aden, B. B. Knowles, and H. R. Colten. 1982. Complement biosynthesis by the human hepatoma-derived cell line HepG2. *J. Clin. Invest.* **70:** 906–913.
35. Morris, K. M., and B. B. Knowles. 1982. Biosynthesis and processing of a human precursor complement protein, pro-C3, in a hepatoma-derived cell line. *Science.* **215:** 399–400.
36. Zannis, V. I., J. L. Breslow, T. R. San Giacomo, D. P. Aden, and B. B. Knowles. 1981. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry.* **20:** 7089–7096.
37. Cheung, M. C., A. C. Wolf, K. D. Lum, J. H. Tollefson, and J. J. Albers. 1986. Distribution and localization of lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity in apoA-I-containing lipoproteins. *J. Lipid Res.* **27:** 1135–1144.
38. Lagrost, L., P. Gambert, M. Boquillon, and C. Lallemant. 1989. Evidence for high density lipoproteins as the major apolipoprotein A-IV-containing fraction in normal human serum. *J. Lipid Res.* **30:** 1525–1534.
39. Patsch, J. R., A. M. Gotto, Jr., T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein-like particles during lipolysis of very low density lipoproteins in vitro. *Proc. Natl. Acad. Sci. USA.* **75:** 4519–4523.
40. Clay, M. A., K-A. Rye, and P. J. Barter. 1990. Evidence in vitro that hepatic lipase reduces the concentration of apolipoprotein A-I in rabbit high density lipoproteins. *Biochim. Biophys. Acta.* **1044:** 50–56.
41. Jenne, D. E., and J. Tschopp. 1989. Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. *Proc. Natl. Acad. Sci. USA.* **86:** 7123–7127.
42. Montpetit, M. L., K. R. Lawless, and M. Tenniswood. 1986. Androgen-repressed messages in the rat ventral prostate. *Prostate.* **8:** 25–36.
43. Griswold, M. D., K. Roberts, and P. Bishop. 1986. Purification and characterization of a sulfated glycoprotein secreted by Sertoli cells. *Biochemistry.* **25:** 7265–7270.
44. Abdullah, M., L. L. Tres, H. Ueda, P-C. Hu, and A. L. Kierszenbaum. 1988. Antigenic homology between rat sperm tail polypeptides and Sertoli cell secretory proteins. *Mol. Cell. Biochem.* **81:** 165–176.
45. Blaschuk, O., K. Burdzy, and I. B. Fritz. 1983. Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. *J. Biol. Chem.* **258:** 7714–7720.
46. Hartmann, K., J. Rauch, J. Urban, K. Parczyk, P. Diel, C. Pilarsky, D. Appel, W. Haase, K. Mann, A. Weller, and C. Koch-Brandt. 1991. Molecular cloning of a glycoprotein complex secreted by kidney cells in vitro and in vivo: a link between the reproductive system and the complement cascade. *J. Biol. Chem.* **266:** 9924–9931.
47. Palmer, D. J., and D. L. Christie. 1990. The primary structure of glycoprotein III from bovine adrenal medullary chromaffin granules: sequence similarity with human serum protein-40,40 and rat Sertoli cell glycoprotein-2. *J. Biol. Chem.* **265:** 6617–6623.
48. Laurell, C-B. 1972. Composition and variation of the gel electrophoretic fractions of plasma, cerebrospinal fluid and urine. *Scand. J. Clin. Lab Invest.* **29:** 71–82.