Synthesis and secretion of B-100 and A-I apolipoproteins in response to the changes of intracellular cholesteryl ester content in chick liver

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Abstract We investigated in the chick whether the diet-induced changes of the hepatic content of cholesteryl esters (CE) influence the synthesis and the secretion of apoB- and apoA-I-containing lipoproteins. Control chicks received a low cholesterol diet for 2 (SD-1), 4 (SD-2), or 7 (SD-3) weeks; the chicks in the experimental groups received a cholesterol-rich diet for 2 weeks and were killed at the end of the cholesterol feeding (CH-F), and after 2 (CH-D) or 5 (CH-DD) weeks of a low cholesterol diet. Hepatic CE content in CH-F chicks was 30-fold that observed in controls, but returned to the control level after 5 weeks of cholesterol depletion (CH-DD). The incorporation of ^{35}S-labeled amino acids into cell and medium apoB and apoA-I was measured in liver slices. Intracellular ^{35}S-labeled apoB was similar in all groups whereas medium ^{35}S-labeled apoB was 2-fold higher in CH-F than in controls (SD-1). Pulse-chase experiments showed that radioactive apoB secreted by CH-F chicks at 120 min of chase was 2 times that of SD-1 chicks. This increased secretion of apoB was not found in CH-D chicks. In CH-F chicks, the intracellular and medium ^{35}S-labeled apoA-I were 2-fold the values found in controls (SD-1); apoA-I production returned to the control level only after 5 weeks of cholesterol depletion (CH-DD). The increased secretion of apoB and apoA-I in CH-F chicks was associated with an increased secretion of very low, intermediate, and low density lipoproteins containing newly synthesized apoB and apoA-I and of high density lipoproteins containing predominantly apoA-I. Thus, in response to hepatic CE accumulation induced by cholesterol feeding, a larger proportion of newly synthesized apoB is driven to the secretory pathway and more apoA-I is synthesized. This promotes an increased secretion of plasma lipoproteins that contribute to the removal of CE from the liver.—**Tarugi, P., S. Nicolini, G. Ballarini, L. Marchi, C. Duvigneau, P. Tartoni, and S. Calandra.** Synthesis and secretion of B-100 and A-I apolipoproteins in response to the changes of intracellular cholesteryl ester content in chick liver. *J. Lipid Res.* 1996. **37:** 493–507.

Supplementary key words cholesterol feeding • hepatic cholesteryl esters • lipoprotein secretion

In recent years there has been renewed interest in the changes of hepatic cholesterol content that occur during late embryonic and early post-natal life of the chick (1–4). A large amount of cholesteryl esters (CE) progressively accumulates in the liver during the embryonic life and the first 2 days after hatching, reaching a level that is 30-fold that found in the adult animal (1–4). It has been suggested that CE accumulation derives from the uptake of remnants of cholesterol-rich lipoproteins secreted by the yolk sac membrane as well as the inefficiency of the embryonic liver in disposing of this excess material (1, 2). This enormous hepatic CE store decreases within a few days (days 2–7) of post-natal life (3, 4). This rapid depletion is thought to be the result of the interaction of several factors, such as the local utilization of cholesterol for growth, the conversion of cholesterol into bile acids, or its direct excretion into the bile, and the secretion of CE-rich lipoproteins into the plasma (1, 2, 4).

In a recent study (4) we demonstrated that the depletion of hepatic CE that occurs from day 2 to day 7 of post-natal life was associated with an increased production of apoB-100 and apoA-I by the liver and an increased secretion of CE-rich lipoproteins containing these apolipoproteins. These results led us to propose that CE accumulation in the liver of the newborn chick might be the trigger for the increased production of apoA-I and apoB (4). Within this context it might be expected that other in vivo conditions known to cause a substantial accumulation of CE in the liver are associ-

Abbreviations: apoB, apolipoprotein B-100; apoA-I, apolipoprotein A-I; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CE, cholesteryl ester; CH, cholesterol; HSS, high-speed supernatant.

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ated with an overproduction of apoB and apoA-I, leading to an enhanced secretion of plasma lipoproteins that, in turn, contributes to the removal of CE from the liver.

To test this hypothesis we have chosen the model of cholesterol feeding, a dietary manipulation known to cause an accumulation of cholesteryl esters in the liver (5, 6) whose magnitude might be comparable to that found in the liver of the newborn chick. In order to mimic the situation found in the newborn chick (accumulation and depletion of hepatic CE), our study was performed in chicks that, after being fed a cholesterol-rich diet, (hepatic cholesterol accumulation), received a low cholesterol diet for variable periods of time (hepatic cholesterol depletion).

MATERIALS AND METHODS

Materials

Pro-Mix (L-[^{35}S]methionine and L-[^{35}S]cysteine > 37 TBq/mM), [^{32}P]dCTP (3000 Ci/mmol), [^{3}H]oleic acid (370 GBq/mmol), Hyperfilm-MP X-ray films, and Hybond-N membranes were obtained from Amersham (U.K.), Protosol and Omnifluor were from DuPont NEN (Milano, Italy), Protein A-Sepharose and protein size markers were from Pharmacia (Upsala, Sweden), and silica gel G plates were from Merck (Darmstadt, Germany). Zeta-probe membranes and molecular weight protein standards were obtained from Bio-Rad (Richmond, CA). Polybrene, leupeptin, aprotinin, soy bean trypsin inhibitor, lima bean trypsin inhibitor, and glutathione were obtained from Sigma (St. Louis, MO). Ketalar (ketamine) was obtained from Parke-Davis (Milano, Italy). Nondenaturing polyacrylamide precast gels (Isophore) were obtained from Isolab (Akron, OH).

Animals

Male chicks (Warren strain) from the same batch of eggs were obtained from a local poultry supplier. Chicks were fed a low cholesterol diet (a standard diet containing 25% soy proteins, 4.5% lipids, 5.5% fiber, 0.045% (w/w) total sterols, and 0.005% (w/w) cholesterol) for 2 weeks. At 2 weeks of age, animals were divided into six groups of six animals each. The three groups of control animals received the standard diet for 2 weeks (SD-1 group), 4 weeks (SD-2 group), or 7 weeks (SD-3 group). The other three groups were fed a cholesterol-rich diet (standard diet supplemented with 2% cholesterol) for 2 weeks and were killed either at the end of the cholesterol feeding period (CH-F group) or after 2 (CH-D group) and 5 weeks (CH-DD group) of feeding the low cholesterol diet (standard diet). In this experimental design the CH-F group represents a model for the hepatic cholesterol accumulation whereas CH-D and CH-DD groups represent models for partial and complete hepatic cholesterol depletion, respectively.

Blood and tissue samples

Chicks were anesthetized by peritoneal injection of Ketalar (5 mg/100 g of body weight). Blood was collected by cardiac puncture using K_3 EDTA as anticoagulant. Livers were excised, washed in cold 0.154 M NaCl, and used for in vitro labeling or immediately frozen in liquid nitrogen and stored at -80°C for subsequent analysis (RNA and lipid extraction).

TABLE 1. Effect of cholesterol feeding on some physiological parameters

Groups	Body Weight	Liver Weight	Plasma TC	Plasma TG
	g	*g*	*mg/dl*	*mg/dl*
SD-1	183.3 ± 40.8	5.17 ± 1.09	184.0 ± 12.6	49.5 ± 1.5
CH-F	205.3 ± 25.6	6.89 ± 1.04	229.0 ± 49.0	57.5 ± 10.6
SD-2	355.0 ± 50.4	9.15 ± 1.13	165.5 ± 18.8	39.8 ± 2.2
CH-D	381.8 ± 45.7	9.54 ± 1.15	183.3 ± 25.4	44.0 ± 12.3
SD-3	862.7 ± 77.1	17.91 ± 1.13	147.5 ± 5.1	103.0 ± 11.6
CH-DD	1015.0 ± 134.0	25.28 ± 4.08	141.5 ± 15.2	137.0 ± 10.8
CH-F vs. SD-1	NS	$P < 0.05$	$P < 0.05$	NS
CH-D vs. SD-2	NS	NS	NS	NS
CH-DD vs. SD-3	NS	$P < 0.05$	NS	$P < 0.05$

Each value represents the mean ± standard deviation for data obtained from six animals per group. SD-1, SD-2, and SD-3: control chicks fed a low cholesterol diet for 2, 4, and 7 weeks respectively; CH-F: chicks fed a cholesterol-rich diet for 2 weeks; CH-D and CH-DD: chicks fed a cholesterol-rich diet for 2 weeks followed by 2 (CH-D) and 5 (CH-DD) weeks of feeding a low cholesterol diet. Abbreviations: TC, total cholesterol; TG, triacylglycerols. Statistical analysis was performed using Student's *t*-test.

TABLE 2. Effect of cholesterol feeding on liver lipids

Groups	CH	CE	TG	PL
		μmol/g of liver		
SD-1	6.47 ± 0.55	1.22 ± 0.33	1.99 ± 0.21	33.91 ± 2.63
CH-F	10.23 ± 1.13	38.52 ± 17.16	2.08 ± 0.28	31.61 ± 1.74
SD-2	5.73 ± 0.62	0.78 ± 0.22	2.15 ± 0.55	34.54 ± 1.26
CH-D	6.65 ± 1.33	6.55 ± 4.51	1.89 ± 0.40	31.76 ± 1.41
SD-3	6.59 ± 0.26	1.96 ± 0.28	2.46 ± 0.52	29.14 ± 2.30
CH -DD	6.39 ± 0.62	2.21 ± 0.81	4.92 ± 1.05	28.17 ± 2.22
CH-F vs. SD-1	$P<0.01$	$P<0.01$	NS	NS
CH-D vs. SD-2	NS	$P<0.05$	NS	NS
CH-DD vs. SD-3	NS	NS	$P<0.05$	NS

Each value represents the mean ± standard deviation for data obtained from six animals per group. Abbreviations: CH, free cholesterol; CE, cholesteryl esters; TG, tricylglycerols and PL, phospholipids. Statistical analysis was performed using Student's *t*-test.

Plasma lipids and lipoproteins

Total plasma cholesterol and triacylglycerols were measured by automated enzymatic methods (Ames Division, Miles, U.K.). Plasma lipoproteins were separated by two procedures.

Continuous density gradient ultracentrifugation. Before the separation of plasma lipoproteins, equal aliquots of plasma taken from six animals per group were pooled. Lipoproteins were isolated from the pooled plasmas by density gradient ultracentrifugation in an SW41 rotor at 38,000 rpm for 38 h at 15°C (7). After centrifugation, aliquots of 500 μl (fraction 1) or 400 μl (fractions 2–24) were collected (7) and their protein (8) and cholesterol concentrations (see above) were measured. Preliminary experiments, in which four replicates of the same chick plasma pool were subjected to density gradient ultracentrifugation, showed that the variation coefficient of lipoprotein-cholesterol values ranged from 1% to 7% for the density fractions 1–11, and from 5% to 15% for the density fractions 12–18.

Sequential density ultracentrifugation. Plasmas of single animals (4 animals per group) were subjected to sequential ultracentrifugation to separate the following lipoprotein fractions: VLDL ($d < 1.006$ g/ml), IDL + LDL (1.006–1.063 g/ml), and HDL (1.063–1.210 g/ml) (9). The animals used for the separation of plasma lipoproteins by sequential ultracentrifugation were the same animals used for the study of the hepatic synthesis of apolipoproteins (experiment 1 in Tables 4 and 5). The protein concentration of plasma lipoproteins was measured by the method of Lowry et al. (8).

Apolipoproteins of VLDL, IDL + LDL, and HDL were analyzed by SDS-polyacrylamide gradient (5–20%) gel electrophoresis (3). Gels were stained with Coomassie Blue R-250. The content of B and A-I apolipoproteins in the various plasma lipoprotein classes was estimated from the 605 nm absorbance of the Coomassie Blue stain eluted from the gel with 25% pyridine in 1% acetic acid (10). Plasma concentration of apoA-I was measured by electroimmunoassay (9).

Liver lipids

Livers were finely minced, suspended in 10 ml of 0.25 M sucrose per g of tissue, and homogenized with three strokes of a Potter homogenizer. Lipids were extracted, separated, and measured as previously described (3).

Synthesis of apolipoproteins in vitro

The right lobe of the livers was washed in cold 0.154 M NaCl and cut into slices (3.36 ± 0.78 mg per slice, 0.5–1 mm thick) (11). In the steady-state incubations, 250 mg of liver slices taken from individual animals (four animals per group) was incubated for 4 h at 40°C in 3 ml of Krebs-Ringer bicarbonate buffer containing 60 μCi/ml of [^{35}S]methionine/[^{35}S]cysteine (Pro-Mix, Amersham), 50 units/ml of penicillin, 50 μg/ml of streptomycin, 100 kallikrein inhibitor units/ml of aprotinin, under an atmosphere of 95% O_2–5% CO_2. At the end of the incubations, media were collected and supplemented with leupeptin (0.1 mM), polybrene (25 μg/ml), Na_2EDTA (0.1 mM), soy bean trypsin inhibitor (20 μg/ml), lima bean trypsin inhibitor (20 μg/ml), glutathione (0.02%), phenylmethylsulfonylfluoride (PMSF) (0.2 mg/ml), and aprotinin (5 μg/ml). This mixture was exhaustively dialyzed against 10 mM NH_4HCO_3, 1 mM Na_2EDTA, Trasylol (100 kallikrein inhibitor units/ml), 1 mM methionine, and 1 mM cysteine and lyophilized. Tissue slices were washed with ice-cold Krebs-Ringer bicarbonate buffer, 1 mM unlabeled methionine and cysteine, and Trasylol (20 kallikrein inhibitor units/ml) and homogenized in 15 volumes (vol/tissue weight) of 20 mM Na-phosphate buffer,

pH 7, 150 mM NaCl, 5 mM Na_2EDTA, 2% Triton X-100, and 200 μg/ml PMSF. This material was centrifuged at 226,000 *g* for 1 h at 4°C to prepare the high speed supernatant (HSS) (12). In pulse-chase experiments, pools of equal amounts (60 mg) of liver slices taken from four animals per group (CH-F and SD-1) were incubated for 20 min at 40°C in 3 ml of Krebs-Ringer bicarbonate buffer (see above) in the presence of 200 μCi/ml of [^{35}S]methionine/[^{35}S]cysteine under an atmosphere of 95% O_2–5% CO_2. These incubations were performed in plastic wells (Netwells, Costar, Cambridge, MA) that are specifically designed to carry out pulse-chase experiments using tissue explants. After the pulse labeling, liver slices were carefully washed with cold Krebs-Ringer bicarbonate buffer (see above) containing 1 mM methionine and 1 mM cysteine and chased for 0, 15, 30, 60, 90, and 120 min in the same medium containing an excess of unlabeled methionine and cysteine. At each time of chase, medium and liver slices were processed as specified above. In both steady-state and pulse-chase experiments, aliquots of incubation media and high speed supernatants (HSS) were immunoprecipitated by anti-chick apoB (4, 11, 12) and anti-chick apoA-I rabbit IgG or nonimmune rabbit IgG (3, 4). The apoB immunocomplexes were dissolved in 100 mM Tris-HCl, pH 7.3, 20% glycerol, 10% SDS, and 5% 2-mercaptoethanol, heated at 100°C for 5 min and applied to a linear 5–10% gradient SDS-polyacrylamide gel. The apoA-I immunocomplexes were dissolved in 2% SDS, 3.5% 2-mercaptoethanol, 50 mM Tris-HCl, pH 7, heated at 95°C for 3 min and applied to a linear 5–20% gradient SDS-polyacrylamide gel (3, 11). Gels were stained with Coomassie Blue R-250, destained, processed for fluorography, and exposed to Hyperfilm-MP X-ray films at -80°C. Radioactive bands corresponding to apoB and apoA-I were excised from the gel, solubilized in 30% hydrogen peroxide, and incubated overnight at 37°C. After the addition of 1 ml Protosol, samples were shaken for 6 h at room temperature and counted in scintillation fluid (Omnifluor 3.8 g, Protosol 25 ml, and water 3.5 ml per liter of toluene) (12). In both steady-state and pulse-chase experiments, the amount of ^{35}S-labeled amino acids incorporated into total cell and medium proteins was measured by trichloroacetic acid precipitation (12).

Isolation of ^{35}S-labeled lipoproteins secreted in vitro

In some experiments, equal amounts of liver slices taken from four animals per group were pooled and incubated in quadruplicate with [^{35}S]methionine/[^{35}S]cysteine for 4 h as specified above (steady-state incubations). At the end of the incubation, each medium was dialyzed against 0.154 M NaCl, 10 mM Na_2EDTA, 1 mM methionine, 1 mM cysteine, and Trasylol (100 kallikrein inhibitor units/ml), and then subjected to density gradient ultracentrifugation to separate ^{35}S-labeled lipoproteins. Density fractions were collected as specified above for plasma lipoproteins and protein radioactivity was measured after precipitation of the samples with 10% trichloroacetic acid. The recovery of total protein radioactivity (24 density fractions) was >95% in all gradients. The corresponding fractions from the four density gradients were pooled and equal volumes of each pooled fraction were applied to a linear 5–10% or 5–20% gradient SDS-polyacrylamide gel for the separation of ^{35}S-labeled apoB and ^{35}S-labeled apoA-I, respectively. Gels were stained with Coomassie Blue R-250, destained, processed for fluorography, and exposed to Hyperfilm-MP X-ray films at -80°C.

[^{3}H]oleic acid incorporation into hepatic cholesteryl esters

In some experiments liver slices taken from four animals per group were pooled and quadruplicate samples were pulse-labeled for 1 h in 3 ml of Krebs-Ringer bicarbonate buffer in the presence of 70 μCi/ml of [^{3}H]oleic acid-albumin complex (4). At the end of the pulse period, two samples of liver slices were homogenized and extracted in chloroform–methanol 2:1 (v/v) and lipids were separated by thin-layer chromatography.

TABLE 3. Effect of cholesterol feeding on plasma lipoprotein concentration

Groups	VLDL	IDL + LDL	HDL
	μg/ml of plasma		
SD-1	33.28 ± 2.34	166.77 ± 19.55	2082.35 ± 142.27
CH-F	160.82 ± 69.83	245.37 ± 33.51	378.66 ± 95.59
SD-2	52.51 ± 12.12	219.08 ± 55.93	2882.50 ± 316.37
CH-D	34.62 ± 9.34	153.15 ± 35.92	2697.50 ± 214.22
CH-F vs. SD-1	$P < 0.01$	$P < 0.01$	$P < 0.01$
CH-D vs. SD-2	NS	NS	NS

Each value represents the mean ± standard deviation for data obtained from four animals per group. VLDL, d < 1.006 g/ml; IDL + LDL, 1.006–1.063 g/ml; and HDL, 1.063–1.210 g/ml. Plasma lipoprotein concentration is given as lipoprotein-protein per ml of plasma. Statistical analysis was performed using Student's *t*-test.

The spots corresponding to cholesteryl esters were scraped and counted in a liquid scintillation counter (4). The other two samples of liver slices were chased for 3 h in Krebs-Ringer bicarbonate buffer. At the end of the chase period incubation media were collected and subjected to density gradient ultracentrifugation (see above). Density fractions 1–9 (VLDL, IDL, and LDL, d < 1.063 g/ml) and 10–18 (HDL, d 1.063–1.210 g/ml) were pooled and lipoprotein lipids were extracted and separated by thin-layer chromatography. The spots corresponding to cholesteryl esters were scraped and counted as specified above. Free fatty acids were extracted from liver homogenate according to Dole and Meinertz (13) and measured by a colorimetric method (14). The analysis of free fatty acid composition was performed by gas–liquid chromatography after diazomethane methylation (15).

Northern blotting and slot-blot analysis

Total cellular RNA was extracted from the liver of individual animals (four animals per group) by the guanidine-HCl method, as previously described (16). Equal aliquots of total liver RNA from each animal per group were pooled before slot-blot or Northern blot hybridization (4, 17). For slot-blot analysis, serial dilutions of RNA (0.5–4 μg/slot) were alkaline-denatured and blotted onto Zeta-probe membranes (18). Membranes were hybridized with ^{32}P-labeled chick apoB and chick apoA-I cDNA probes and subsequently reprobed with ^{32}P-labeled human β-actin cDNA (4, 18). Conditions of pre-hybridization and hybridization for Northern blot and slot-blot were as previously described (17). Membranes were exposed to Hyperfilm-MP X-ray films for autoradiography at -80°C. Densitometric analysis of autoradiograms was performed using an LKB 2202 Ultroscan Laser Densitometer.

Statistical analysis

Values were given as the mean ± standard deviation of the mean (SD). Statistical comparison between

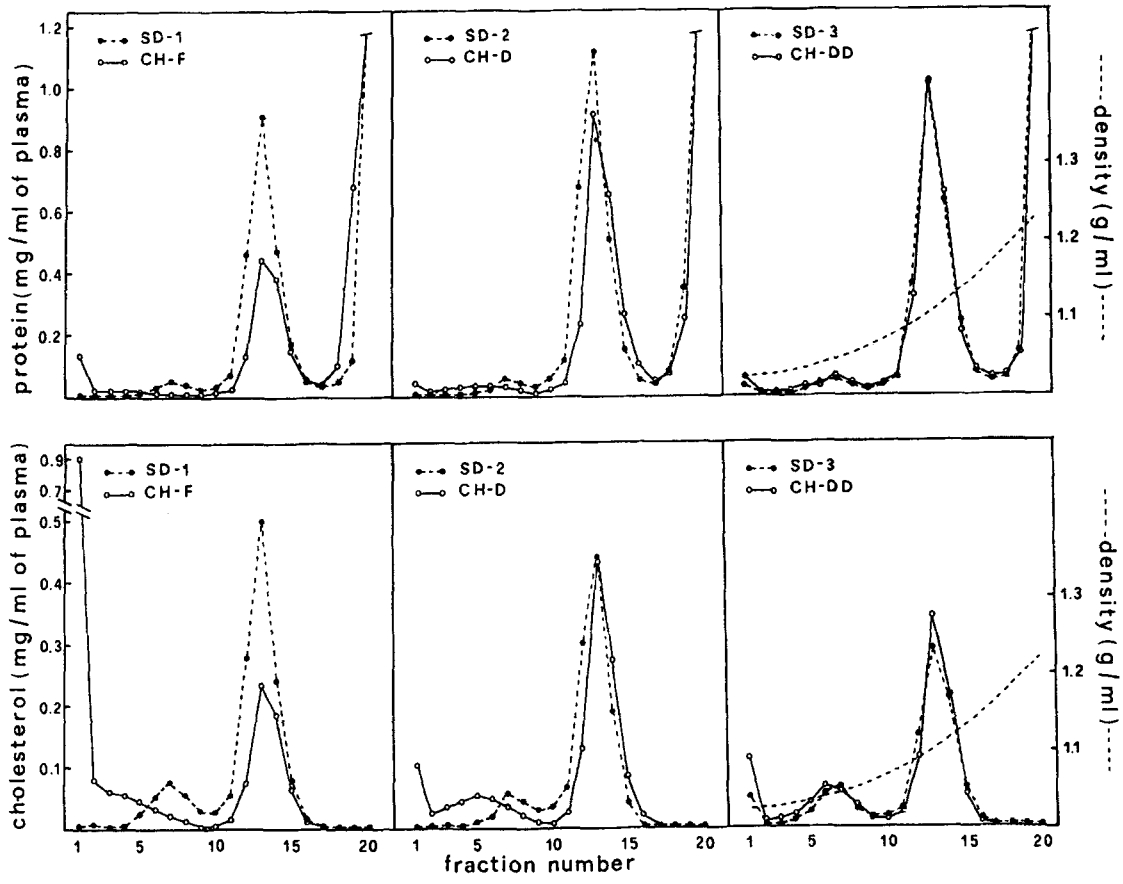

Fig. 1. Plasma lipoproteins in cholesterol-fed and cholesterol-depleted chicks. Plasma lipoproteins were separated by density gradient ultracentrifugation from pools of plasmas taken from six animals per group. VLDL, fractions 1–2; IDL + LDL, fractions 3–9; HDL, fractions 10–18. Lipoprotein concentration is given as lipoprotein-protein (upper panels) and lipoprotein-cholesterol (lower panels). SD-1, SD-2, and SD-3: control chicks fed a low cholesterol diet for 2, 4, and 7 weeks, respectively. CH-F: chicks fed a cholesterol-rich diet for 2 weeks. CH-D and CH-DD: chicks fed a cholesterol-rich diet for 2 weeks followed by 2 (CH-D) and 5 (CH-DD) weeks of feeding a low cholesterol diet.

TABLE 4. Per cent of total protein radioactivity incorporated into apoB by liver slices

Groups	Experiment 1		Experiment 2	
	Cell	Medium	Cell	Medium
SD-1	0.241 ± 0.040	0.157 ± 0.039	0.232 ± 0.097	0.183 ± 0.035
CH-F	0.241 ± 0.005	0.435 ± 0.147	0.252 ± 0.046	0.423 ± 0.065
SD-2	0.222 ± 0.058	0.104 ± 0.063	0.272 ± 0.036	0.129 ± 0.041
CH-D	0.250 ± 0.081	0.105 ± 0.041	0.247 ± 0.067	0.115 ± 0.028
CH-F vs. SD-1	NS	$P < 0.05$	NS	$P < 0.05$
CH-D vs. SD-2	NS	NS	NS	NS

Liver slices taken from single animals (4 animals per group) were incubated for 4 h (steady-state incubation) in the presence of [^{35}S]methionine/[^{35}S]cysteine. ^{35}S-labeled apoB was immunoprecipitated from cell homogenate and incubation media and separated by 5–10% linear gradient SDS-PAGE. ApoB bands were excised from the gel and the amount of incorporated label was determined by scintillation counting. The radioactivity incorporated into cell and medium apoB is given as the percentage of total ^{35}S-labeled cell and medium proteins, respectively. In each experiment, each value represents the mean ± standard deviation for data obtained from 4 animals per group (see Methods). Statistical analysis was performed using Student's *t*-test.

groups (CH-F vs. SD-1; CH-D vs. SD-2 and CH-DD vs. SD-3) was made using the Student's *t*-test. Data were checked for validity of the test and in case of breach in the assumption of homoscedasticity, variance stabilizing transformation of the data or separate variance *t*-test were used.

RESULTS

Physiological parameters

Table 1 shows the changes of some physiological parameters in cholesterol-fed (CH-F) and cholesterol-depleted (CH-D and CH-DD) chicks. While plasma cholesterol was higher (+ 25%) in CH-F chicks than in controls (SD-1), no difference was found between cholesterol-depleted chicks (CH-D and CH-DD) and the corresponding controls (SD-2 and SD-3). No difference in the plasma concentration of triacylglycerols (TG) was found between CH-F or CH-D and the corresponding controls (SD-1 and SD-2). However, plasma TG level of CH-DD chicks was slightly higher than that found in the controls (SD-3).

As shown in **Table 2,** the levels of liver free cholesterol (CH) and cholesteryl esters (CE) in CH-F chicks were 1.6- and 31-fold, respectively, those found in the corresponding controls (SD-1). After 2 weeks of cholesterol depletion, the hepatic level of CE in CH-D chicks was approximately 8-fold that found in the corresponding controls (SD-2), whereas that of CH was similar in the two groups. After 5 weeks of cholesterol depletion, the hepatic levels of CH and CE were similar in the two groups (CH-DD vs. SD-3). There was no difference in the level of hepatic TG between CH-F or CH-D chicks and the corresponding controls (SD-1 and SD-2). However, the level of liver TG in CH-DD chicks was 2-fold that found in the controls (SD-3).

Plasma lipoproteins

Table 3 shows the concentration of plasma lipoproteins isolated by sequential ultracentrifugation. In CH-F chicks the concentrations of VLDL and IDL + LDL were higher and that of HDL was lower than those found in the control chicks (SD-1). After 2 weeks of cholesterol depletion, the lipoprotein levels of CH-D chicks were similar to those of the corresponding controls (SD-2). In view of the latter finding, lipoproteins were not isolated by sequential ultracentrifugation from the plasma of CH-DD and SD-3 chicks (see below).

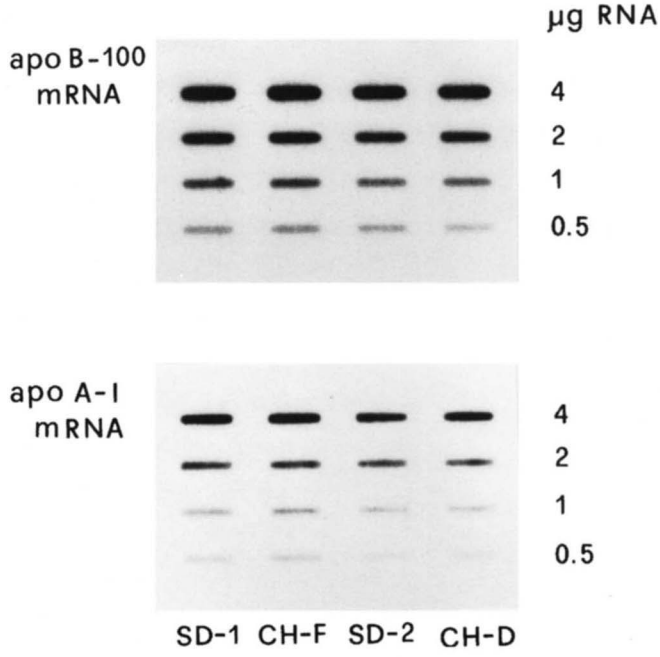

Fig. 2. Slot-blot hybridization analysis of B and A-I apolipoprotein mRNAs. Total RNA isolated from the livers of four animals per group was pooled and blotted onto Zeta-probe membranes that were hybridized with ^{32}P-labeled chick apoB and chick apoA-I cDNA probes and exposed to X-ray films.

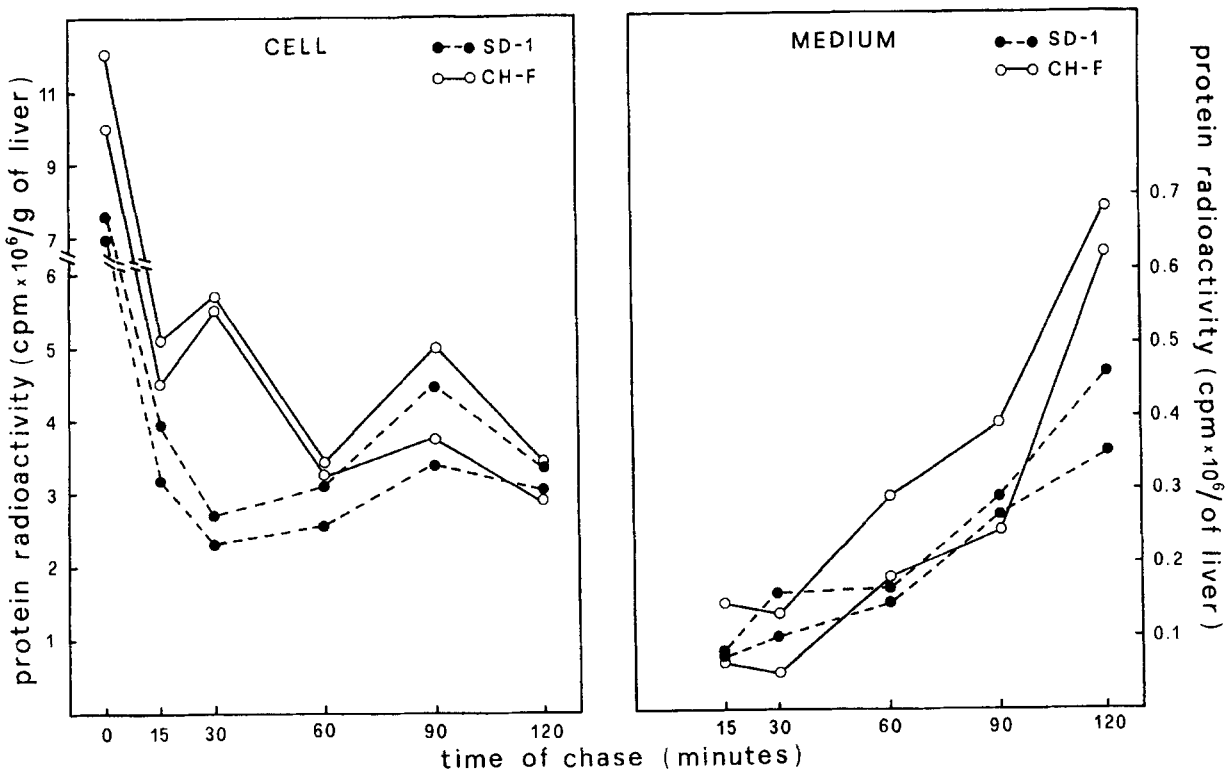

Fig. 3. Cell and medium protein radioactivity in pulse-chase experiments. Pools of equal amounts of liver slices taken from four animals per group were incubated with [^{35}S]methionine/[^{35}S]cysteine for 20 min (pulse) and chased for various periods of time. At each chase period, cell and medium ^{35}S-labeled proteins were precipitated with trichloroacetic acid and the radioactivity was counted in a liquid scintillation counter. The data obtained from two individual experiments are shown.

In order to analyze more closely the lipoprotein changes shown in Table 3, plasma lipoproteins were also separated by density gradient ultracentrifugation. In **Figure 1** the plasma concentration of each density fraction is given as lipoprotein-protein (upper panels) and lipoprotein-cholesterol (lower panels). The main changes of the plasma lipoprotein profile induced by cholesterol feeding (CH-F vs. SD-1 chicks) were: *a)* the appearance of a major peak in the VLDL region (fractions 1–2); *b)* the decrease of the LDL peak (fractions 5–9) which was balanced by an increase of the IDL peak (fractions 3–4); and *c)* a substantial decrease of the HDL peak (fractions 10–18). After 2 weeks of cholesterol depletion, the lipoprotein profile of CH-D chicks was characterized by: *a)* a high VLDL peak; *b)* a broad peak encompassing the IDL-LDL region (fractions 3–9); and *c)* an HDL peak similar to that found in the corresponding controls (SD-2). After 5 weeks of cholesterol depletion, the plasma lipoprotein profile of CH-DD chicks resembled that of the corresponding controls (SD-3) apart from a higher VLDL peak and a slight shift of the LDL peak toward the lower densities.

The apolipoprotein composition of plasma lipoproteins isolated by density gradient ultracentrifugation demonstrated that in all groups apoB was the main peptide of VLDL, IDL, and LDL, whereas apoA-I was the main peptide of HDL (data not shown) (3). As reported previously, apoA-I was present also in VLDL, IDL, and LDL (data not shown) (3, 7). The distribution of apoA-I among plasma lipoproteins was affected by cholesterol feeding. The percentage of plasma apoA-I present in VLDL, IDL, and LDL was 0.64% and 4.5% in SD-1 and CH-F chicks, respectively. After cholesterol depletion, the percentage of plasma apoA-I present in VLDL, IDL, and LDL of CH-D and CH-DD chicks (1.3%) was similar to that found in the corresponding controls (SD-2 and SD-3) (1.2%). Cholesterol feeding induced a 25% reduction of plasma apoA-I (198 ± 12.3 vs. 264 ± 22.0 mg/dl in CH-F and SD-1, respectively, $P < 0.01$). After cholesterol depletion, no difference was observed between CH-D and SD-2 chicks (357 ± 22 vs. 323 ± 23 mg/dl) and between CH-DD and SD-3 chicks (364 ± 17 vs. 373 ± 17 mg/dl).

^{35}S-labeled amino acid incorporation into apoB by liver slices in steady-state incubations

In CH-F, CH-D, and CH-DD chicks, the rates of incorporation of ^{35}S-labeled amino acids into total cell and medium proteins by liver slices were similar to those found in the corresponding controls (data not shown).

The percentage of total protein radioactivity incorporated into apoB is shown in **Table 4.** There was no difference in cell apoB radioactivity between CH-F or CH-D chicks and the corresponding controls (SD-1 and SD-2). In contrast, the radioactive apoB present in the medium of CH-F chicks was 2.5-times that found in controls (SD-1). This difference, however, was not found in the case of CH-D chicks and the corresponding controls (SD-2).

Slot blot (**Fig. 2**) and Northern blot determinations (data not shown) showed that hepatic cholesterol accumulation and depletion did not change the steady-state level of apoB mRNA in the liver.

^{35}S-labeled amino acid incorporation into apoB by liver slices in pulse-chase experiments

To investigate the mechanism responsible for the increased secretion of newly synthesized apoB in CH-F chicks (Table 4), liver slices from CH-F and SD-1 chicks were pulsed for 20 min and chased for various periods of time (0, 15, 30, 60, 90, and 120 min). The uptake of ^{35}S-labeled amino acids by liver slices (measured after 20-min pulse as the radioactivity of trichloroacetic acid-soluble material) was similar in the two groups (67.6 ± 0.68 cpm × 10^6 in SD-1 vs. 63.9 ± 8.5 cpm × 10^6 in CH-F chicks). At each time point ^{35}S-labeled apoB was immunoprecipitated from liver slice homogenates and incubation media. **Figure 3** shows the profile of total protein radioactivity in cell and medium during the chase period and **Fig. 4** shows the radioactivity incorporated into cell and medium apoB. In both CH-F and SD-1 chicks, ^{35}S-labeled apoB present in the medium increased almost linearly up to 90–120 min of chase. At 120 min of chase the amount of radioactive apoB found in the medium of CH-F chicks was approximately 2.0-fold that found in the medium of SD-1 chicks (**Fig. 5**).

^{35}S-labeled amino acid incorporation into apoA-I by liver slices in steady-state incubations

Table 5 shows that in CH-F chicks the percentage of total protein radioactivity incorporated into cell and medium apoA-I was 2.1- and 2.5-fold, respectively, that found in the controls (SD-1). After 2 weeks of cholesterol depletion, the percentage of total protein radioactivity incorporated into cell and medium apoA-I of CH-D chicks was 1.7- and 1.9-fold, respectively, that found in the corresponding controls (SD-2). After 5 weeks of cholesterol depletion, there was no difference

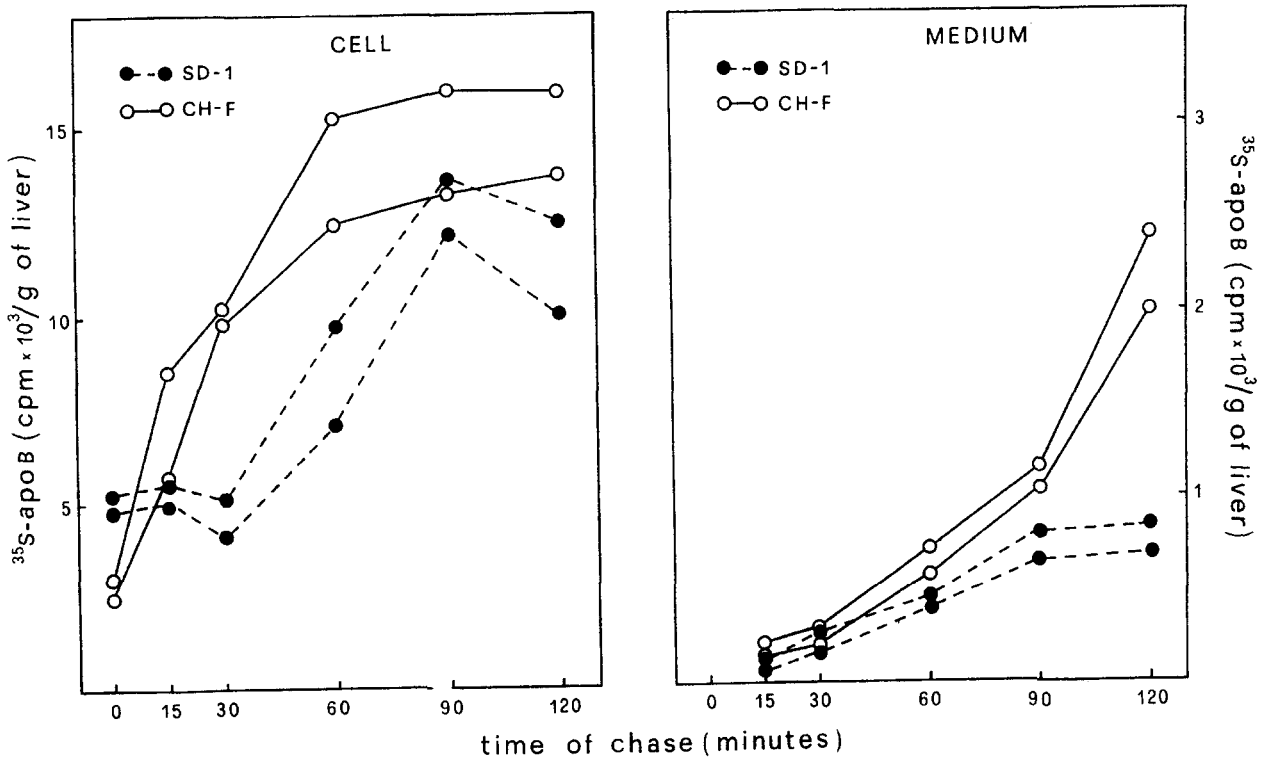

Fig. 4. Cell and medium ^{35}S-labeled apoB in pulse-chase experiments. Pools of equal amounts of liver slices taken from four animals per group (see Fig. 3) were incubated with [^{35}S]methionine/[^{35}S]cysteine for 20 min (pulse) and chased for various periods of time. At each chase period ^{35}S-labeled apoB was immunoprecipitated from cell homogenates and incubation media and separated by 5–10% linear gradient SDS-PAGE. ApoB bands were excised from the gel and the amount of incorporated label was determined by scintillation counting. The data obtained from two individual experiments are shown.

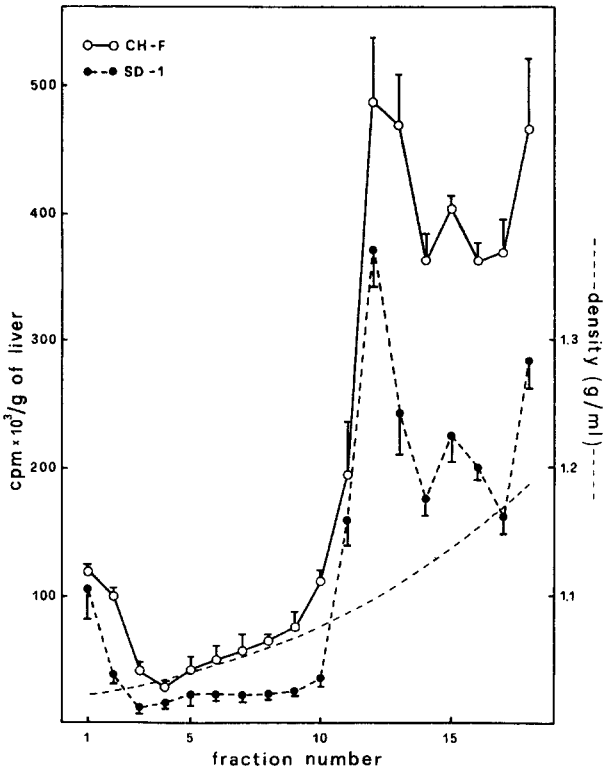

Fig. 5. Effect of cholesterol feeding on the ^{35}S-labeled lipoproteins secreted into the medium by liver slices. Equal amounts of liver slices taken from four animals per group were pooled and incubated in quadruplicate in the presence of [^{35}S]methionine/[^{35}S]cysteine for 4 h (steady-state incubation). At the end of the incubation, each medium was subjected to density gradient ultracentrifugation. The protein radioactivity of each fraction was measured after trichloroacetic acid precipitation. Each value represents the mean ± standard deviation from four density gradient fractions.

between CH-DD chicks and the corresponding controls (SD-3).

Slot blot (Fig. 2) and Northern blot (data not shown) determinations showed that hepatic cholesterol accumulation and depletion did not change the steady-state level of apoA-I mRNA in the liver.

Characterization of ^{35}S-labeled lipoproteins isolated from the incubation medium

To analyze the ^{35}S-labeled lipoproteins secreted by liver slices during steady-state incubations, the incubation media were subjected to density gradient ultracentrifugation (Figs. 5–6). In CH-F chicks, the radioactivity incorporated into VLDL (fractions 1–2), IDL + LDL (fractions 3–10), and HDL (fractions 11–18) was 1.5-, 2.6-, and 1.7-fold, respectively, that found in the controls (SD-1) (Fig. 5). After 2 weeks of cholesterol depletion (CH-D chicks), the radioactivity incorporated into VLDL (fractions 1–2) and into HDL (fractions 11–18) was 1.5-fold that found in controls (SD-2) whereas the radioactivity incorporated into IDL + LDL was similar in the two groups (**Fig. 6**).

The apolipoprotein analysis of ^{35}S-labeled medium lipoproteins is shown in **Fig. 7** and **Fig. 8.** In view of the low radioactivity, some density fractions were pooled before SDS-PAGE. In both CH-F and SD-1 chicks radioactive apoB was present only in VLDL, IDL, and LDL (fractions 1–10 of the density gradient shown in Fig. 5), being most abundant in the density fractions 1 and 2. In CH-F chicks the intensity of the apoB band in the density fractions 3–10 was more pronounced than that found in the corresponding density fractions of the controls (SD-1) (Fig. 7). After 2 weeks of cholesterol depletion, the apoB band in VLDL (fractions 1–2) of CH-D chicks was more intense than that of the controls (SD-2) (Fig. 7).

Figure 8 shows that in all animal groups ^{35}S-labeled apoA-I was the main peptide of HDL (fractions 11–18 of the density gradient shown in Figs. 5–6) but was also present in substantial amount in the lipoproteins of density less than 1.063 g/ml. In all lipoprotein fractions of the CH-F chicks, the intensity of apoA-I band was more pronounced than that found in the lipoproteins of the controls (SD-1). Figure 8 also shows that the intensity of some minor components of HDL apolipoproteins (12 kD and 9 kD, respectively), present in the density fractions 17–18 (Fig. 5), was higher in CH-F than in SD-1 chicks. After 2 weeks of cholesterol depletion, the distribution of radioactive apoA-I in the lipoproteins of CH-D chicks was similar to that found in controls (SD-2) but the overall intensity of the apoA-I band was higher in all density fractions.

[^{3}H]oleic acid incorporation into hepatic cholesteryl esters

To determine whether the accumulation and depletion of hepatic cholesteryl esters observed in CH-F and CH-D chicks were associated with changes in the hepatic production and secretion of this lipid class, the incorporation of [^{3}H]oleic acid into CE was measured in liver slices. As the hepatic content of free oleic acid was similar in all groups (4.16 ± 0.25, 3.85 ± 0.42, 3.90 ± 0.26, and 3.75 ± 0.31 μmol/g of liver in CH-F, SD-1, CH-D, and SD-2 chicks, respectively) we assumed that the specific activity of [^{3}H]oleic acid present in liver slices was the same in all groups.

Table 6 shows that in CH-F chicks the radioactivity incorporated into hepatic CE was 3.4-fold that found in controls (SD-1), whereas after 2 weeks of cholesterol depletion there was no difference between CH-D and SD-2 chicks. To investigate to what extent labeled intra-

TABLE 5. Per cent of total protein radioactivity incorporated into apoA-I by liver slices

	Experiment 1		Experiment 2	
Groups	Cell	Medium	Cell	Medium
SD-1	0.242 ± 0.036	0.081 ± 0.021	0.285 ± 0.071	0.078 ± 0.010
CH-F	0.582 ± 0.087	0.219 ± 0.049	0.540 ± 0.046	0.188 ± 0.015
SD-2	0.345 ± 0.022	0.053 ± 0.009	0.380 ± 0.050	0.083 ± 0.021
CH-D	0.637 ± 0.225	0.104 ± 0.039	0.650 ± 0.160	0.158 ± 0.039
SD-3	0.452 ± 0.138	0.186 ± 0.024	Not done	Not done
CH-DD	0.338 ± 0.078	0.202 ± 0.050	Not done	Not done
CH-F vs. SD-1	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
CH-D vs. SD-2	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
CH-DD vs. SD-3	NS	NS	–	–

Liver slices taken from single animals (4 animals per group) were incubated for 4 h (steady-state incubation) in the presence of [^{35}S]methionine/[^{35}S]cysteine. ^{35}S-labeled apoA-I was immunoprecipitated from cell homogenate and incubation media and separated by 5–20% linear gradient SDS-PAGE. ApoA-I bands were excised from the gel and the amount of incorporated label was determined by scintillation counting. The radioactivity incorporated into cell and medium apoA-I is given as the percentage of total ^{35}S-labeled cell and medium proteins, respectively. In each experiment, each value represents the mean ± standard deviation from 4 animals per group (see Methods). Statistical analysis was performed using Student's *t*-test.

cellular CE were incorporated into newly synthesized lipoproteins, liver slices were pulse-labeled with [^{3}H]oleic acid for 1 h and then chased for 3 h. At the end of the chase period, medium ^{3}H-labeled VLDL-LDL and ^{3}H-labeled HDL were isolated by ultracentrifugation and their ^{3}H-labeled CE content was measured. Table 6 shows that the amount of ^{3}H-labeled CE incorporated into VLDL-LDL and HDL secreted by liver slices of CH-F chicks was 3-fold that found in controls (SD-1). After 2 weeks of cholesterol depletion, the amount of ^{3}H-labeled CE present in VLDL-LDL and HDL of CH-D chicks was 116% and 135%, respectively, that found in the corresponding lipoproteins of the control chicks (SD-2).

DISCUSSION

In a previous study we demonstrated that the accumulation of cholesteryl esters (CE) observed in the liver of the newborn chick was associated with an increased production of apoB and apoA-I (4). We suggested that the hepatic CE overload stimulated the synthesis and/or the secretion of apoB and apoA-I that were incorporated into CE-rich lipoproteins, the secretion of which contributed to the rapid depletion of hepatic CE observed during the first week of post-natal life (4).

In the present study we investigated whether the hepatic production of apoB and apoA-I changed in response to dietary manipulations designed to induce an accumulation and a depletion of hepatic CE comparable to that observed in the newborn chick. We found that after a 2-week administration of a cholesterol-rich diet (CH-F chicks) the hepatic CE content increased almost 30-fold but it progressively decreased to the normal levels during a 5-week administration of a low cholesterol diet (CH-DD chicks).

In our experimental setting, hepatic apolipoprotein production was measured by the incorporation of ^{35}S-labeled amino acids into apoB and apoA-I by liver slices during a 4-h incubation. The steady-state levels of hepatic apoB and apoA-I mRNA were also measured. We found that the accumulation of hepatic CE induced by cholesterol feeding (CH-F chicks) had no effect on the amount of radioactive apoB found intracellularly (Table 4) nor on the apoB mRNA level (Fig. 2). These findings are in agreement with the observations in several mammalian species such as rat (19, 20), rabbit (21), and nonhuman primates (22–26) that the hepatic level of apoB mRNA and the rate of hepatic apoB synthesis are not affected by cholesterol feeding. We found, however, that cholesterol feeding induced an increase of the radioactive apoB present in the incubation medium (CH-F vs. SD-1 chicks, Table 4) suggesting that this dietary manipulation increases the amount of newly synthesized apoB that is available for secretion (23). Several studies conducted in rat hepatocytes (27–29) and human HepG2 cells (30) have demonstrated that a significant proportion of newly synthesized apoB is degraded intracellularly (see ref. 31 for review) and the rate of apoB degradation is modulated by the amount of triacylglycerols (31–36) and/or cholesteryl esters (37–40) synthesized in the hepatocytes. As the amount of these lipids increases, a larger proportion of newly synthesized apoB molecule is incorporated into lipoprotein particles and driven to the secretory pathway. In this context it is conceivable that the 2-fold increase of medium apoB found in CH-F chicks reflects a reduced

intracellular degradation of newly synthesized apoB caused by the large availability of cholesteryl esters in the endoplasmic reticulum. The result of the pulse-chase experiment (Fig. 4) showing that at 120 min of chase the amount of radioactive apoB present in the medium of CH-F chicks was approximately 2.0-fold that found in controls (SD-1), appears to be consistent with this interpretation. Finally, the observation that when the hepatic CE content decreases as in CH-D chicks, the secretion of newly synthesized apoB becomes comparable to that found in controls (SD-2) (Tables 2 and 4) reinforces the idea that the hepatic CE content plays an important role in modulating hepatic apoB secretion in chick liver. Our results seem to suggest that the hepatic CE content must reach a threshold level in order to increase the incorporation of newly synthesized apoB into lipoprotein particles. It is likely that this threshold level is related to the size of a specific pool of cellular CE, presumably located in the endoplasmic reticulum, where it is generated by the action of acylCoA-acyl transferase (ACAT) (41), an enzyme whose activity, at least in mammalian species, is increased in response to cholesterol feeding (42). As we found that the increased secretion of apoB in CH-F chicks was associated with a 3-fold increase in the incorporation of [^{3}H]oleic acid into hepatic cholesteryl esters, it is likely that the amount of newly synthesized CE is a key factor in driving newly synthesized apoB into the secretory pathway. This idea is supported by the observation that after cholesterol depletion both the secretion of apoB and the hepatic CE synthesis in CH-D chicks returned to the level found in the control animals (SD-2) (Table 6). Taken together, our results are in agreement with previous reports indicating that the rate of secretion of apoB-100 by human hepatocytes and HepG2 cells increases in response to an increased intracellular cholesterol content (43, 44).

We also found that the hepatic CE accumulation induced by cholesterol feeding (CH-F chicks) was associated with a 2-fold increase of the ^{35}S-labeled apoA-I present in cell and medium. Only after the complete depletion of hepatic CE (CH-DD chicks) (Table 2) did the amount of cell and medium ^{35}S-labeled apoA-I reach the levels found in the control chicks (SD-3) (Table 5). As previously reported in some mammalian species (45, 46), the changes of the hepatic apoA-I production observed in cholesterol-fed chicks were not accompanied by parallel changes in the steady-state level of apoA-I mRNA, suggesting that under these circumstances apoA-I synthesis might be regulated at a post-transcriptional level (45). A recent study by Azrolan et al. (47) shed some light on the mechanism involved in the regulation of apoA-I synthesis in animals fed a high fat–high cholesterol diet (HF/HC diet). By using a human apoA-I transgenic mouse model they showed that the increased hepatic apoA-I production induced by HF/HC diet occurs through an increase in the percentage of apoA-I mRNA present in the polysomal pool (i.e., that pool of mRNA which is actively translated). Whether this change in apoA-I mRNA distribution in the cytoplasm is related to the lipid (such cholesteryl ester) content in the hepatocytes or to some other factors is not clear at present. Although it is conceivable that in CH-F and CH-D chicks the amount of apoA-I mRNA bound to polysomal is increased, other factors such as an increased initiation rate of translation of apoA-I mRNA or a reduction of the intracellular degradation of newly synthesized apoA-I may play a role in increasing the hepatic production of apoA-I.

The increased hepatic secretion of apoB and apoA-I by CH-F chicks and of apoA-I by CH-D chicks was associated with an increased secretion of lipoproteins (Figs. 5, 6). These lipoproteins contain radioactive apoB (VLDL, IDL, and LDL) (Fig. 7) and apoA-I (VLDL, IDL + LDL, and HDL) (Fig. 8). These findings support the notion that in response to the CE accumulation induced by cholesterol feeding, the increased availability of apoB

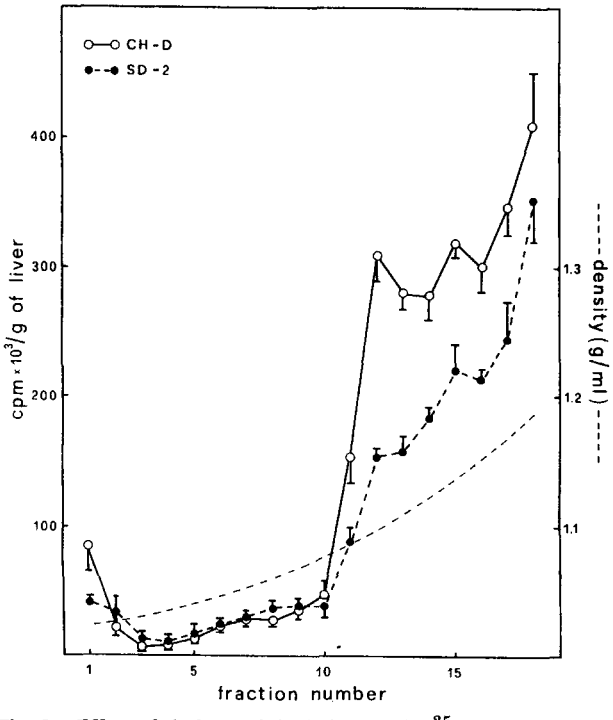

Fig. 6. Effect of cholesterol depletion on the ^{35}S-labeled lipoproteins secreted into the medium by liver slices. Equal amounts of liver slices taken from four animals per group were pooled and incubated in quadruplicate in the presence of [^{35}S]methionine/[^{35}S]cysteine for 4 h (steady-state incubation). At the end of the incubation, each medium was subjected to density gradient ultracentrifugation. The protein radioactivity of each fraction was measured after trichloroacetic acid precipitation. Each value represents the mean ± standard deviation from four density gradient fractions.

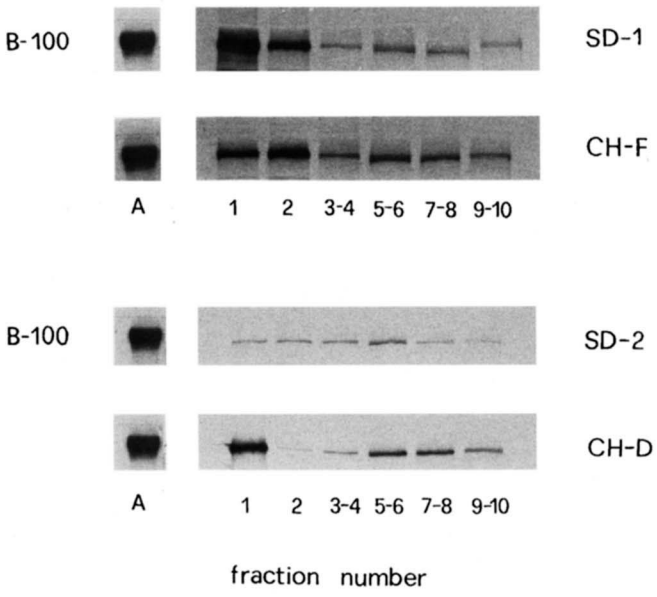

Fig. 7. Fluorograms of ^{35}S-labeled apoB of medium lipoproteins. ^{35}S-labeled lipoprotein fractions isolated from the incubation media by density gradient ultracentrifugation (as shown in Figs. 5, 6) were pooled and analyzed on a 5–10% linear gradient SDS-PAGE (see Methods). Equal volumes of each density fraction were applied to each lane. Fraction number refers to the density fractions 1–10 of the density gradient profiles shown in Figs. 5 and 6. Lane A: chick plasma apoB-100 (Coomassie staining).

and, above all, of apoA-I represents a "protective" mechanism whereby chick liver increases its capacity for reducing the cholesterol overload by secreting a large amount of CE-containing lipoprotein particles over a wide density range. In this context the increased secretion of apoB and apoA-I observed in CH-F chicks resembles the situation we previously described in the newborn chicks (4) where the increased secretion of apoB- and apoA-I-containing lipoproteins represents one of the mechanisms involved in the rapid depletion of the large hepatic CE pool present at the time of hatching (4).

In the present study we confirmed that cholesterol feeding (CH-F chicks) increased the plasma levels of VLDL and IDL and reduced that of LDL and HDL as previously reported by Hermier and Dillon (48). This lipoprotein pattern progressively changed during the depletion period and after 5 weeks of cholesterol depletion it returned within the values found in control animals. Surprisingly, the levels of HDL and plasma apoA-I were found to be decreased in CH-F chicks despite a substantial increase of the hepatic apoA-I production and secretion of apoA-I-containing HDL. Decreased plasma levels of HDL and/or apoA-I have been previously reported in rabbits (49) and in some susceptible mouse strain (C57BL/6) (50) after the administration of a cholesterol-rich diet. In the rabbit, hepatic apoA-I mRNA was found to increase 5-fold in response to a cholesterol-rich diet (51) whereas in C57BL/6 mice the rate of synthesis of apoA-I and the level of apoA-I mRNA were unchanged (50). Thus, in some cholesterol-fed animals, including the chick, there appears to be no direct correlation between the hepatic apoA-I synthesis (and/or apoA-I mRNA) and the plasma concentrations of HDL and apoA-I. The reasons for the decreased plasma level of HDL and apoA-I in cholesterol-fed chicks are poorly understood. One explanation may be a reduced production of apoA-I by the intestine that in the chick is a main source of apoA-I (52). This hypothesis seems unlikely for several reasons: *a)* in cholesterol-fed rats apoA-I mRNA translation activity in the intestine is increased approximately 2-fold (53); *b)* in various strains of cholesterol-fed mice there are no changes in the rate of intestinal synthesis of apoA-I (54); and *c)* no reduction of intestinal apoA-I mRNA level was reported in human apoA-I transgenic mice fed a HF/HC diet (47). A second explanation for the de-

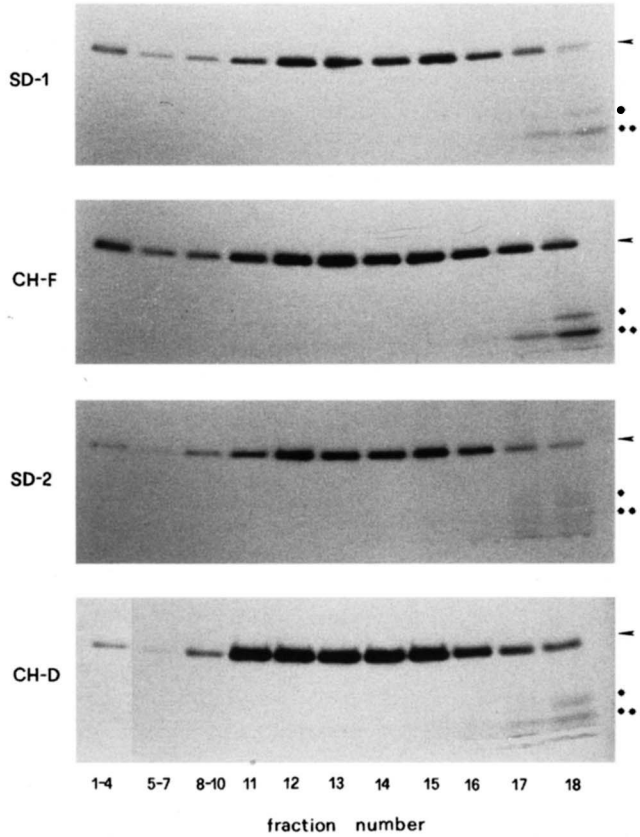

Fig. 8. Fluorograms of ^{35}S-labeled apoA-I of medium lipoproteins. ^{35}S-labeled lipoprotein fractions isolated from the incubation media by density gradient ultracentrifugation (as shown in Figs. 5, 6) were pooled and analyzed on a 5–20% linear gradient SDS-PAGE (see Methods). Equal volumes of each density fraction were applied to each lane. Fraction number refers to the density fractions 1–18 of the density gradient profiles shown in Figs. 5 and 6. The arrow indicates the migration of plasma apoA-I and the stars indicate some minor peptides (12 kD* and 9 kD**) of HDL.

TABLE 6. Incorporation of [^{3}H]oleic acid into cholesteryl esters

Groups	Experiment 1			Experiment 2		
	Hepatic ^{3}H-CE	^{3}H-CE VLDL-LDL	^{3}H-CE HDL	Hepatic ^{3}H-CE	^{3}H-CE VLDL-LDL	^{3}H-CE HDL
	dpm × 10^3/g/h	*dpm* × 10^3/*g*	*dpm* × 10^3/*g*	*dpm* × 10^3/g/h	*dpm* × 10^3/*g*	*dpm* × 10^3/*g*
SD-1	1430.1	40.6	10.8	1504.3	32.9	9.5
CH-F	5603.8	117.8	33.7	4504.9	105.8	24.3
SD-2	1212.8	27.6	10.2	1208.5	22.8	9.7
CH-D	1101.4	30.1	12.6	967.9	28.0	14.4

Liver slices taken from 4 animals per group were pooled and pulse-labeled with [^{3}H]oleic acid-albumin complex for 1 h and chased for 3 h. At the end of the pulse period, hepatic ^{3}H-labeled cholesteryl esters (CE) were measured in liver slice homogenates. At the end of the chase period, the medium was subjected to density gradient ultracentrifugation to isolate labeled VLDL-LDL (d < 1.063 g/ml) and labeled HDL (d 1.063–1.210 g/ml). The content of ^{3}H-labeled CE in lipoprotein fractions was measured (see Methods). In each experiment, each value represents the average of duplicate incubations.

creased HDL level in CH-F chicks may be a reduced intravascular lipolysis of VLDL (lipoprotein lipase-resistant β-VLDL) that are a poor substrate for lipoprotein lipase in view of their low triglyceride content (48). This hypothesis is supported by the observation that the presence of lipoprotein lipase-resistant VLDL in the laying hen (55) is accompanied by a reduced level of plasma HDL (56). Finally, it is possible that in cholesterol-fed chicks there is an increased catabolism of HDL. High density lipoproteins of CH-F chicks are smaller in size (Tarugi, P., unpublished observation), have a higher density and a reduced phospholipid content (48) as compared to HDL of control chicks. These changes may render these lipoproteins more susceptible to tissue uptake and catabolism.

Taken together, the evidence from the present study indicates that in response to CE accumulation in chick liver induced by cholesterol feeding, a larger proportion of newly synthesized apoB is available for secretion as a constituent of VLDL, IDL, and LDL, and more apoA-I is synthesized and incorporated in all lipoprotein density classes. The increased secretion of apoB- and apoA-I-containing lipoproteins presumably contributes to the removal of CE from the liver (Table 6).

Our results underline the role of hepatic cholesteryl ester content in the regulation of apoB and apoA-I production by chick liver.

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