

Low density and high density lipoprotein turnover following portacaval shunt in swine

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Abstract Turnover of ¹²⁵I-low density lipoprotein (LDL) and of ¹³¹I-high density lipoprotein (HDL) was determined before and after end-to-side portacaval shunt in eight swine. LDL (d 1.019–1.063) and HDL (d 1.09–1.21) were isolated by ultracentrifugation and iodinated by the iodine monochloride technique. Immediately postoperatively there was no consistent change in the fractional catabolic rate (FCR) of LDL compared to preoperative control values, while in all animals FCR of HDL was significantly increased (by as much as 300%). After recovery from surgery, neither LDL nor HDL catabolic rates were significantly elevated above control values in four swine. However, plasma levels of LDL and HDL protein, and of LDL and HDL cholesterol were significantly reduced 10–12 weeks after the portacaval shunt. The reduced levels of LDL and HDL associated with normal fractional clearance rates imply a reduction in synthesis of LDL and HDL following portal diversion.

Supplementary key words radioiodine · metabolism · cholesterol · apoprotein turnover · liver · lipoprotein degradation · lipoprotein synthesis · kinetics

Starzl and coworkers (1–3) recently reported a dramatic decrease in plasma cholesterol levels in a patient with homozygous familial hypercholesterolemia (HFH) after end-to-side portacaval shunting. Preoperatively, cholesterol levels ranged from 600 to 800 mg/dl and were unresponsive to diet or drug therapy; postoperatively, cholesterol levels fell progressively to a mean value of approximately 300 mg/dl by six months and there was striking clinical improvement. At least six additional homozygous familial hypercholesterolemia cases have been similarly treated (4, 5) and all have shown a decrease in cholesterol levels, although less dramatic, and varying degrees of clinical improvement.

The mechanism underlying these responses to portacaval shunting has not been established. Preoperatively the patients have shown a significant response to sustained intravenous alimentation but how this relates to the effects of shunting is not clear. We have previously reported that LDL re-

moval from the plasma continues after total hepatectomy in swine and in dogs (6), implying that peripheral tissues play a quantitatively important role in LDL removal. Moreover, the fractional rate of removal after hepatectomy was actually greater than it was prior to hepatectomy. Since the hepatectomy was accompanied by the creation of a portacaval shunt, we considered the possibility that the increased removal rate might be attributable in part to the portacaval shunting, rather than the absence of the liver per se. The diversion of portal blood (containing high concentrations of bile acids, insulin, glucagon, gastroduodenal hormones and nutrients) might significantly affect the rate of removal of LDL by tissues in the periphery. Accordingly we have measured the fractional catabolic rate (FCR) of ¹²⁵I-LDL before and after end-to-side portacaval shunting in swine. The FCR of ¹³¹I-HDL was determined simultaneously. Total plasma cholesterol levels and plasma LDL and HDL levels were determined serially before and after shunting. Preliminary reports of some of these results have appeared elsewhere (7).

MATERIALS AND METHODS

Duroc or Hampshire swine (20–40 kg) were maintained on a standard chow diet ad libitum (Purina Complete Sow Chow, Ralston-Purina Co., St. Louis, Mo.).

Preparation and labeling of LDL and HDL

Lipoproteins of density 1.019–1.063 and 1.09–1.21 were isolated from swine plasma (containing

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Abbreviations: ¹²⁵I-LDL, ¹²⁵I-labeled low density lipoprotein; ¹³¹I-HDL, ¹³¹I-labeled high density lipoprotein; FCR, fractional catabolic rate.

1 mg/ml EDTA) essentially as described by Havel, Eder, and Bragdon (8) using a 60 Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.). The isolated lipoprotein fractions were diluted with salt solutions of density 1.063 and 1.21, respectively, and centrifuged again in a 50 Ti rotor for 40 hr at 40,000 rpm.

The purified LDL fraction was iodinated with ^{125}I by a modification of the method of McFarlane (9) using glycine-NaOH buffer, pH 10. Ten moles of ICl were added per mole of LDL protein (assumed mol wt 200,000) yielding preparations with about 1 atom of iodine per LDL protein molecule. The purified HDL fraction was iodinated with ^{131}I as described above, except that five moles of ICl were added per mole of HDL protein (assumed mol wt 50,000) but again yielding preparations with about 1 atom of iodine per HDL protein molecule.

Purification of iodinated lipoproteins

Unbound iodide was removed from the ^{125}I -LDL and ^{131}I -HDL by dialysis against 0.1% EDTA-0.9% NaCl. After dialysis, less than 1% of the radioiodine in the sample remained unbound as determined by precipitation with 10% trichloroacetic acid. Prior to injection, the ^{125}I -LDL and ^{131}I -HDL were sterilized by passage through a 0.45 μm Millipore filter (Millipore Corp., Bedford, Mass.). The ^{125}I -LDL and ^{131}I -HDL migrated with freshly prepared, unlabeled swine LDL and HDL on agarose gel electrophoresis.

In this study and in previous studies of LDL turnover in intact and hepatectomized swine (6, 10), we have attempted to assess the integrity of the ^{125}I -LDL by several means. The final ^{125}I -LDL preparation was analyzed by sucrose density-gradient centrifugation to equilibrium. The radioactivity showed a symmetrical peak centered at d 1.04, the distribution being comparable to that of native LDL. At all times after injection of ^{125}I -LDL, more than 95% of the ^{125}I activity in plasma could be recovered in the $d < 1.063$ supernatant. Plasma was taken from primary recipients 4-6 hrs after injection of ^{125}I -LDL, by which time the major part of the rapid initial disappearance had already been completed. When this plasma was injected into a secondary recipient, a biphasic disappearance curve similar in all respects to that seen in the primary recipient was duplicated.

Similar "biological screening" experiments were done with ^{125}I - or ^{131}I -HDL. Again, the disappearance of plasma labeled HDL was biphasic in the secondary recipient and similar in all respects to the curve in the primary recipient. After injection of ^{131}I -HDL less than 3% of the plasma radioactivity could be re-

covered in the $d < 1.063$ supernatant, and 5-10% was recovered in the $d > 1.21$ infranatant fraction. This appeared to be independent of time after injection.

^{125}I -LDL and ^{131}I -HDL turnover studies

Under general anesthesia (sodium pentobarbital) and using aseptic techniques, a polyvinyl catheter was inserted into each external jugular vein and the distal end was brought out through a separate stab incision at the rear of the neck. The animal was allowed to recover from the anesthetic and given free access to food and water (containing 3 drops of Lugol's iodine solution per liter). The following day purified autologous ^{125}I -LDL and ^{131}I -HDL (20-40 μCi) were administered simultaneously to the animal. Plasma samples drawn from the contralateral catheter were obtained at appropriate time intervals up to 79 hr.

Portacaval shunting was performed 6-7 days after the control lipoprotein turnover study. Using aseptic techniques and pentobarbital anesthesia, a right subcostal incision was made and the portal vein and infrahepatic inferior vena cava were isolated. A side-to-side portal vein to vena cava anastomosis was performed using 6-0 Deknatel sutures. The portal vein was then doubly ligated and divided as close to the body of the liver as possible, creating a functional end-to-side portacaval anastomosis. Care was taken to identify pancreatic veins to insure that they were intact and that their outflow was diverted via the portal shunt. Polyvinyl catheters were replaced in the external jugular veins. Plasma samples drawn from the contralateral catheter was obtained at appropriate intervals for periods up to 71 hr following the injection of labeled lipoproteins.

In six animals, ^{125}I -LDL and ^{131}I -HDL (20-40 μCi) were administered simultaneously immediately following surgery and again 5-14 days postoperatively. In two other animals, ^{125}I -LDL and ^{131}I -HDL turnover were determined 120 and 141 days following portacaval shunting. Previous studies have shown that ^{125}I -LDL preparations stored up to 10 days at 4°C in 0.9% NaCl-0.01% EDTA yield disappearance curves identical in all respects to those obtained with the fresh preparation (tested in the same animal) (6). Therefore, studies done up to 10 days postshunt employed the same lipoprotein preparations used for the preshunt studies. For the studies done at later times, fresh lipoproteins were prepared and results were compared to those in an intact control animal studied simultaneously using the same lipoprotein preparations.

Plasma radioiodine measurements and kinetic analysis

Plasma ^{125}I and ^{131}I were separately determined using a dual channel gamma ray spectrometer. Spillover of ^{131}I activity into the ^{125}I channel was corrected by the channels ratio method. The fractional catabolic rate of ^{125}I -LDL and ^{131}I -HDL were determined from analysis of the ^{125}I and ^{131}I plasma radioactivity decay curves, respectively (11). The validity of using total plasma ^{125}I or ^{131}I radioactivity to measure the disappearance of ^{125}I -LDL or ^{131}I -HDL was supported by the following observations. (1) More than 99% of ^{125}I and ^{131}I radioactivity could be precipitated from plasma by 10% trichloroacetic acid in all samples obtained up to 79 hr following isotope injection (the longest time of sampling); (2) at all times following injection of ^{125}I -LDL more than 95% of ^{125}I radioactivity could be recovered in the $d < 1.063$ supernatant fraction; after injection of ^{131}I -HDL more than 85% of ^{131}I -HDL activity was recoverable in the $d 1.063$ – 1.21 density fraction. At all times following injection of ^{131}I -HDL from 5–10% of plasma ^{131}I radioactivity was recovered in the $d 1.21$ infranatant fraction. In this regard, Levy and Fredrickson (12) have demonstrated the presence of HDL apoprotein in the $d 1.21$ infranatant fraction and Roheim et al (13) have observed that following injection of ^{125}I -HDL into rats a small fraction of the plasma radioactivity was recoverable in the $d 1.21$ infranatant fraction. The small but constant fraction of radioactivity recoverable in the $d > 1.21$ fraction by ultracentrifugation suggests either rapid equilibration of HDL protein with free HDL apoprotein in the 1.21 infranatant fraction or dissociation of apoprotein from HDL in the presence of the high salt concentration during ultracentrifugation. The latter explanation is made more likely by our finding that recentrifugation of freshly labeled HDL (without injection into animals) yields radioactivity in the $d 1.21$ infranatant fraction. In any case, the ratio of ^{131}I radioactivity in the $d 1.21$ infranatant fraction to that in the supernatant fraction was constant at all times following injection. Only 1% or less of injected ^{131}I -HDL radioactivity was recoverable in the $d < 1.006$ supernatant fraction. This is consistent with the finding of Eisenberg, Windmueller and Levy (14) that there was less than 1% transfer of radioactivity to the $d < 1.006$ fraction in plasma of rats injected with ^{125}I -labeled human HDL.

The plasma radioactivity decay curve was fitted to a biexponential function using curve-peeling techniques and least squares-fitting with a Burroughs 6700 computer. The slopes (b_1 and b_2) and the

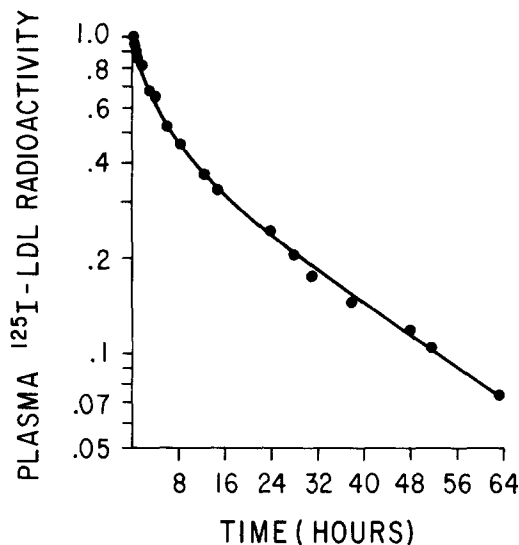


Fig. 1. Representative plasma disappearance curve for ^{125}I -LDL. The plasma radioactivity decay curve was biexponential and could be resolved into separate monoexponential components by curve peeling. The data shown are those of swine 76 studied 141 days following portacaval shunt. All data have been normalized so the 10 min value = 1.0. The solid curve represents the biexponential function, obtained by curve peeling, which best fits the data. The calculated fractional catabolic rate is 0.055 hr^{-1} .

normalized ordinate intercepts (C_1 and C_2 ; $C_1 + C_2 = 1.0$) of the first and second exponentials were used to calculate the fractional catabolic rate (FCR) of irreversible removal of LDL from plasma. The equation for FCR is (11):

$$\text{FCR} = (C_1/b_1 + C_2/b_2)^{-1}$$

Plasma cholesterol and apolipoprotein levels

Plasma cholesterol and lipoprotein-protein levels were determined before and after portacaval shunting in ten swine. In addition to the eight swine in which lipoprotein catabolism studies were performed as described above, plasma cholesterol and lipoprotein levels were determined serially in two other swine that had undergone portacaval shunting. Under light anesthesia (ketamine hydrochloride, ca. 10 mg/kg body wt) blood samples were drawn from fasted animals by venipuncture. Total plasma cholesterol and HDL cholesterol levels were determined by the methods of the Lipid Research Clinics (15). Lipoprotein subfractions of density < 1.006 , < 1.063 , and < 1.21 from three aliquots of plasma were prepared using a 50 Ti rotor at 40,000 rpm for 60 hr. The supernatant fractions were removed by pipette and protein concentrations were measured in each fraction by the method of Lowry et al. (16). Lipoprotein-protein levels in the density ranges < 1.006 (VLDL), 1.006 – 1.063 (LDL), and 1.063 – 1.21 (HDL) were calculated by subtraction.

TABLE 1. ¹²⁵I-LDL Catabolism before and after portacaval shunting: fractional catabolic rate (FCR), slopes and intercepts of biexponential plasma disappearance curves^a

Animal No.	Preshunt			Immediately postshunt			Later postshunt		
	FCR	Slope b_1	Inter- cept C_1	FCR	Slope b_1	Inter- cept C_1	FCR	Slope b_1	Inter- cept C_1
<i>Short-term Studies</i>	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}
38	0.072	0.057	0.211	0.074	0.048	0.235	0.074	0.048	0.444
55	0.047	0.026	0.185	0.057	0.042	0.309	0.057	0.042	0.309
54	0.069	0.038	0.260	0.063	0.050	0.351	0.063	0.050	0.750
72	0.078	0.056	0.202	0.138	0.095	0.598	0.138	0.095	0.627
57	0.054	0.050	0.722	0.066	0.053	0.477	0.066	0.053	0.781
58	0.053	0.044	0.570	0.052	0.040	0.337	0.052	0.040	0.730
<i>Long-term Studies</i>									
Mean	0.062	0.045	0.348	0.075	0.054	0.384	0.075	0.054	0.689
± SD	0.012	0.011	0.219	0.032	0.020	0.130	0.032	0.020	0.084
A ^c	0.065	0.028	0.191	0.333	0.667				
57				0.067 (14d)	0.030	0.184	0.335	0.665	
58				0.065 (14d)	0.030	0.163	0.338	0.662	
B ^c	0.054	0.033	0.287	0.550	0.450				
85				0.055 (120d)	0.031	0.282	0.514	0.486	
76				0.055 (140d)	0.031	0.243	0.502	0.498	

^a Kinetic analysis after Matthews (11) as described in Methods. Plasma radioactivity (normalized so zero time value equals units) = $C_1e^{-b_1t} + C_2e^{-b_2t}$.
^b Number of days post-shunt in parentheses.
^c A is nonoperated animal studied concurrently using the same ¹²⁵I-LDL preparation used in shunted animals 57 and 58; B is nonoperated animal studied concurrently with shunted animals 85 and 76.

Plasma cholesterol and apolipoprotein data were obtained in six swine up to three weeks following shunting (in the earliest studies, the animals were killed immediately following the isotope kinetic studies). Data were obtained from four of the animals for up to 8 weeks, in three animals up to 12 weeks, and in two for up to 17 weeks postoperatively.

One animal was killed at 8 weeks because it became anemic and showed occult blood in the stool. Autopsy revealed evidence of fresh and healed gastric and duodenal ulceration. Another animal died 12 weeks postoperatively and also had evidence of gastric ulcers as well as intestinal parasites (*ascaris*). The portacaval anastomosis was examined at autopsy in each animal studied and was patent in every case.

RESULTS

¹²⁵I-LDL Turnover

The turnover of ¹²⁵I-LDL was determined before and immediately after portacaval shunting in six swine. In all cases the disappearance of ¹²⁵I-LDL from plasma was biexponential over the period of observation. A representative disappearance curve is shown in **Fig. 1**. From 70 to 95% of the injected dose had disappeared from plasma over this period. The biexponential disappearance curves were analyzed as described under Methods to obtain the slopes and intercepts of the individual exponential components for use in calculating FCR (**Table 1**). The FCR of ¹²⁵I-LDL immediately postshunt remained unchanged in three animals, increased by about 20% in two, and 77% in one. The mean value immediately postshunt was 21% above the preshunt value. However, this difference was not statistically significant (*t* test for paired data) and stands in marked contrast to the uniform 50–70% increase in FCR following total hepatectomy in swine (6).

Three of these animals were restudied 5–8 days postshunt using the same ¹²⁵I-LDL preparation used initially. Animal 72, whose FCR immediately postshunt had been 77% above the control value, showed at 8 days an FCR within 10% of the preshunt value. The value in animal 54 remained essentially unchanged from the control value, while that in animal 55 was still 22% above the initial value.

The FCR of ¹²⁵I-LDL was determined in four swine 14–141 days following portacaval shunt (**Table 1**, long-term studies). The FCR in the shunted swine was compared to that of an unoperated control animal injected with the same ¹²⁵I-LDL preparation. In those animals studied at least 14 days postopera-

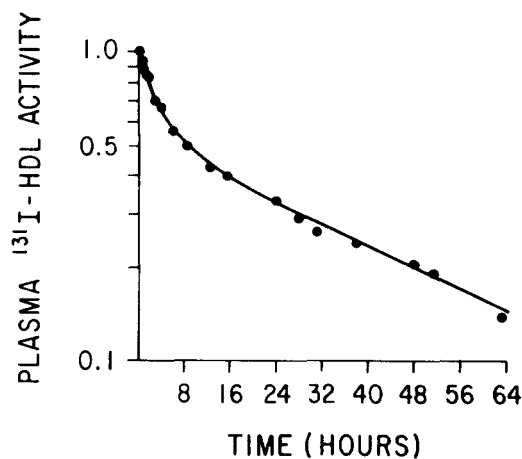


Fig. 2. Representative plasma disappearance curve for ¹³¹I-HDL. Plasma decay of ¹³¹I HDL (d 1.090–1.21) was biexponential as in the case of ¹²⁵I-LDL decay. The solid curve is the best-fit exponential function obtained by curve peeling for the normalized data shown (10 min value = 1.0).

tively, there was in no case a significant difference in the fractional catabolic rate of LDL between shunted and unoperated animals.

¹³¹I-HDL Turnover

In most animals the turnover of ¹³¹I-HDL (d 1.090–1.21) was determined simultaneously with that of ¹²⁵I-LDL before and after portacaval shunting. Plasma ¹³¹I-HDL disappeared in a biexponential fashion and a representative disappearance curve is shown in **Fig. 2**. The calculated FCR for HDL in intact animals was approximately one-half that for LDL (FCR for HDL, 0.029 hr⁻¹; for LDL 0.062 hr⁻¹).

Table 2 shows the FCR values determined before shunt, immediately after shunt, and at later times postshunt in five swine. In every animal there was a marked increase in FCR immediately after the shunt, the mean HDL FCR rising to 0.079 hr⁻¹ at that time. In three swine studies 5–8 days after operation (nos. 54, 55, and 72) the FCR was still elevated but to a considerably lesser degree than in the immediate postshunt period.

FCR was determined in three swine at 14, 120, and 141 days following shunt (**Table 2**, long-term studies). Swine no. 58 was studied 14 days postshunt using a different ¹³¹I-HDL preparation than that used in the preoperative and immediate postoperative studies (short-term studies). The FCR was 0.020 hr⁻¹ in swine no. 58 14 days postshunt. The FCR of HDL in an unoperated control animal studied simultaneously with the same ¹³¹I-HDL preparation was 0.022 hr⁻¹. Thus in this animal (no. 58) the 14 day postshunt value of FCR was slightly below the preoperative control value in the same animal studied

TABLE 2. ¹³¹I-HDL Catabolism before and after portacaval shunting: fractional catabolic rate (FCR), slopes and intercepts of biexponential plasma disappearance curves^a

Animal No.	Preshunt				Immediately postshunt				Later postshunt				
	FCR	Slope b_1	Slope b_2	Inter- cept C_1	FCR	Slope b_1	Slope b_2	Inter- cept C_1	FCR	Slope b_1	Slope b_2	Inter- cept C_1	Inter- cept C_2
<i>Short-term Studies</i>	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}	hr^{-1}
38	0.030	0.024	0.199	0.764	0.084	0.052	0.357	0.552	0.448				
55	0.028	0.017	0.306	0.600	0.044	0.028	0.212	0.586	0.414	0.033 (5d) ^b	0.022	0.213	0.623
54	0.022	0.014	0.277	0.589	0.411	0.066	0.040	0.505	0.495	0.048 (7d)	0.031	0.549	0.621
72	0.037	0.024	0.223	0.604	0.396	0.141	0.090	0.577	0.423	0.058 (8d)	0.029	0.275	0.453
58	0.026	0.019	0.627	0.736	0.264	0.033	0.337	0.710	0.291				
Mean	0.029	0.019	0.326	0.658	0.079	0.018	0.356	0.586	0.414	0.046	0.027	0.345	0.565
± SD	0.006	0.004	0.173	0.084	0.040	0.024	0.190	0.076	0.075	0.010	0.004	0.178	0.097
<i>Long-term Studies</i>													
A ^c	0.022	0.013	0.182	0.587	0.413								
58					0.020 (14d)	0.013	0.207	0.602	0.398				
B ^c	0.032	0.022	0.333	0.661	0.339								
85					0.035 (120d)	0.024	0.313	0.656	0.342				
76					0.038 (140d)	0.021	0.296	0.533	0.467				

^a Kinetic analysis after Matthews (11) as described in Methods section. Plasma radioactivity (normalized so zero time value equals unity) = $C_1e^{-b_1t} + C_2e^{-b_2t}$.

^b Number of days post-shunt given in parentheses.

^c A is nonoperated animal studied concurrently using the same ¹³¹I-HDL preparation used in shunted animal 58; B is nonoperated animal studied concurrently with shunted animals 85 and 76.

with a different HDL preparation, but it did not differ significantly from that determined simultaneously in a control animal using the same ^{131}I -HDL preparation. In swine 85 and 76, studied 120 and 141 days postshunt, the FCR values were 0.035 and 0.038 hr^{-1} , respectively. These were only slightly higher than the value of 0.032 hr^{-1} in an unoperated control animal studied simultaneously. The small differences in FCR between the shunted animals and the control animals are probably within the experimental error of the method.

Plasma cholesterol and apolipoprotein levels

Fig. 3 shows the changes in fasting cholesterol levels (expressed as a percentage of preoperative values) as a function of time after portacaval shunting for seven animals. The control values for total plasma cholesterol ranged 71–93 mg/dl, while those for HDL and LDL cholesterol ranged 25–36 mg/dl and 45–57 mg/dl, respectively. LDL cholesterol was estimated as total cholesterol minus HDL cholesterol (12); in samples from four swine in which VLDL cholesterol was separately determined, the VLDL cholesterol represented less than 10% of the sum of LDL plus VLDL cholesterol.

Cholesterol levels were determined in seven animals over a two week period following portacaval shunting. Four animals were followed for 8 weeks, three animals for 12 weeks, and two animals for 17 weeks. During the first two weeks postoperatively, total plasma cholesterol in seven animals (means of three determinations each) fell on average to $73 \pm 12\%$ (SD) of their respective control values ($P < 0.02$). During the fourth to the sixth and the sixth to the eighth postoperative weeks, the four animals studied showed some recovery, but cholesterol levels were still below preoperative values ($85.2 \pm 4.1\%$ and $84.6 \pm 6.8\%$ of control levels; significant at the $P < 0.01$ and $P < 0.02$ levels, respectively.²) In the three animals studied during the 10th to the 12th postoperative weeks, total plasma cholesterol values had decreased to $66.9 \pm 4.0\%$ of control ($P < 0.01$). HDL and estimated LDL cholesterol levels followed the same pattern as did the total plasma cholesterol, although the reduction in estimated LDL cholesterol levels during the 10th to the 12th postoperative week ($51.0 \pm 18.5\%$; $P < 0.02$ with respect to control levels; $n = 3$) was relatively much larger than the reduction in HDL cholesterol levels during this later period ($87 \pm 5.3\%$ of control levels; $P < 0.05$ with respect to controls; $n = 3$).

² *t* Test for paired data. For each animal, mean control level was compared to mean of two or more levels measured during time intervals given.

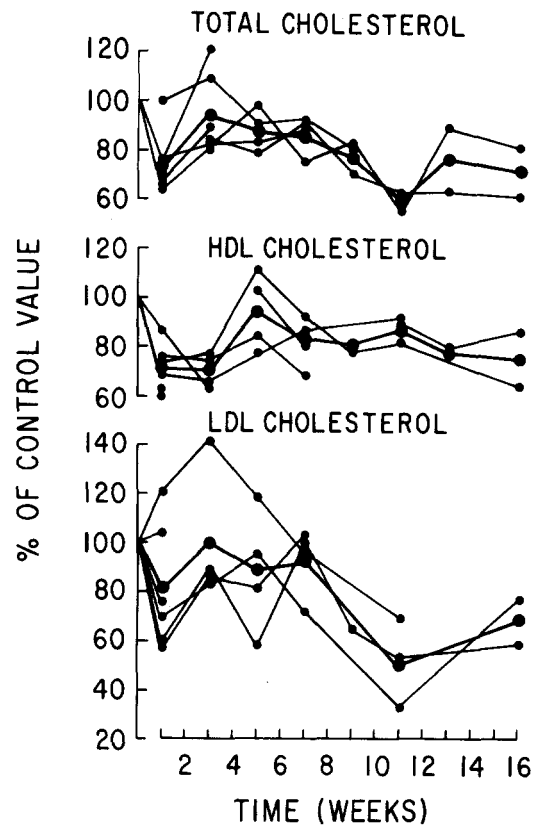


Fig. 3. Plasma cholesterol levels following portacaval shunt. The data represent values of total plasma cholesterol, HDL cholesterol, and estimated LDL cholesterol as functions of time following portacaval shunting in six swine. Values are expressed as a percentage of the preoperative control values which ranged 71–93 mg/dl for total plasma cholesterol, 25–36 mg/dl for HDL cholesterol, and 45–57 mg/dl for estimated LDL cholesterol. (●—●) Values for individual animals; (●—●) mean values for all animals represented. Six animals were followed for 3 weeks postshunt; four of these were studied to 8 weeks postshunt; three for 12 weeks and two for the full 16 weeks. The values for LDL cholesterol were estimated by subtracting the HDL cholesterol values from the total cholesterol values (see text).

The postoperative changes in plasma LDL and HDL cholesterol were closely paralleled by changes in the levels of isolated LDL and HDL apoprotein (**Fig. 4**). The absolute preoperative control values ranged 377–450 μg of LDL apoprotein/ml and 732–1380 μg of HDL apoprotein/ml. A postoperative nadir in apoLDL and apoHDL levels was reached in the first week or two following portacaval shunting in five of the six animals in which apoprotein measurements were made. The sixth animal actually showed an *increase* in apoLDL and apoHDL levels with respect to its control value and thus the mean reduction was not statistically significant ($86 \pm 17\%$ of control values). At six to eight weeks postoperatively, apoLDL levels were reduced significantly ($71.0 \pm 13.6\%$ of control; $P < 0.05$; $n = 4$) while reductions in apoHDL were not significant at the

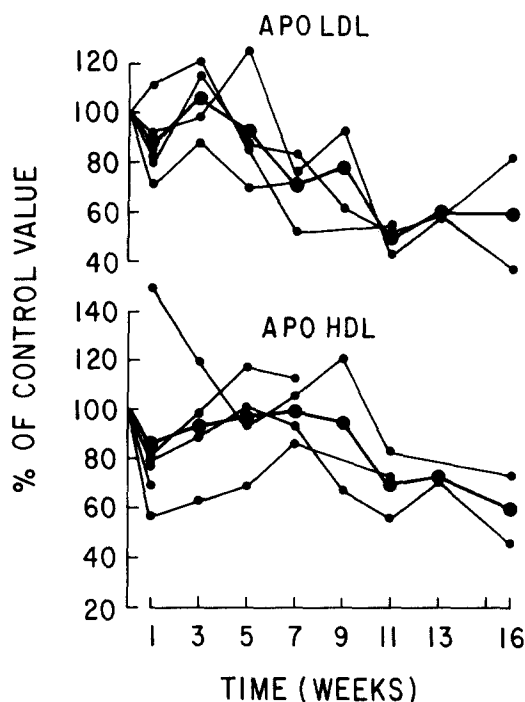


Fig. 4. Lipoprotein protein levels in plasma following portacaval shunt. Ultracentrifugally isolated LDL (d 1.006–1.063) and HDL (d 1.063–1.21) protein concentrations were determined as a function of time following portacaval shunting (see text for details). The data are shown as a percentage of the preoperative control values (preoperative LDL protein, 377–450 $\mu\text{g}/\text{ml}$; HDL protein, 732–1380 $\mu\text{g}/\text{ml}$). Data from six swine are shown as in Figure 3: (●—●) values for individual animals; (●—●) mean values for all animals.

0.05 level. By the 10th to 12th week, apoLDL was reduced to $50.3 \pm 6.4\%$ of control ($P < 0.01$; $n = 3$) and apoHDL reduced to $70.0 \pm 13.1\%$ of control ($P = 0.058$; $n = 3$).

Strict comparison of the plasma LDL and HDL apoprotein levels with HDL and estimated LDL cholesterol levels and the calculation of cholesterol-to-protein ratio in the two lipoproteins were not attempted because of the differing isolation techniques used in the cholesterol and apolipoprotein determinations. The changes in mean levels of LDL and HDL cholesterol (Fig. 3) correspond reasonably well with changes in mean levels of apoLDL and apoHDL, respectively (Fig. 4). It was not possible from the present data, however, to rule out slight changes in cholesterol-to-protein ratio in the two lipoprotein fractions, because sterol and protein measurements were not carried out on aliquots of the same fraction (see Methods).

DISCUSSION

Except for some transient changes in the immediate postoperative period, portacaval shunting did not

significantly affect the fractional catabolic rate of LDL in these studies. In the four animals studied 2–20 weeks following shunting, there was no detectable difference in the fractional catabolic rate of LDL compared to that measured simultaneously in unoperated control animals. Even immediately postshunt the changes were minimal except in one of six animals. This is in contrast to the consistent and marked increase ($67.1 \pm 16.4\%$) always seen after portacaval shunting followed by total removal of the liver (6). Thus it is clear that the increased fractional rate of LDL catabolism after hepatectomy is not simply due to the shunting of portal blood around the liver.

The FCR for HDL immediately postshunt was elevated in every case, in some by as much as 300%. However, the values dropped sharply toward normal 5–8 days postshunt and, in the three animals studied 2–20 weeks postshunt, the FCR was well within normal limits. The cause of the immediate but transient increase in HDL removal rate after shunting is not clear, and may reflect merely an effect of the surgical trauma. Certainly transient alterations in serum lipid levels following stress are known to occur. If HDL is predominantly cleared by the liver, however, as has been proposed (17), one might have expected a *decrease* in removal rate since total hepatic blood flow is reduced (18). On the other hand, the substantive metabolic changes occurring with shunting might very well enhance hepatic removal even in the face of reduced blood flow. Nevertheless, these results raise the question of whether or not the liver is the only organ involved in HDL catabolism.

The decrease in cholesterol levels (total, HDL, and LDL) during the first week or two postoperatively is difficult to interpret because of the changes in nutritional and hormonal status produced by the surgery per se. In fact, most animals showed a return toward preshunt values by the 3rd or 4th week, suggesting that the early fall in levels was a transient effect of shunting or was related in some way to the stress of surgery. Then, however, levels declined slowly so by the tenth to the twelfth week total cholesterol level was $66.9 \pm 4.1\%$, ($P < 0.01$), LDL cholesterol $51.0 \pm 18.5\%$ ($P < 0.02$), and HDL cholesterol $87 \pm 5.3\%$ ($P < 0.05$) of control values, respectively. The apoprotein levels in LDL and HDL fell in similar fashion. Since the FCR for both LDL and HDL had returned to normal values by 2 weeks and remained normal thereafter, net flux or turnover of both lipoproteins was reduced. Since the animals were not in an absolute steady state with regard to their lipoprotein levels, even in the

later studies, one must ask to what extent the calculated parameters may be in error as a consequence, i.e., would changes in pool size during a kinetic study affect the result significantly? First, the rate of change in lipoprotein levels was quite slow in the long-term studies. Second, the FCR of LDL is probably independent of plasma LDL pool size as suggested by the results of the present study and those of previous studies (6, 19). In the four swine studied 2–20 weeks following portacaval shunt, LDL fractional catabolic rate did not differ from control despite a 20–50% decrease in plasma LDL levels. In hepatectomized swine (6) plasma LDL levels fell mono-exponentially following removal of the liver, indicating that the fractional catabolic rate did not change as LDL levels fell, but instead remained strictly first order. Recently, Thompson and Myant (19) have shown that LDL fractional catabolic rate was unaltered in homozygous familial hypercholesterolemic patients during and after reduction in LDL levels by means of plasmaphoresis.

It would seem, therefore, that the estimates of fractional catabolic rate obtained in the present study are valid. However it does not necessarily follow that these values can be used to derive valid data for the synthetic rate. The complication that arises in determining the synthetic rate in the non-steady state condition can be easily seen by considering a one pool system with a mass M , an input rate U (not necessarily constant) and a fractional catabolic rate K (presumed constant). We can ignore for the moment the additional complication of an equilibrating extravascular pool. The rate of change of mass in the pool (e.g. plasma) would be

$$dM/dt = -kM + U$$

assuming that the system is first order. It is clear that when the system is in steady state (i.e. $dM/dt \equiv 0$), $kM = U$ (flux out = flux in). In the non-steady state case where dM/dt is not zero, this term must be included in the calculation of the synthetic rate, U . The correction will be of negligible magnitude if the rate of change of the pool size (dM/dt) is small compared to the flux of material out of the pool ($-kM$). Such appears to be the case in the present study. Taking the mean FCR for LDL to be 0.062 hr^{-1} and multiplying by the estimated plasma pool size ($400 \mu\text{g LDL protein/ml} \times 1500 \text{ ml}$), we obtain an hourly flux of LDL leaving plasma irreversibly of $\sim 36 \text{ mg/hr}$. This is to be compared to a rate of change in LDL pool size dM/dt which at most was 0.9 mg/hr (i.e. $200 \mu\text{g/ml}/2 \text{ weeks} \times 1500 \text{ ml}$). For practical purposes this small correction is probably insignificant and thus we can interpret the changes in LDL pool

size (shown in Fig. 4) to directly reflect alteration in synthetic rate.³

Starzl and coworkers (20) have recently reported a small (25%) but significant reduction in plasma cholesterol and phospholipid levels in dogs over a 2-month period after portacaval shunting. In baboons, anastomosis of the superior mesenteric vein to the vena cava did not depress plasma lipid levels whereas a subsequent end-to-side complete portacaval shunt did depress them (20), implying that shunting of pancreatic hormones to the periphery may be of critical importance (21). Examination of the livers in the animals subjected to portal vein–vena cava shunting showed a reduction in liver mass, reduction in the size of the hepatocytes with depletion of glycogen, swelling of mitochondrial cisternae, and reduction in the amount of rough endoplasmic reticulum. Similar histologic changes were seen in a liver biopsy taken from a patient with familial hypercholesterolemia 6 months after portacaval shunting (1).

Although there was no systematic attempt in the present study to delineate changes in growth or hepatocellular morphology following portacaval shunting, the animals studied showed reduced liver mass and did not maintain the growth rate normally associated with swine of this age (4–6 months old at the time of operation). Cuschieri et al. (22) and Baker, Cuschieri, and Hanson (23), have reported detailed findings on growth, nutrition, and liver function in portacaval shunted swine. They reported a loss of liver mass and evidence of hepatocellular damage (in swine) similar to that observed by Starzl et al. (1, 2, 20) in humans, dogs, and baboons.

Bilheimer et al. (5) have measured FCR of iodinated LDL in a patient with familial hypercholesterolemia before and again 5 months after portacaval shunting. FCR did not increase despite a net fall in LDL cholesterol level from 801 to 492 mg/dl. All of these findings, together with the present experimental results on LDL turnover, support the conclusion that changes in hepatic function with a reduction in the rate of lipoprotein production accounts for the decreases in plasma lipoprotein levels produced by portacaval shunting. ■

³ We have ignored in this discussion the contribution of the extravascular pool(s). Analysis of the plasma decay curve (data shown in Tables 1 and 2) enables us to calculate exchange rate of the plasma LDL with the extravascular LDL pools (see Matthews (11)). It can be shown that this, too, is rapid compared to the rate of change in plasma pool size and thus should not significantly affect the close relationship between changes in plasma pool size and changes in synthetic rate.

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