Bile acid kinetics in relation to endogenous tryglyceride metabolism in various types of hyperlipoproteinemia

Bo Angelin, Kurt Einarsson, Kjell Hellström, and Barbro Leijd

Department of Medicine, Karolinska Institutet at Serafimerlasarettet, Stockholm, Sweden

Abstract Bile acid and plasma endogenous triglyceride kinetics were determined under standardized dietary conditions in 47 hyperlipidemic subjects with the aid of [^{14}C]cholic acid, [^{14}C]chenodeoxycholic acid, and [^{3}H]glycerol, respectively. On the basis of their lipoprotein pattern the patients were separated into three groups characterized by hyperlipoproteinemia (HLP) type IIa ($n = 19$), type IIb ($n = 6$), and type IV ($n = 22$). In keeping with previous reports from this laboratory the total bile acid formation in HLP type IV (19.5 ± 2.2 μmol kg^{-1}d^{-1}, mean ± SEM) exceeded that encountered in type IIa (10.7 ± 0.9 μmol kg^{-1}d^{-1}, $P < 0.005$). This difference was mainly due to an increased synthesis of cholic acid in type IV HLP (12.7 ± 1.7 μmol kg^{-1}d^{-1} vs. 6.1 ± 0.5 μmol kg^{-1}d^{-1}, $P < 0.005$). Bile acid formation in type IIb HLP was essentially within the limits recorded for type IIa. Apparent plasma triglyceride formation (as calculated from the 10-hr radioactivity decay curve) averaged 10.5 ± 0.7 μmol kg^{-1}hr^{-1} in type IIa HLP and was significantly higher in type IIb (20.7 ± 1.9 μmol kg^{-1}hr^{-1}, $P < 0.001$) and in type IV (22.1 ± 1.4 μmol kg^{-1}hr^{-1}, $P < 0.001$). The apparent fractional turnover rate of plasma triglyceride in type IV HLP (0.147 ± 0.011 hr^{-1}) was lower than that encountered in type IIa (0.188 ± 0.008, $P < 0.01$) and in type IIb (0.177 ± 0.011 hr^{-1}). The apparent production of plasma triglycerides and the formation of cholic acid correlated in type IIa ($r = +0.69$, $P < 0.001$) and in type IV HLP ($r = +0.70$, $P < 0.001$). A similar pattern was seen for total bile acid formation, while chenodeoxycholic acid showed a correlation to apparent triglyceride synthesis only in type IV HLP. It is suggested that an increased formation of plasma triglycerides—monitoring very low density lipoprotein synthesis—is linked to an enhanced degradation of cholesterol to bile acids and that there is an integrated regulation of the metabolism of these two parameters.

Supplementary key words cholesterol · cholic acid · chenodeoxycholic acid · low density lipoprotein · very low density lipoprotein

Hyperlipoproteinemia (HLP) is the result of an imbalance between the formation and degradation of either the lipoprotein in toto or any of its constituents. The type IV lipoprotein pattern (1) has been reported to be due to overproduction of very low density lipoprotein (VLDL) (2–5), decreased clearance of VLDL (6–10), or both (11–13). As evidenced by studies in heterozygous familial hypercholesterolemia (HLP type IIa), elevation of the serum low density lipoprotein (LDL) level is linked to an impaired fractional turnover of the LDL particles (14, 15).

Several studies have indicated abnormalities in cholesterol and bile acid metabolism in primary HLP (16–26). Type IIa and type IIb HLP are both associated in general with a subnormal production of cholic acid (C), while total bile acid formation and the net cholesterol "balance" remain within normal limits (22, 26). A different pattern is often encountered in type IV HLP, which is frequently though not invariably characterized by an enhanced formation of bile acids, particularly cholic acid, and an increased net steroid balance (22, 26). The heterogeneous pattern of bile acid (22) and VLDL (2–13) formation in patients with type IV HLP initiated the present investigation, in which the metabolisms of both bile acids and plasma endogenous triglyceride were studied simultaneously under standardized conditions in patients with three types of HLP. The heterogeneity concerning these two variables in type IV patients is confirmed. The data also suggest the existence of a close correlation between the two parameters, indicating an integrated regulation of their metabolism. A preliminary report of some of these findings has been given (27).

METHODS

Subjects

The patients ($n = 47$) were those consecutively admitted because of primary HLP at the time of the in-

Abbreviations: C, cholic acid; CD, chenodeoxycholic acid; GBD, gallbladder disease (cholelithiasis, cholecystitis, cholecystectomy); HLP, hyperlipoproteinemia; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

vestigation. They were separated into three groups on the basis of their lipoprotein pattern (see below). None showed evidence of intestinal, hepatic, or renal disease, hyper- or hypothyroidism, or addiction to alcohol or narcotics. Patients with obesity (body weight exceeding 120% of ideal), HLP type III or type V were excluded. A few patients were not included due to nonacceptability of their triglyceride elimination curves (see below). An oral cholecystogram was obtained in all noncholecystectomized subjects. Basal data and clinical diagnoses of the patients are listed in **Table 1** and lipid levels in **Table 2.** Genetic analysis (28), carried out in some of the patients, revealed familial hypercholesterolemia in patients no. 2, 8, 9, 11, 18, and 19, familial combined hyperlipidemia in patients no. 20 and 25, and HLP of polygenetic origin in patient no. 13. Patient no. 27 had familial type IV HLP.

Experimental procedure

The patients were hospitalized during the study. For 4–7 days before and during the investigation they were maintained on a standardized diet of natural type. About 40% of the energy content was supplied as fat, most of which contained saturated fatty acids. The major part of the carbohydrates, which accounted for 39% of the calories, was supplied as starch. The energy intake, calculated from standard foodstuff tables,[1] was adjusted to keep the body weight constant. The intake of cholesterol was about 0.5 mmol/day in each subject.

The first part of the experimental protocols covered the determination of bile acid kinetics. The sodium salts of [^{14}C]cholic acid (C) (4 μCi) and [^{14}C]chenodeoxycholic acid (CD) (4 μCi) dissolved in water were administered orally as a mixture in the morning before breakfast. Four to five samples of duodenal bile were collected from each subject at intervals of 2–4 days. Cholecystokinin was administered intravenously and samples of concentrated bile (5–10 ml) were obtained through a thin polyvinyl tube. The specific radioactivities of C and CD were determined in each sample.

A few days after the end of the bile sampling period, the subjects received an intravenous injection of [^{3}H]glycerol (40 μCi), dissolved in 10 ml of saline, in the morning after an overnight fast. Venous blood samples were drawn from an indwelling catheter in the opposite arm at intervals of 30 min from 2 to 6 hr and then at 7, 8, and 10 hr after the injection of the isotope. A meal was served after the 8 hr sample. The radioactivity in plasma triglycerides was determined in all samples, and the level of the plasma triglycerides was measured in specimens obtained at 0, 2, 4, 6, and 8 hr.

Venous blood samples were drawn several times during the experimental period and analyzed for cholesterol, triglycerides, and lipoprotein pattern.

Material

[24-^{14}C]Cholic acid (138 μCi/mg) and [24-^{14}C]chenodeoxycholic acid (138 μCi/mg) were purchased from New England Nuclear Corp., Boston, MA. The radiochemical purity of the labeled bile acids was ascertained by radioautography of thin-layer chromatograms. [2-^{3}H]Glycerol (200 μCi/μmol) was obtained from the Radiochemical Centre, Amersham, England. Prior to use, the material was evaporated to dryness in vacuo in order to eliminate [^{3}H]water. Cholecystokinin was obtained from the Gastrointestinal Hormone Research Group, Department of Chemistry, Karolinska Institutet, Stockholm, Sweden.

Methods

Cholesterol and triglycerides were measured with a Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, NY). Lipoprotein phenotyping was performed according to WHO recommendations (1) as described earlier (22). The upper normal limit was set at 7.2 mmol/l for total cholesterol, at 5.2 mmol/l for β-lipoprotein cholesterol, and at 2.0 mmol/l for triglycerides. Glucose tolerance was determined with an intravenous glucose tolerance test (29), k values below 0.9 being considered abnormal.

Measurements of bile acid kinetics

The duodenal bile samples were hydrolyzed with 1 M KOH in closed steel tubes for 12 hr at 110°C. The deconjugated bile acids were extracted with ethyl ether after acidification, and their methyl esters were separated by thin-layer chromatography (30). The separation of bile acids was complete, and only trace amounts of deoxycholic acid contaminant were occasionally observed in the CD fraction. One aliquot was then quantitated by gas–liquid chromatography after preparation of the trimethylsilyl derivatives. A 1.5% SE-30 column was used. Another aliquot from this extract was analyzed for radioactivity by liquid scintillation spectrometry. On the basis of the specific radioactivity curve from the 4–5 time points obtained, the fractional turnover rate, pool size, and rate of synthesis were determined for C and CD according to the principles described by Lindstedt (31). The mean standard error of the fractional turnover rate was 7% and 8% for C and CD, respectively, and the correlation coefficients ranged from 0.961 ($P < 0.05$) to 1.000 ($P < 0.001$) for both bile acids. All studies were

[1] Abramson, E. 1971. Kosttabell. Läromedelsförlagen AB, Stockholm, Sweden.

TABLE 1. Basal data on the individual patients

Patient Number	Sex, Age	Body Weight		Glucose Tolerance, k-value	Previous History, Present Symptoms[b]
		kg	Relative (% of ideal)[a]		
Hyperlipoproteinemia type IIa					
1. KL	M 65	82	110	0.6	
2. KE	M 55	76	113	2.9	IHD, GBD, xanthoma
3. KJ	M 57	73	99	1.3	
4. KH	M 49	65	93	1.4	
5. ES	M 48	68	88	1.4	
6. EH	M 43	86	105	1.3	IHD, HT
7. BJ	M 34	83	117	0.7	IHD
8. BLg	M 33	70	93	1.3	
9. BLn	M 32	66	83	1.5	
10. EA	F 63	73	120	2.4	IHD, xanthelasma
11. AR	F 58	53	86	1.8	IHD, xanthoma, xanthelasma
12. AW	F 58	46	77	1.3	IHD, GBD
13. KW	F 57	73	109	2.1	HT
14. GB	F 56	52	87	1.4	
15. GN	F 64	55	82	0.8	
16. IB	F 54	70	111		GBD
17. MM	F 48	63	95	1.1	GBD
18. EHn	F 41	46	92	1.6	
19. IE	F 24	60	95	1.6	
Hyperlipoproteinemia type IIb					
20. PP	M 65	70	96	1.0	IHD
21. EO	M 57	73	94		DM
22. HR	M 30	83	115	1.3	
23. VL	F 56	81	114	1.6	HT, GBD
24. MD	F 54	59	92	1.4	HT, GBD
25. LP	F 54	68	111	1.0	Xanthelasma
Hyperlipoproteinemia type IV					
26. HH	M 66	87	109	1.2	IHD, GBD
27. HE	M 61	87	110	0.9	HT
28. JM	M 57	82	91	0.8	IHD, GBD
29. TL	M 55	84	111	1.4	HT
30. KP	M 55	89	120	1.6	IHD
31. KB	M 54	69	91	1.2	
32. GG	M 51	80	99	1.8	
33. SG	M 50	91	108	0.9	
34. PK	M 50	76	109	0.7	
35. KEd	M 44	64	93	1.4	HT
36. GL	M 42	73	106	1.4	
37. HL	M 39	84	111	0.8	
38. RK	M 54	76	109	1.4	GBD
39. SM	M 49	83	114	1.0	
40. LA	M 58	83	106	0.6	IHD, GBD
41. NH	M 53	77	100		DM, HT, IHD
42. SR	M 68	69	109	0.5	IHD
43. EC	F 65	60	97	1.5	HT, GBD
44. MJ	F 69	60	98	0.9	HT, GBD
45. AG	F 68	71	101	1.3	IHD, HT, GBD
46. JT	F 59	60	94		IHD, HT, DM
47. AF	F 51	66	110	1.0	

thus adequately fitted with a single exponential function. Further details of this method have been given in a previous paper (22).

Measurements of plasma triglyceride turnover

The method used was described originally by Farquhar et al. (32) and later by Nikkilä and Kekki

TABLE 1. (*Continued*)

Patients (Number)	Age	Body Weight kg	Body Weight Relative (% of ideal)[a]	Abnormal Glucose Tolerance (% of ideal)	(% of ideal)
Hyperlipoproteinemia type IIa					
M (9)	46 ± 4[c]	74 ± 3[c]	100 ± 4[c]	2	
F (10)	52 ± 4	59 ± 3[e]	96 ± 4	1	
Total (19)	49 ± 3	66 ± 3	98 ± 3	3	
Hyperlipoproteinemia type IIb					
M (3)	51 ± 11	75 ± 4	102 ± 7	1	
F (3)	55 ± 1	69 ± 6	106 ± 7		
Total (6)	53 ± 5	72 ± 4	104 ± 4	1	
Hyperlipoproteinemia type IV					
M (17)	53 ± 2	80 ± 2	106 ± 3	6	
F (5)	62 ± 3[d]	63 ± 2[f]	100 ± 3	1	
Total (22)	55 ± 2	76 ± 2[g]	105 ± 2	7	

[a] Calculated as $\frac{\text{weight (kg)}}{\text{height (cm)} - 100} \times 100\%$.

[b] IHD, ischemic heart disease; HT, hypertension; DM, diabetes mellitus; GBD, gallbladder disease.

[c] Mean ± SEM.

[d] Significantly different from males with the same lipoprotein pattern, $P < 0.05$.

[e] Significantly different from males with the same lipoprotein pattern, $P < 0.01$.

[f] Significantly different from males with the same lipoprotein pattern, $P < 0.001$.

[g] Significantly different from type IIa patients, $P < 0.01$.

(11, 33, 34). Plasma was separated immediately by centrifugation and 2-ml samples of each specimen were extracted with 25 ml of chloroform–methanol 2:1 (v/v). NaCl (0.5%, 5 ml) was added, the tubes were shaken vigorously for 5 min and left at 4°C overnight. The chloroform phase (separated by centrifugation) was washed once with 5 ml of a mixture containing chloroform–methanol–0.5% NaCl 3:47:50 (v/v/v), and subsequently treated with silicic acid to eliminate the phospholipids. The residue obtained after filtration and evaporation of the solvent was analyzed for radioactivity by liquid scintillation counting. As evidenced by thin-layer chromatography, more than 95% of this label accumulated in the triglyceride fraction.

Radioactivity was plotted against time on a semilogarithmic scale and the curve was analyzed with a digital computer. Curves clearly composed of more than one exponential slope and cases where the triglyceride concentration showed systemic changes (more than ±5% of initial value) during the investigation were rejected, as recommended by Nikkilä and Kekki (33, 34). Altogether three patients with type IIa, one with type IIb, and three with type IV HLP were excluded from the study for such reasons. It is worth noting that the bile acid kinetics in these subjects showed a pattern similar to that of the other subjects with corresponding types of HLP.

The apparent fractional turnover rate of endogenous plasma triglyceride (k, hr^{-1}) was determined from the exponential descending slope of the plasma radioactivity curve. Ten to twelve time points were obtained in each study, and the standard error of the individual fractional turnover rate determinations averaged 0.011 (6%), 0.012 (6%), and 0.010 (7%) in HLP type IIa, IIb, and IV, respectively. The correlation coefficients ranged from 0.914 to 0.989 ($P < 0.001$ in all cases) and thus these patients displayed single-exponential decay curves (with the exception of the subjects excluded as mentioned above). Neglecting the errors that might be attributed to the possible emergence of a slow exponential component after 10 hr (cf Discussion), a value for endogenous plasma triglyceride production (the apparent triglyceride production rate) was calculated as described by others (11, 33, 34). The apparent plasma triglyceride production rate (V), expressed in μmol $kg^{-1}hr^{-1}$ was obtained from the equation $V \times 45 \cdot k \cdot s \cdot b$. The factor 45 represents the distribution space of plasma triglycerides, supposed to equal the total plasma volume, i.e., 4.5% of ideal body weight. Factor s is the mean plasma triglyceride concentration (mmol/l) during the experiment; factor b is used to correct for overweight and is calculated from the equation $b = [(4500 + a \cdot c)/(100 + c)]/45$. In this expression a represents the plasma volume per kg adipose tissue (9.7 ml for males and 10.8 ml for females) (35) and c is the excess of relative body weight (RBW − 100) (11, 32, 33). RBW was calculated as [body weight (kg)/(height (cm) − 100)] × 100. The

TABLE 2. Plasma lipids and triglyceride kinetics in the individual patients

Patient Number, Sex	Plasma Cholesterol Concentration mmol·l^{-1} [a]	Plasma Triglyceride		
		Concentration mmol·l^{-1} [a]	Apparent Fractional Turnover Rate hr^{-1}	Apparent Formation μmol·kg^{-1}·hr^{-1}
Hyperlipoproteinemia type IIa				
1. M	8.5	1.6	0.232	15.5
2. M	12.0	1.8	0.170	12.5
3. M	8.9	1.5	0.206	13.9
4. M	9.1	1.3	0.187	10.9
5. M	8.5	1.2	0.159	8.6
6. M	8.4	1.5	0.179	12.4
7. M	7.8	1.3	0.225	11.7
8. M	7.8	1.0	0.131	5.9
9. M	9.6	1.0	0.208	9.9
10. F	9.1	1.3	0.165	8.4
11. F	11.2	1.5	0.224	15.1
12. F	6.3	1.2	0.234	12.7
13. F	8.7	1.1	0.157	7.3
14. F	8.7	0.7	0.215	6.8
15. F	7.5	1.0	0.174	7.8
16. F	10.4	1.1	0.234	10.7
17. F	8.0	1.5	0.218	14.7
18. F	8.6	1.9	0.113	9.7
19. F	8.4	0.8	0.147	5.3
Hyperlipoproteinemia type IIb				
20. M	7.5	2.4	0.213	23.0
21. M	8.6	4.0	0.153	27.5
22. M	7.6	3.9	0.146	23.1
23. F	8.1	2.1	0.190	16.3
24. F	7.9	2.1	0.192	18.2
25. F	7.9	2.3	0.168	16.0
Hyperlipoproteinemia type IV				
26. M	6.9	3.7	0.149	23.2
27. M	7.8	4.5	0.166	31.2
28. M	5.6	2.1	0.202	19.2
29. M	8.6	4.1	0.167	28.5
30. M	6.5	2.1	0.185	15.3
31. M	5.3	3.4	0.135	20.6
32. M	5.8	4.0	0.121	21.8
33. M	7.6	3.0	0.195	24.8
34. M	7.2	4.3	0.063	11.5
35. M	7.1	3.2	0.194	28.0
36. M	6.4	2.2	0.170	15.9
37. M	6.5	3.2	0.153	20.4
38. M	6.9	5.0	0.059	12.3
39. M	7.7	3.8	0.192	29.6
40. M	7.1	3.1	0.202	32.1
41. M	7.7	3.1	0.157	21.9
42. M	4.1	2.3	0.204	19.9
43. F	5.8	3.8	0.210	35.8
44. F	5.1	4.6	0.112	23.3
45. F	6.1	8.3	0.061	16.8
46. F	5.8	5.9	0.072	19.2
47. F	7.9	6.7	0.056	15.8

standard error of the individual triglyceride synthesis calculation (considering both error in fractional turnover rate and triglyceride variability) averaged 9%, 9%, and 10% in HLP type IIa, IIb, and IV, respectively.

In seven patients—two with type IIa and five with type IV HLP—VLDL was isolated by ultracentrifugation at d 1.006 g/ml (36), and the radioactivity decay in this fraction was analyzed in parallel to that of total plasma. The apparent formation of VLDL triglyceride

TABLE 2. (*Continued*)

Patients (Number)	Plasma Cholesterol Concentration mmol·l^{-1} [a]	Plasma Triglyceride		
		Concentration mmol·l^{-1} [a]	Apparent Fractional Turnover Rate hr^{-1}	Apparent Formation μmol·kg^{-1}·hr^{-1}
Hyperlipoproteinemia type IIa				
M (9)	9.0 ± 0.4[b]	1.4 ± 0.1[b]	0.189 ± 0.011[b]	11.3 ± 1.0[b]
F (10)	8.7 ± 0.4	1.2 ± 0.1	0.188 ± 0.013	9.9 ± 1.1
Total (19)	8.9 ± 0.3	1.3 ± 0.1	0.188 ± 0.008	10.5 ± 0.7
Hyperlipoproteinemia type IIb				
M (3)	7.9 ± 0.4	3.4 ± 0.5[g]	0.171 ± 0.021	24.5 ± 1.5[g]
F (3)	8.0 ± 0.1	2.2 ± 0.1[g]	0.183 ± 0.008	16.8 ± 0.7[c,e]
Total (6)	8.0 ± 0.2	2.8 ± 0.4[g]	0.177 ± 0.011	20.7 ± 1.9[g]
Hyperlipoproteinemia type IV				
M (17)	6.7 ± 0.3[g]	3.4 ± 0.2[g]	0.160 ± 0.011[e]	22.1 ± 1.5[g]
F (5)	6.1 ± 0.5[f,h]	5.9 ± 0.8[d,g,h]	0.102 ± 0.029[f]	22.2 ± 3.6[f]
Total (22)	6.6 ± 0.2[g,i]	3.9 ± 0.3[g]	0.147 ± 0.011[f]	22.1 ± 1.4[g]

[a] To convert mmol/l to mg/dl multiply cholesterol concentrations by 38.7 and triglyceride concentrations by 0.1 × mol wt of triglyceride (e.g., 88.5 for triolein).
[b] Mean ± SEM.
[c] Significantly different from males with the same lipoprotein pattern, $P < 0.05$.
[d] Significantly different from males with the same lipoprotein pattern, $P < 0.01$.
[e] Significantly different from corresponding group with type IIa pattern, $P < 0.05$.
[f] Significantly different from corresponding group with type IIa pattern, $P < 0.01$ or $P < 0.005$.
[g] Significantly different from corresponding group with type IIa pattern, $P < 0.001$.
[h] Significantly different from corresponding group with type IIb pattern, $P < 0.05$.
[i] Significantly different from corresponding group with type IIb pattern, $P < 0.005$.

averaged 18.9 ± 4.5 μmol kg^{-1}hr^{-1} and that of total triglyceride 20.4 ± 4.0 μmol kg^{-1}hr^{-1}. There was a highly significant correlation between the two determinations ($r = +0.98$, $P < 0.001$).

Statistical analysis

Data are presented as mean ± SEM. Significances of differences were evaluated by Student's t test. Linear regressions were calculated by the method of least squares, and their significances were tested by estimating the correlation coefficient, r (37).

RESULTS

Bile acid kinetics

Data on the formation, pool size, and fractional turnover of the bile acids are listed in **Table 3.** The mean synthesis of cholic acid in the 22 patients with type IV HLP (0.95 ± 0.11 mmol d^{-1}) exceeded that in the 19 patients with type IIa (0.42 ± 0.04, $P < 0.001$). Although less pronounced, a difference was found also in the formation of chenodeoxycholic acid, which averaged 0.52 ± 0.05 mmol d^{-1} in type IV and 0.30 ± 0.04 mmol d^{-1} in type IIa HLP ($P < 0.001$). Total bile acid production (C + CD) was twice as high on the average in type IV as in type IIa patients. The values recorded in type IIb HLP were similar to those in type IIa. The fractional turnover of C and CD did not vary significantly in relation to sex or type of HLP. The formation of C and that of C and CD combined, expressed in absolute amounts, were higher in males than in females with the type IIa pattern, while no such sex differences were seen among the patients with a type IIb or type IV lipoprotein pattern (Table 3).

In the patients with type IIa HLP, body weight correlated positively with the formation of C ($r = +0.65$, $P < 0.01$) and (C + CD) ($r = +0.54$, $P < 0.05$) but not with that of CD. No such relationships were found in HLP type IIb or IV. In all the subgroups, the formation of C, CD, and (C + CD) was unrelated to plasma concentrations of cholesterol and triglycerides, and to the presence of glucose intolerance or gallbladder disease (GBD).

The pool size of the two primary bile acids (C + CD) was significantly larger in type IV HLP (4.01 ± 0.32 mmol) than in type IIa HLP (2.52 ± 0.41 mmol, $P < 0.01$) and type IIb HLP (2.43 ± 0.48 mmol, $P < 0.025$). This was true also when comparing type IV and type IIa patients without gallbladder disease ($P < 0.02$), and was mainly due to an augmented pool size of C (Table 3).

Bile acid synthesis was also calculated as μmol formed per kg body weight per day. As is evident from Table 3, the differences between type IIa and type IV HLP in

TABLE 3. Bile acid kinetics, individual data

Patient Number, Sex	Cholic Acid				Chenodeoxycholic Acid				Total Bile Acid Formation	
	Pool Size mmol	Formation mmol·d^{-1}	Fractional Turnover Rate, d^{-1}	Formation μmol·kg·d^{-1}	Pool Size mmol	Formation mmol·d^{-1}	Fractional Turnover Rate, d^{-1}	Formation μmol·kg^{-1}·d^{-1}	mmol·d^{-1}	μmol·kg^{-1}·d^{-1}
Hyperlipoproteinemia type IIa										
1. M	2.50	0.83	0.334	10.3	0.92	0.37	0.397	4.5	1.20	14.8
2. M	1.13	0.66	0.580	8.6	1.04	0.43	0.409	5.6	1.08	14.2
3. M	3.60	0.63	0.174	8.6	5.08	0.71	0.139	9.7	1.33	18.2
4. M	0.61	0.46	0.753	7.0	0.59	0.29	0.489	4.4	0.75	11.5
5. M	0.50	0.21	0.418	3.1	0.88	0.25	0.288	3.7	0.46	6.8
6. M	3.51	0.64	0.181	7.4	1.52	0.42	0.274	4.8	1.05	12.2
7. M	1.72	0.53	0.309	6.4	1.11	0.29	0.260	3.5	0.82	9.9
8. M	0.97	0.20	0.209	2.9	0.53	0.17	0.321	2.4	0.37	5.3
9. M	1.19	0.50	0.417	7.5	0.92	0.36	0.387	5.4	0.85	12.9
10. F	1.86	0.55	0.298	7.6	1.30	0.25	0.195	3.5	0.81	11.0
11. F	1.67	0.31	0.183	5.8	1.38	0.20	0.143	3.7	0.50	9.5
12. F	0.60	0.30	0.502	6.5	1.01	0.29	0.286	6.3	0.59	12.8
13. F	0.71	0.18	0.253	2.5	0.68	0.11	0.165	1.6	0.29	4.0
14. F	1.31	0.30	0.231	5.8	1.35	0.52	0.386	10.1	0.83	15.9
15. F	0.85	0.17	0.198	3.1	0.67	0.14	0.205	2.5	0.31	5.6
16. F	0.89	0.44	0.496	6.3	0.95	0.41	0.433	5.9	0.86	12.2
17. F	1.15	0.42	0.362	6.6	1.33	0.31	0.231	4.9	0.72	11.5
18. F	0.28	0.29	1.036	5.9	0.45	0.18	0.389	3.6	0.47	9.5
19. F	0.85	0.29	0.346	4.9	0.29	0.09	0.308	1.5	0.38	6.4
Hyperlipoproteinemia type IIb										
20. M	1.01	0.55	0.538	7.8	0.68	0.35	0.522	5.0	0.90	12.8
21. M	2.60	0.59	0.226	8.1	2.09	0.29	0.138	4.0	0.88	12.0
22. M	1.66	0.41	0.249	5.0	0.65	0.23	0.352	2.8	0.64	7.7
23. F	1.11	0.93	0.838	11.5	0.53	0.37	0.697	4.5	1.30	16.0
24. F	1.68	0.32	0.189	5.4	0.92	0.07	0.074	1.2	0.39	6.5
25. F	0.59	0.41	0.695	6.0	1.08	0.44	0.407	6.4	0.85	12.5
Hyperlipoproteinemia type IV										
26. M	1.32	1.37	1.036	15.7	0.71	0.62	0.880	7.2	1.99	22.9
27. M	2.40	1.47	0.614	16.9	1.80	0.75	0.415	8.6	2.22	25.5
28. M	1.33	0.40	0.303	4.9	1.31	0.20	0.154	2.5	0.61	7.4
29. M	0.76	0.91	1.197	10.8	0.54	0.59	1.103	7.0	1.50	17.9
30. M	2.04	0.59	0.287	6.6	1.85	0.46	0.249	5.2	1.05	11.7
31. M	2.10	0.72	0.341	10.4	1.57	0.43	0.273	6.2	1.14	16.6
32. M	2.50	0.97	0.388	12.1	2.44	0.70	0.287	8.7	1.67	20.8
33. M	2.24	0.72	0.320	7.9	3.43	0.57	0.166	6.3	1.29	14.1
34. M	2.08	0.58	0.278	7.6	1.90	0.49	0.256	6.4	1.07	14.0
35. M	3.37	0.83	0.245	12.9	1.80	0.43	0.237	6.7	1.26	19.6
36. M	2.07	0.84	0.406	11.5	2.07	0.51	0.246	7.0	1.35	18.5
37. M	1.65	0.64	0.387	7.6	0.98	0.24	0.242	2.8	0.88	10.5
38. M	2.62	0.69	0.264	9.1	1.69	0.40	0.238	5.3	1.10	14.4
39. M	3.90	1.52	0.388	18.3	2.87	1.02	0.354	12.3	2.54	30.6
40. M	2.91	2.11	0.724	25.4	2.14	0.89	0.416	10.7	3.00	36.1

41. M		3.59	0.58	0.160	7.6	2.31	0.48	0.208	6.3	1.06	13.9	
42. M		3.54	1.13	0.320	16.4	2.37	0.62	0.261	9.0	1.75	25.4	
43. F		3.61	2.41	0.668	40.2	1.22	0.79	0.642	13.1	3.20	53.3	
44. F		1.29	0.48	0.372	8.0	1.07	0.32	0.294	5.3	0.79	13.2	
45. F		1.07	0.90	0.836	12.6	0.84	0.47	0.555	6.6	1.36	19.2	
46. F		2.74	0.33	0.120	5.5	1.70	0.20	0.118	3.3	0.53	8.8	
47. F		1.67	0.74	0.440	11.1	0.80	0.27	0.335	4.1	1.00	15.2	
Hyperlipoproteinemia type IIa												
M	(9)	1.75 ± 0.40[a]	0.52 ± 0.07[a]	0.375 ± 0.065[a]	6.9 ± 0.8[a]	1.40 ± 0.47[a]	0.37 ± 0.05[a]	0.329 ± 0.034[a]	4.9 ± 0.7[a]	0.88 ± 0.11[a]	11.8 ± 1.3[a]	
F	(10)	1.02 ± 0.15	0.33 ± 0.04[b]	0.391 ± 0.080	5.5 ± 0.5	0.94 ± 0.13	0.25 ± 0.04	0.274 ± 0.032	4.4 ± 0.8	0.58 ± 0.07[b]	9.8 ± 1.2	
Total	(19)	1.36 ± 0.22	0.42 ± 0.04	0.383 ± 0.051	6.1 ± 0.5	1.16 ± 0.23	0.30 ± 0.04	0.300 ± 0.024	4.6 ± 0.5	0.72 ± 0.07	10.7 ± 0.9	
Hyperlipoproteinemia type IIb												
M	(3)	1.76 ± 0.46	0.52 ± 0.05	0.338 ± 0.101	7.0 ± 1.0	1.14 ± 0.48	0.29 ± 0.03	0.338 ± 0.111	3.9 ± 0.6	0.81 ± 0.08	10.8 ± 1.6	
F	(3)	1.13 ± 0.31	0.55 ± 0.19	0.574 ± 0.197	7.6 ± 1.9	0.84 ± 0.16	0.29 ± 0.11	0.392 ± 0.180	4.0 ± 1.5	0.85 ± 0.26	11.7 ± 2.8	
Total	(6)	1.44 ± 0.29	0.54 ± 0.09	0.456 ± 0.112	7.3 ± 1.0	0.99 ± 0.23	0.29 ± 0.05	0.365 ± 0.095	4.0 ± 0.7	0.83 ± 0.12	11.3 ± 1.4	
Hyperlipoproteinemia type IV												
M	(17)	2.38 ± 0.21	0.95 ± 0.11[c]	0.450 ± 0.069	11.9 ± 1.3[c]	1.87 ± 0.18	0.55 ± 0.05[c,f]	0.352 ± 0.062	7.0 ± 0.6[c]	1.50 ± 0.15[c]	18.8 ± 1.8[c]	
F	(5)	2.08 ± 0.48[c]	0.97 ± 0.37[c]	0.487 ± 0.123	15.5 ± 6.3[c]	1.13 ± 0.16	0.41 ± 0.10	0.389 ± 0.094	6.5 ± 1.7	1.38 ± 0.48[c]	21.9 ± 8.0	
Total	(22)	2.31 ± 0.19[d,f]	0.95 ± 0.11[e]	0.459 ± 0.059	12.7 ± 1.7[d]	1.70 ± 0.16[f]	0.52 ± 0.05[e,f]	0.360 ± 0.051	6.8 ± 0.6[d,f]	1.43 ± 0.16[e,f]	19.5 ± 2.2[d]	

[a] Means ± SEM. [b] Significantly different from males with the same lipoprotein pattern, $P < 0.05$. [c] Significantly different from corresponding group with type IIa pattern, $P < 0.05$ or $P < 0.02$. [d] Significantly different from corresponding group with type IIa pattern, $P < 0.01$ or $P < 0.005$. [e] Significantly different from corresponding group with type IIa pattern, $P < 0.001$. [f] Significantly different from corresponding group with type IIb pattern, $P < 0.05$.

the formation of C (6.1 ± 0.5 vs. 12.7 ± 1.7 μmol $kg^{-1}d^{-1}$, $P < 0.005$), CD (4.6 ± 0.5 vs. 6.8 ± 0.6 μmol $kg^{-1}d^{-1}$, $P < 0.01$), and (C + CD) (10.7 ± 0.9 vs. 19.5 ± 2.2 μmol $kg^{-1}d^{-1}$, $P < 0.005$) are still present after correction for body weight.

Triglyceride kinetics

The plasma triglyceride concentration averaged 1.3 ± 0.1, 2.8 ± 0.4, and 3.9 ± 0.3 mmol/l in the patients with type IIa, IIb, and IV lipoprotein pattern, respectively. Apparent triglyceride formation in HLP type IIa (10.5 ± 0.7 μmol $kg^{-1}hr^{-1}$) was significantly less than that recorded in HLP type IIb (20.7 ± 1.9 μmol $kg^{-1}hr^{-1}$, $P < 0.001$) and in HLP type IV (22.1 ± 1.4 μmol $kg^{-1}hr^{-1}$, $P < 0.001$) (Table 2). As a general tendency, the apparent biosynthesis of triglycerides was higher in males than in females. The apparent fractional turnover rate of serum triglycerides as determined over 10 hr was on the average approximately the same in HLP type IIa (0.188 ± 0.008) and type IIb (0.177 ± 0.011), while it was significantly lower in the patients with the type IV lipoprotein pattern (0.147 ± 0.011, $P < 0.01$) vs. those with type IIa (Table 2).

The apparent triglyceride production correlated significantly with the triglyceride plasma concentration in HLP type IIa ($r = +0.70$, $P < 0.001$) and type IIb ($r = +0.83$, $P < 0.05$), but not in type IV ($r = -0.18$). No relationship to serum cholesterol, glucose intolerance, or the presence of GBD was found for apparent triglyceride synthesis or fractional turnover rate.

Relationship between apparent triglyceride formation and bile acid synthesis

In **Fig. 1,** C production in μmol $kg^{-1}d^{-1}$ is plotted against apparent triglyceride formation in μmol $kg^{-1}hr^{-1}$. These two parameters correlated significantly in type IIa ($r = +0.69$, $P < 0.001$), type IV ($r = +0.70$, $P < 0.001$), and in the total series of patients ($r = +0.70$, $P < 0.001$), but not in type IIb HLP. As is evident from Fig. 1, however, the lack of correlation in type IIb is mainly caused by patient no. 23, whose exclusion yields a correlation coefficient of +0.61. A correlation was also found between the apparent biosynthesis of triglyceride and CD in type IV HLP and in the combined series (**Table 4**). Total bile acid production was significantly related to apparent triglyceride formation in HLP type IIa, type IV, and in the total number of patients. As seen in Table 4, the relationship between apparent triglyceride synthesis and C formation also applies in the male subgroups of type IIa and type IV patients.

A slight correlation between C pool size and ap-

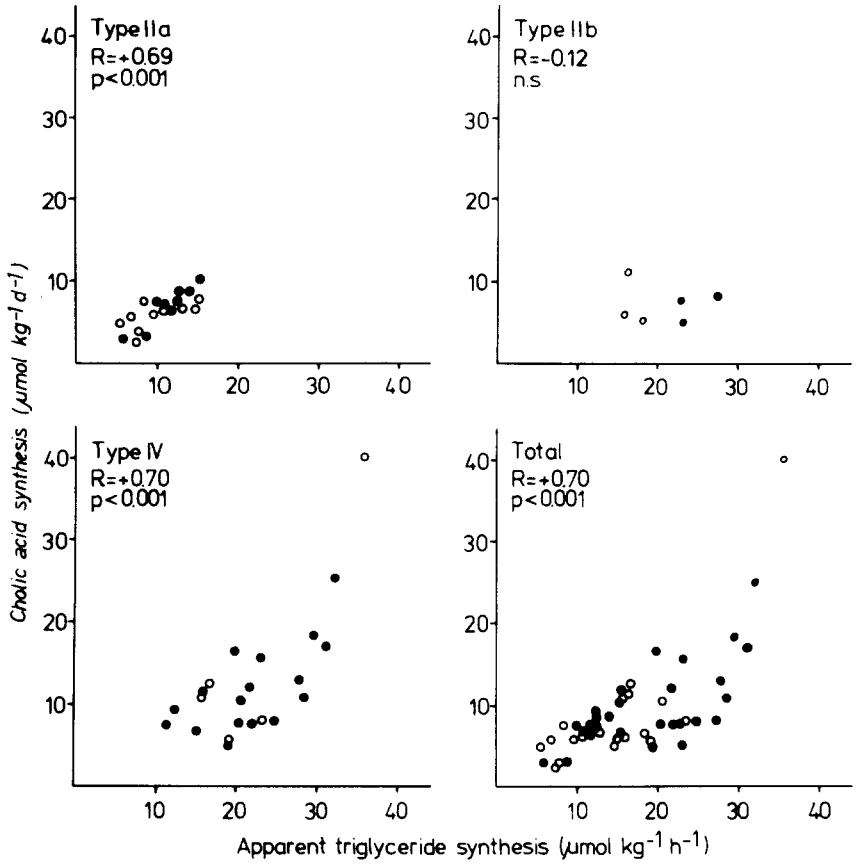

Fig. 1. Relationship between apparent triglyceride synthesis (μmol $kg^{-1}hr^{-1}$) and cholic acid formation (μmol $kg^{-1}d^{-1}$) in HLP type IIa (upper left, $n = 19$), type IIb (upper right, $n = 6$), type IV (lower left, $n = 22$), and the total series (lower right, $n = 47$). R indicates the calculated correlation coefficient, n.s. = not significant. Equations of the regression lines are given in Table 4.

parent triglyceride synthesis was seen in type IIa HLP ($r = +0.48$, $P < 0.05$), otherwise no significant relationships between bile acid pool size and triglyceride formation were seen in the different types of HLP.

DISCUSSION

In the present work, [24-^{14}C]-labeled C and CD were used in the determination of bile acid kinetics, whereas in a previous study (22) [24-^{14}C]-labeled C and randomly tritiated CD were used. This change in experimental design was made to avoid the small errors inherent in using [^{3}H]-labeled CD (38), and probably explains the slightly lower figures for CD formation seen in the present investigation. However, the findings on bile acid kinetics in HLP in the present study are in good agreement with those seen earlier (22), with a higher elimination of cholesterol as bile acid in type IV compared to type IIa HLP. The two groups also differed with regard to the composition of the bile acids synthesized, the formation and pool size of C being more dominant in type IV HLP. Total bile acid production was about the same in HLP type IIa and type IIb. Under the conditions used, differences in bile acid formation have been found to reflect reciprocal differences in net steroid balance, i.e., cholesterol biosynthesis (26).

The possibility of performing detailed studies concerning bile acid kinetics in relation to VLDL metabolism is hampered by the difficulties inherent in the estimation of VLDL production. VLDL synthesis in the liver is influenced by factors such as plasma free fatty acid and insulin levels, which show diurnal variations. No method so far available gives a proper evaluation of the 24-hr VLDL formation under physiological conditions, i.e., when the subjects have an ordinary intake of food. With most techniques used, the subjects have been studied in the postabsorptive state or when fed a low-fat diet. Furthermore, the studies in general only measure the synthesis during part of the day.

In the present study, the endogenous [^{3}H]glycerol labeling technique originally described by Farquhar et al. (32) was used to estimate plasma triglyceride

formation. As the patients were studied in the postabsorptive state, the newly synthesized triglycerides should represent VLDL triglycerides. In accordance with this contention, formation of total plasma triglyceride and VLDL triglyceride correlated closely (cf Methods), as has been described previously by others (39, 40). The validity and reliability of the [^{3}H]glycerol labeling technique have been supported (32, 33, 39–41) as well as questioned (42, 43). According to Havel (42) and Havel and Kane (43) the [^{3}H]glycerol technique underestimates VLDL triglyceride formation in the normal range but gives reliable data at high levels of plasma triglycerides. Techniques based on the incorporation of radioactive glycerol into triglyceride appear to be less affected by the re-entry of labeled molecules (recycling) than methods based on fatty acid incorporation (44). Thus, in agreement with earlier studies (32–34, 39–41), the radioactivity curves of the present investigation with very few exceptions closely adhered to a log-linear decay (cf. Methods). However, the present studies were carried out over a 10-hr time period, and the possible emergence of a slow component of the curve after about 15 hr as has been suggested by some preliminary reports (45–47) cannot be excluded. Such data would require multicompartmental analysis for resolution as has been discussed for fatty acid labeling techniques (48). If so, the current data concerning apparent triglyceride production rate may not be a true index of VLDL triglyceride formation. The magnitudes of such errors inherent to the present technique are difficult to evaluate. The glycerol labeling technique as used in the present study has however been found to give data comparable with those obtained by an independent lipolytic rate procedure (40, 41). Furthermore, preliminary data on VLDL triglyceride formation in normo- and hypertriglyceridemic patients as calculated from multicompartmental analysis after glycerol labeling (47) appear to be in reasonable agreement with those seen in the present work.

The apparent plasma triglyceride turnover rate as determined during 10 hr in the patients with HLP type IIa was within the range reported for healthy controls (11, 13, 39). The values obtained in type IIb and type IV HLP were higher and similar to those observed for other patients with these disorders (11). However, the pattern seen in type IV patients was heterogeneous, with some patients exhibiting low fractional turnover rates, and no overall positive relationship between triglyceride concentration and formation, suggesting the presence of subpopulations with different triglyceride kinetics. The metabolism of VLDL has also been studied by means of autologous radioiodinated VLDL particles (13). It was clear that the turnover of the VLDL apoB, which correlated positively to that of the VLDL triglyceride, was nor-

TABLE 4. Correlations between the apparent synthesis of plasma triglycerides (TG) and the formation of cholic acid (C), chenodeoxycholic acid (CD), and (C + CD)

Patient Subgroup	x	y	Correlation Coefficient r	Significance of Correlation, P	Equation of the Regression Line
Hyperlipoproteinemia type IIa (n = 19)	TG[a]	C[b]	+0.694	<0.001	$y = 0.47x + 1.25$
	TG	CD[b]	+0.341	N.S.[c]	
	TG	(C + CD)[b]	+0.588	<0.01	$y = 0.72x + 3.21$
Hyperlipoproteinemia type IIb (n = 6)	TG	C	−0.119	N.S.	
	TG	CD	−0.171	N.S.	
	TG	(C + CD)	−0.183	N.S.	
Hyperlipoproteinemia type IV (n = 22)	TG	C	+0.701	<0.001	$y = 0.84x - 5.89$
	TG	CD	+0.667	<0.01	$y = 0.28x + 0.63$
	TG	(C + CD)	+0.714	<0.001	$y = 1.12x - 5.28$
Total (n = 47)	TG	C	+0.702	<0.001	$y = 0.59x - 0.82$
	TG	CD	+0.546	<0.001	$y = 0.20x + 2.20$
	TG	(C + CD)	+0.690	<0.001	$y = 0.79x + 1.37$
Hyperlipoproteinemia type IIa, males (n = 9)	TG	C	+0.920	<0.001	$y = 0.79x - 2.04$
	TG	CD	+0.577	N.S.	
	TG	(C + CD)	+0.861	<0.01	$y = 1.20x - 1.76$
Hyperlipoproteinemia type IV, males (n = 17)	TG	C	+0.665	<0.01	$y = 0.56x - 0.44$
	TG	CD	+0.562	<0.05	$y = 0.22x + 2.08$
	TG	(C + CD)	+0.656	<0.01	$y = 0.78x + 1.61$

[a] Expressed as μmol kg^{-1} hr^{-1}.
[b] Expressed as μmol kg^{-1} d^{-1}.
[c] N.S., not significant.

mal in HLP type IIa (heterozygous familial hypercholesterolemia) and significantly less than in HLP types IIb, IV, and V. The combined evidence from the reports cited above (including the present study) indicates that overproduction of VLDL is of major importance for the development of hypertriglyceridemia in most patients; however, in others a defective clearance may be the determining factor.

In the two major groups of patients studied (HLP type IIa and IV), the apparent formation of plasma triglycerides in the postabsorptive state as determined over 10 hr correlated closely to the production of bile acids as determined under ordinary dietary conditions during the preceding week. With the limitations inherent to the techniques used as discussed above, the combined evidence from this study strongly indicates that an increased formation of VLDL is associated with an enhanced degradation of cholesterol as bile acids. The mechanism(s) behind this relationship cannot be decided from the present study, but some alternative explanations may be considered.

The liver continuously delivers cholesterol to be incorporated into lipoproteins, excreted in the bile, or converted to bile acids. The outflow of cholesterol from the liver is balanced by hepatic cholesterogenesis and by an inflow of lipoprotein cholesterol. On the basis of data reported for the VLDL production and the triglyceride/cholesterol ratio of the VLDL particles (49), it appears that several mmol of VLDL cholesterol enter the circulation each day. Unless lipoprotein cholesterol is effectively reutilized in the hepatic formation of VLDL, an enhanced biosynthesis of such particles may result in an increased hepatic cholesterogenesis and an elevated cholesterol balance. In agreement with this contention we have recently shown that the activity of 3-hydroxy-3-methylglutaryl CoA reductase—the rate-limiting enzymatic step in hepatic cholesterol biosynthesis—is increased in most patients with type IV HLP, whereas the enzyme activity in HLP type IIa is within the normal range.[2] As mentioned above, previous studies from our laboratory have also shown an enhanced net steroid balance in patients with type IV HLP (26). Thus, an increased hepatic formation of VLDL cholesterol may be associated with an enhanced degradation of lipoprotein cholesterol.

As suggested by Sodhi (50, 51) there may be at least two pools of hepatic cholesterol, one anabolic containing cholesterol aimed for lipoprotein synthesis and one catabolic containing cholesterol from lipoprotein breakdown aimed for elimination as bile acids and neutral steroids. This hypothesis is however not fully supported by the observations that de novo synthesized cholesterol is preferred for the synthesis of bile acids in the rat (52, 53) as well as in man (54)[3]; this seems to be more pronounced for C than for CD (55).[3] Thus, the covariation between the apparent formation of plasma triglyceride and the synthesis of bile acids—especially that of C—may at least in part be due to the sharing of a pool of newly synthesized cholesterol, available both for lipoprotein synthesis and bile acid formation. Some support for this view is also gained from the results reported in the accompanying paper (56), suggesting that experimentally induced alterations primarily affecting bile acid metabolism appear to be associated with changes in plasma triglyceride metabolism. Finally, the possibility that the formation of plasma triglycerides and the bile acid production vary independently but in parallel because of the influence of a common regulatory factor, e.g., insulin or free fatty acid concentration, must of course be considered.

To summarize, the results of the present study suggest an integrated regulation of the synthetic rate of VLDL triglyceride and the degradation of cholesterol to bile acids. Such a relationship may offer an explanation for the high bile acid biosynthesis often seen in patients with HLP type IV. Moreover, it may account for the reduced production of bile acids seen in hypertriglyceridemic patients treated with clofibrate (57), nicotinic acid (58), or by weight reduction (22), conditions reported to be associated with decreased VLDL formation (11, 59, 60). Further studies are however needed to characterize this apparent relationship and may lead to a better understanding of some of the mechanisms involved in the development of hyperlipoproteinemia.

The skillful technical assistance of Mrs. Maria Dery, Mrs. Kerstin Hedström, and Mrs. Margret Wahlström is gratefully acknowledged. The study was supported by grants from the Swedish National Association against Heart and Chest Diseases and from the Swedish Medical Research Council (Project no. 19X-3397). The ethical aspects of the study were approved by the Ethical Committee of Karolinska Institutet.

Manuscript received 25 October 1977; accepted 4 April 1978.

REFERENCES

1. Beaumont, J. L., L. A. Carlson, G. R. Cooper, Z. Fejfar, D. S. Fredrickson, and T. Strasser. 1970. Clas-

[2] Ahlberg, J., B. Angelin, I. Björkhem, K. Einarsson, and B. Leijd. Hepatic cholesterol metabolism in normo- and hyperlipidemic patients with cholesterol gallstones. *J. Lipid Res.*, in press.

[3] Ahlberg, J., B. Angelin, and K. Einarsson. Manuscript in preparation.

sification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. WHO.* **43:** 891–915.
2. Reaven, G. M., D. B. Hill, R. C. Gross, and J. W. Farquhar. 1965. Kinetics of triglyceride turnover of very low density lipoproteins of human plasma. *J. Clin. Invest.* **44:** 1826–1833.
3. Porte, D., Jr., and E. L. Bierman. 1969. The effect of heparin infusion on plasma triglyceride in vivo and in vitro with a method for calculating triglyceride turnover. *J. Lab. Clin. Med.* **73:** 631–648.
4. Olefsky, J., J. W. Farquhar, and G. M. Reaven. 1974. Sex difference in the kinetics of triglyceride metabolism in normal and hypertriglyceridaemic human subjects. *Eur. J. Clin. Invest.* **4:** 121–127.
5. Adams, P. W., A. H. Kissebah, P. Harrigan, T. Stokes, and V. Wynn. 1974. The kinetics of plasma free fatty acid and triglyceride transport in patients with idiopathic hypertriglyceridaemia and their relation to carbohydrate metabolism. *Eur. J. Clin. Invest.* **4:** 149–161.
6. Sailer, S., F. Sandhofer, and H. Braunsteiner. 1966. Umsatzraten für freie Fettsäuren und Triglyceride im Plasma bei essentieller Hyperlipämie. *Klin. Wochenschr.* **44:** 1032–1035.
7. Havel, R. J., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49:** 2017–2035.
8. Quarfordt, S. H., A. Frank, D. M. Shames, M. Berman, and D. Steinberg. 1970. Very low density lipoprotein triglyceride transport in type IV hyperlipoproteinemia and the effects of carbohydrate-rich diets. *J. Clin. Invest.* **49:** 2281–2297.
9. Eaton, R. P. 1971. Synthesis of plasma triglycerides in endogenous hypertriglyceridemia. *J. Lipid Res.* **12:** 491–497.
10. Boberg, J., L. A. Carlson, U. Freyschuss, B. W. Lassers, and M. L. Wahlqvist. 1972. Splanchnic secretion rates of plasma triglycerides and total and splanchnic turnover of plasma free fatty acids in men with normo- and hypertriglyceridaemia. *Eur. J. Clin. Invest.* **2:** 454–466.
11. Nikkilä, E. A., and M. Kekki. 1972. Plasma endogenous triglyceride transport in hypertriglyceridaemia and effect of a hypolipidaemic drug (SU-13437). *Eur. J. Clin. Invest.* **2:** 231–238.
12. Kaye, J. P., and D. J. Galton. 1975. Triglyceride-production rates in patients with type IV hypertriglyceridaemia. *Lancet* **1:** 1005–1007.
13. Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. Metabolism of very low density lipoproteins in hyperlipidaemia: Studies of apolipoprotein B kinetics in man. *Eur. J. Clin. Invest.* **6:** 167–177.
14. Langer, T., W. Strober, and R. I. Levy. 1972. The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. *J. Clin. Invest.* **51:** 1528–1536.
15. Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. The metabolism of low density lipoprotein in endogenous hypertriglyceridaemia. *Eur. J. Clin. Invest.* **6:** 151–158.
16. Kottke, B. A. 1969. Differences in bile acid excretion. Primary hypercholesterolemia compared to combined hypercholesterolemia and hypertriglyceridemia. *Circulation.* **40:** 13–20.
17. Einarsson, K., and K. Hellström. 1972. The formation of bile acids in patients with three types of hyperlipoproteinaemia. *Eur. J. Clin. Invest.* **2:** 225–230.
18. Wollenweber, J., and A. Stiehl. 1972. Grösse des Pools und Turnover der primären Gallensäuren bei Hyperlipoproteinämien: Unterschiedliche Befunde bei Typ II und Typ IV Hyperlipoproteinämie. *Klin. Wochenschr.* **50:** 33–38.
19. Grundy, S. M., E. H. Ahrens, Jr., G. Salen, P. H. Schreibman, and P. J. Nestel. 1972. Mechanisms of action of clofibrate on cholesterol metabolism in patients with hyperlipidemia. *J. Lipid Res.* **13:** 531–551.
20. Sodhi, H. S., and B. J. Kudchodkar, 1973. Catabolism of cholesterol in hypercholesterolemia and its relationship to plasma triglycerides. *Clin. Chim. Acta.* **46:** 161–171.
21. Sodhi, H. S., and B. J. Kudchodkar. 1973. Synthesis of cholesterol in hypercholesterolemia and its relationship to plasma triglycerides. *Metabolism.* **22:** 895–912.
22. Einarsson, K., K. Hellström, and M. Kallner. 1974. Bile acid kinetics in relation to sex, serum lipids, body weights, and gallbladder disease in patients with various types of hyperlipoproteinemia. *J. Clin. Invest.* **54:** 1301–1311.
23. Nestel, P. J., and J. D. Hunter. 1974. Differences in bile acid excretion in subjects with hypercholesterolaemia, hypertriglyceridaemia and overweight. *Aust. N.Z.J. Med.* **4:** 491–496.
24. Miettinen, T. A. 1975. Bile acid metabolism. *In* Hypolipidemic Agents. D. Kritchevsky, editor. Springer-Verlag, Berlin. 109–150.
25. Grundy, S. M. 1975. Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. *J. Clin. Invest.* **55:** 269–282.
26. Angelin, B., K. Einarsson, K. Hellström, and M. Kallner. 1976. Elimination of cholesterol in hyperlipoproteinaemia. *Clin. Sci. Mol. Med.* **51:** 393–397.
27. Hellström, K., B. Angelin, K. Einarsson, M. Kallner, and B. Leijd. 1977. Regulation of bile acid synthesis in hyperlipoproteinemia. *In* Bile Acid Metabolism in Health and Disease. G. Paumgartner and A. Stiehl, editors. MTP Press Ltd, London. 235–239.
28. Goldstein, J. L., H. B. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II: Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52:** 1544–1568.
29. Ikkos, D., and R. Luft. 1957. On the intravenous glucose tolerance test. *Acta Endocrinol.* (Copenhagen). **25:** 312–334.
30. Eneroth, P. 1963. Thin-layer chromatography of bile acids. *J. Lipid Res.* **4:** 11–16.
31. Lindstedt, S. 1957. Turnover of cholic acid in man. *Acta Physiol. Scand.* **40:** 1–9.
32. Farquhar, J. W., R. C. Gross, R. M. Wagner, and G. M. Reaven. 1965. Validation of an incompletely coupled two-compartment nonrecycling catenary model for turnover of liver and plasma triglyceride in man. *J. Lipid Res.* **6:** 119–134.
33. Nikkilä, E. A., and M. Kekki. 1971. Polymorphism of plasma triglyceride kinetics in normal human adult subjects. *Acta Med. Scand.* **190:** 49–59.
34. Nikkilä, E. A., and M. Kekki. 1972. Plasma triglyceride

metabolism in thyroid disease. *J. Clin. Invest.* **51:** 2103–2114.
35. Alexander, J. K., E. W. Dennis, W. G. Smith, K. H. Amad, W. C. Duncan, and R. C. Austin. 1962. Blood volume cardiac output and distribution of systemic blood flow in extreme obesity. *Cardiovascular Research Bulletin* (Baylor University). **1:** 39. Quoted in *Medical Physiology.* **1:** 250. 1968. V. B. Mountcastle, editor. The C. V. Mosby Co., St. Louis, MO.
36. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. J. Nelson, editor. Wiley, New York. 181–274.
37. Snedecor, G. W., and W. G. Cochran. 1974. Statistical Methods, 6th edition. Iowa State University Press, Ames, IA.
38. Einarsson, K., K. Hellström, and M. Kallner. 1974. Randomly tritium-labelled chenodeoxycholic acid as tracer in the determination of bile acid turnover. *Clin. Chim. Acta.* **56:** 235–239.
39. Kekki, M., and E. A. Nikkilä. 1975. Turnover of plasma total and very low density lipoprotein triglyceride in man. *Scand. J. Clin. Lab. Invest.* **35:** 171–179.
40. Steiner, G., and T. Murase. 1975. Triglyceride turnover: a comparison of simultaneous determinations using the radioglyceride and the lipolytic rate procedures. *Federation Proc.* **34:** 2258–2262.
41. Chait, A., J. J. Albers, and J. D. Brunzell. 1977. Comparison of methods of plasma triglyceride turnover. *In* Atherosclerosis IV. G. Schettler, Y. Goto, Y. Hata, G. Klose, editors. Springer-Verlag, New York. 132–137.
42. Havel, R. J. 1970. Metabolism of plasma triglycerides. *In* Proc. II. Intern. Symp. on Atherosclerosis. R. J. Jones, editor. Springer-Verlag, New York. 209–220.
43. Havel, R. J., and J. P. Kane. 1975. Quantification of triglyceride transport in blood plasma: a critical analysis. *Federation Proc.* **34:** 2250–2257.
44. Nikkilä, E. A. 1971. Measurement of plasma triglyceride turnover in the study of hyperglyceridemia. *Scand. J. Clin. Lab. Invest.* **27:** 97–104.
45. Nestel, P. J., and S. M. Grundy. 1976. Changes in plasma triglyceride metabolism during withdrawal of bile. *Metabolism.* **25:** 1259–1268.
46. Zech, L. A., S. M. Grundy, and M. Berman. 1976. Triglyceride synthesis and metabolism in man: a model based on glycerol kinetics. (abstr.) *Circulation.* **53–54:** II-5.
47. Grundy, S. M., H. Y. I. Mok, L. Zech, M. Berman, and D. Steinberg. 1977. Flux of very low density lipoprotein-triglycerides (VLDL-TG) in hypertriglyceridemia. (abstr.) *Circulation.* **55–56:** III-5.
48. Shames, D. M., A. Frank, D. Steinberg, and M. Berman. 1970. Transport of plasma free fatty acids and triglycerides in man. A theoretical analysis. *J. Clin. Invest.* **49:** 2298–2314.
49. Eisenberg, S., and R. I. Levy. 1975. Lipoproteins and lipoprotein metabolism. *In* Hypolipidemic Agents. D. Kritchevsky, editor. Springer-Verlag, Berlin. 191–213.
50. Sodhi, H. S., and B. J. Kudchodkar. 1973. Correlating metabolism of plasma and tissue cholesterol with that of plasma lipoproteins. *Lancet* **1:** 513–519.
51. Sodhi, H. S. 1975. Cholesterol metabolism in man. *In* Hypolipidemic agents. D. Kritchevsky, editor. Springer-Verlag, Berlin. 29–107.
52. Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat-liver microsomes. *Eur. J. Biochem.* **34:** 77–83.
53. Cronholm, T., A. L. Burlingame, and J. Sjövall. 1974. Utilization of the carbon and hydrogen atoms of ethanol in the biosynthesis of steroids and bile acids. *Eur. J. Biochem.* **49:** 497–510.
54. Schwartz, C. C., Z. R. Vlahcevic, L. G. Halloran, D. H. Gregory, J. B. Meek, and L. Swell. 1975. Evidence for the existence of definitive hepatic cholesterol precursor compartments for bile acids and biliary cholesterol in man. *Gastroenterology.* **69:** 1379–1382.
55. Mitropoulos, K. A., N. B. Myant, G. F. Gibbson, S. Balasubramaniam, and B. Reeves. 1974. Cholesterol precursor pools for the synthesis of cholic and chenodeoxycholic acids in rats. *J. Biol. Chem.* **249:** 6052–6056.
56. Angelin, B., K. Einarsson, K. Hellström, and B. Leijd. 1978. Effects of cholestyramine and chenodeoxycholic acid on the metabolism of endogenous triglyceride in hyperlipoproteinemia. *J. Lipid Res.* **19:** 1017–1024.
57. Einarsson, K., K. Hellström, and M. Kallner. 1973. The effect of clofibrate on the elimination of cholesterol as bile acids in patients with hyperlipoproteinaemia type II and IV. *Eur. J. Clin. Invest.* **3:** 345–351.
58. Einarsson, K., K. Hellström, and B. Leijd. 1977. Bile acid kinetics and steroid balance during nicotinic acid therapy in patients with hyperlipoproteinemia type II and IV. *J. Lab. Clin. Med.* **90:** 613–622.
59. Kissebah, A. H., P. W. Adams, P. Harrigan, and V. Wynn. 1974. The mechanism of action of clofibrate and tetranicotinoylfructose (Bradilan) on the kinetics of plasma free fatty acid and triglyceride transport in type IV and type V hypertriglyceridaemia. *Eur. J. Clin. Invest.* **4:** 163–174.
60. Olefsky, J., G. M. Reaven, and J. W. Farquhar. 1974. Effects of weight reduction on obesity. Studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects. *J. Clin. Invest.* **53:** 64–76.