Studies on the Turnover of Triglyceride and Esterified Cholesterol in Subjects with the Nephrotic Syndrome

I. F. C. McKenzie and P. J. Nestel

From the University of Melbourne Department of Medicine, The Royal Melbourne Hospital Post Office, Victoria, Australia

ABSTRACT Factors involved in the hyperlipidemia of nephrosis have been studied in seven patients. The turnover of triglyceride was measured in plasma very low density lipoproteins after the injection of glycerol-^{14}C. The turnover of esterified cholesterol was measured in whole plasma and in very low density lipoproteins after the injection of mevalonic acid-2-^{3}H.

Urine protein loss was found to be significantly correlated with the plasma concentrations of triglyceride and free cholesterol, suggesting that increasing loss of protein is associated with the formation of larger lipoproteins. Lactescent plasmas were found in the subjects with the greatest protein loss.

The turnover rate of triglyceride tended to be higher among subjects with higher than with lower triglyceride concentrations and was on the average higher than among six normotriglyceridemic subjects. However, there was also evidence for decreased clearance of glyceride from plasma. The hypertriglyceridemia of nephrosis appeared to reflect both increased formation of glyceride and decreased removal of glyceride from plasma.

The turnover of esterified cholesterol was significantly higher in whole plasma of nephrotic subjects than in normocholesterolemic nonnephrotic patients. Esterified cholesterol turnover in very low density lipoproteins was raised in the two subjects in whom a major part of total esterified cholesterol was carried in this lipoprotein fraction.

These studies were repeated in one subject after remission was induced. The cessation of urinary loss of protein was associated with reductions in the concentrations and turnover of triglyceride and esterified cholesterol.

The increased turnover of plasma lipids in nephrosis may reflect the general increase in the formation of protein.

Send reprint requests to Dr. P. J. Nestel, Department of Clinical Science, Australian National University, Canberra, Australia.

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INTRODUCTION

High concentrations of cholesterol and triglyceride are commonly found in the plasma of human subjects and experimental animals with the nephrotic syndrome. The hyperlipidemia may be present as a predominant hypercholesterolemia, a predominant hypertriglyceridemia, or a combination of the two (1–4).

The mechanism of the hyperlipidemia is unknown, but the major possibilities must include an increased influx of lipid or lipoprotein into the plasma, reduced removal from the plasma, or both. Increased influx into the plasma may reflect increased synthesis in the liver or extrahepatic sites, or a redistribution of lipid between plasma and other sites. Decreased removal from the plasma could be due to reduced utilization or to abnormal clearance mechanisms.

In the experimentally induced nephrotic syndrome in the rat, studies have shown both in-

creased synthesis and decreased removal of lipid from the plasma. Increased incorporation of acetate-^{14}C into cholesterol has been demonstrated (5, 6), and Bar-On and Shafrir have shown increased conversion of glucose-^{14}C into both lipids and proteins (4). Marsh and Drabkin have reported an increased secretion of low density proteins by the isolated perfused rat liver (7) and increased production of albumin by rat liver slices (8). There is also evidence to favor reduced removal of chylomicron lipid from the plasma in nephrotic rats (6, 9).

Few studies have been carried out in human subjects in recent years. There have been reports of reduced removal of fat from the plasma (10, 11), but this may merely reflect a relative saturation of the clearing mechanism by the high concentration of endogenous plasma lipids. The lipid clearing effect of an albumin infusion (12) and the demonstration of lipoprotein lipase loss in the urine of nephrotic children (13) suggest that other mechanisms may play a role in the causation of the hyperlipidemia.

An increased conversion of carbohydrate to lipid has also been suggested in nephrotic human subjects by the finding of an accentuation of the hyperlipidemia after glucose infusions (14).

Although cholesterol and triglyceride are both frequently elevated in nephrosis, the factors which lead to this may be quite distinct. In this paper we therefore report studies in which the turnover of plasma esterified cholesterol and triglyceride have been studied separately in a group of nephrotic patients.

METHODS

The relevant clinical details of the seven nephrotic subjects are shown in Table I. One subject (E.G.) was studied when nephrotic and also some months later when in remission. No subject had diabetes or severe renal failure, although R.R. had some renal impairment. A renal biopsy was done to establish the nature of the underlying renal disease. All subjects were ambulatory before the investigation but were rested in bed during the 4 days of the turnover studies. Body weight and lipid levels were stable for at least 2 wk before the study. One subject only (R.R.) had an unusual diet that was rich in fats. Other subjects ate normal diets. The subjects were fasted overnight immediately before the investigation and then given light, fat-free meals on the 1st day of the study. Corticosteroids had not been used for at least 6 wk before the investigation.

Each subject was given an intravenous dose of 300 μc of DL-mevalonic acid-2-^{3}H and 50 μc of glycerol-1-^{14}C.[1] Tritiated mevalonic acid was also given to seven normocholesterolemic men with untreated coronary artery disease aged 38–51 yr. Triglyceride turnover was also measured in six healthy men.

Samples of venous blood were drawn into heparinized tubes at 2-hr intervals for 16 hr and then thrice daily for 4 days. Plasma was immediately separated at 4°C. Aliquots of whole plasma were then extracted with a chloroform : methanol mixture (2 : 1 v/v). Very low density lipoproteins (density < 1.006) were separated from aliquots of whole plasma by centrifuging at 100,000 *g*

[1] Radiochemical Centre, Amersham, England.

TABLE I

Clinical Details of the Nephrotic Subjects

Subject	Age	Sex	Body weight	Serum total cholesterol	Serum triglyceride	Serum albumin	Urine protein	Blood urea nitrogen	Renal biopsy
	yr		*kg*	*mg/100 ml*	*mg/100 ml*	*g/100 ml*	*mg/100 ml*	*mg/100 ml*	
E. G., nephrotic	60	F	66	650	245	1.4	602	17	Focal glomerulonephritis
V. H.	34	F	57	534	340	1.2	798	17	Proliferative glomerulonephritis
A. S.	17	M	65	800	380	1.3	749	18	Proliferative glomerulonephritis
A. H.	42	M	56	620	312	1.4	728	30	Membranous glomerulonephritis
P. R.	50	M	72	840	580	0.9	1085	50	Minimal change
R. R.	30	M	60	800	906	1.6	980	63	Chronic glomerulonephritis
L. D.	34	M	73	640	750	1.2	980	14	Proliferative glomerulonephritis
E. G. remission	60	F	65	250	60	3.4	7	15	Normal

for 15 hr in the 40.3 rotor of a Spinco Model L ultracentrifuge, Beckman Instruments Inc., Spinco Div., Palo Alto, Calif. (15). The very low density lipoproteins were then removed by slicing the tubes below the supernatant layer and the lipids extracted by the Folch procedure (16).

Aliquots of the chloroform phase, containing the lipids of whole plasma and very low density lipoprotein, were separated on silicic acid columns into two fractions, one containing esterified cholesterol and the other triglyceride and free cholesterol, by eluting with 30 ml of 1% diethyl ether in heptane and with 50 ml of chloroform respectively. The method has been described in detail elsewhere (17).

Radioactivity was measured in a Packard Tri-Carb scintillation counter, Packard Instrument Co., Inc., Downer's Grove, Ill., the triglyceride and free cholesterol radioactivity being determined simultaneously. The esterified cholesterol fraction which had become labeled with tritium was found to be free of ^{14}C radioactivity. The in vivo degradation of the glycerol-^{14}C therefore did not result in detectable labeling of cholesterol or of cholesterol ester fatty acids. The chemical concentrations of free and esterified cholesterol were determined by the method of Sperry and Webb (18) and triglyceride by the method of Carlson (19).

Calculations. The specific activity time curves for the triglycerides of very low density lipoproteins were plotted semilogarithmically. The rate of fall was linear from the time when peak specific activity was reached at 2–5 hr until 30–60 hr later. The fractional turnover rates were calculated as: $1/1.44 \times 1/t_{\frac{1}{2}}$ (20), in which $t_{\frac{1}{2}}$ is the half-time of disappearance of radioactivity in glyceride in the plasma very low density lipoprotein fraction. Farquhar, Gross, Wagner, and Reaven (20) have suggested that turnover rates may be calculated from data such as ours by multiplying the fractional turnover rate by the triglyceride content of plasma very low density lipoprotein since they found that the pool of triglyceride within the plasma very low density lipoproteins turned over in a volume approximating that of plasma. These calculations are shown in the Results although they are based on an assumption which has yet to be confirmed.

The rise and decline in the specific activities of free and esterified cholesterol were plotted on Cartesian graph paper, and the fractional turnover rate of esterified cholesterol was calculated graphically (Fig. 1).

The model used for the calculation is that proposed by

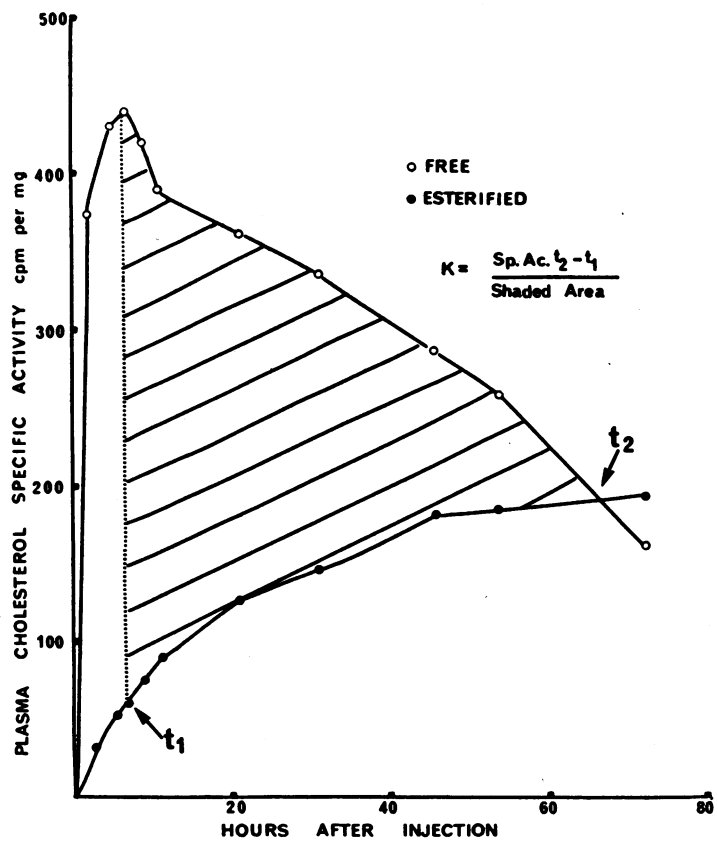

FIGURE 1 The specific activity curve of plasma free and esterified cholesterol after intravenous injection of mevalonic acid-2-^3H in subject P. R. The method of calculating the fractional turnover rate (K) is shown.

Zilversmit (21), in which the algebraic expression for the specific activity of the precursor (free cholesterol) need not be known. The assumptions underlying this model have been presented before (22) and require (*a*) that free cholesterol within the liver or plasma is the major precursor of plasma esterified cholesterol (17), and (*b*) that the esterified cholesterol in plasma is quantitatively very much greater than that present in other tissues including liver (17). The expression of the fractional turnover rate may then be derived from the equation:

$$\text{Fractional turnover rate} = \frac{SA(EC)t_2 - SA(EC)t_1}{\text{shaded area}},$$

in which SA(EC) t_2 and SA(EC) t_1 refer to the specific activities of esterified cholesterol at the times t_2 and t_1, chosen at any point along the curve. The curves intersect at the point of the maximum specific activity of esterified cholesterol. The shaded area in Fig. 1 was calculated by counting squares.

The turnover rate of esterified cholesterol is calculated as the product of the fractional turnover rate and the plasma content [2] of esterified cholesterol. Nestel and Monger (22) had previously shown in a normal subject that the fractional turnover rate of whole plasma represents the average fractional turnover rate of esterified cholesterol in the different lipoproteins, and that the turnover rate in whole plasma equals the sum of the separate turnover rates of esterified cholesterol in the different lipoproteins. The interpretation of the turnover rate of plasma esterified cholesterol is discussed in a later section.

RESULTS

Concentrations of plasma triglyceride and esterified cholesterol and urinary protein. In the nephrotic subjects the plasma triglyceride concentration varied from 245 to 906 mg/100 ml, and the plasma cholesterol concentration varied from 534 to 840 mg/100 ml (Table II). These values are the averages of fasting samples taken daily for several days before and during the study. In the first four subjects listed in Table II, the fasting serum was clear, and in these subjects the triglyceride concentration in density < 1.006 lipoproteins was 285 mg/100 ml or less (Table II). In the remaining three subjects the fasting plasmas were lactescent, especially that of subject R.R. In the first five subjects listed in Table II more than 75% of the total esterified cholesterol was carried in lipoprotein classes of density > 1.006. However, in two of the three subjects with lactescent plasma, much of the esterified cholesterol was carried in the density < 1.006 fraction of the plasma.

The loss of protein in the urine was greatest

[2] The determination of plasma volume poses several problems in the nephrotic subject. Obviously if the patient is grossly edematous, then 5% of the body weight will give an erroneous plasma volume. Also in the nephrotic, the plasma volume tends to be less than normal (23). In such edematous states, the use of albumin-^{131}I to measure plasma volume also leads to erroneous results, since the volume of distribution of labeled albumin is greater than that of plasma (13). However, due to the use of diuretic drugs, none of the patients had more than minimal edema, and body weight had been steady for several weeks, so that 5% of the body weight was probably a close approximation of the true plasma volume. In four subjects the plasma volume was measured by the albumin-^{131}I technique, and this value was found to be similar to the calculated value. (Observed plasma volumes were: 3.4, 3.4, 3.6, and 3.6 liters; ^{131}I-calculated plasma volumes were 3.3, 3.25, 3.55, and 3.7 liters respectively.)

TABLE II

Relationship between Protein Loss in Urine and the Concentrations of Plasma Lipids

Subject	Urine protein	Plasma	Whole plasma cholesterol			d < 1.006 Lipoprotein	
			Free	Esterified	FC/EC	Esterified cholesterol	Triglyceride
	g/day		*mg/100 ml*			*mg/100 ml*	
E. G.	8.6	Clear	180	450	0.40	97	117
V. H.	11.4	Clear	143	371	0.38	54	150
A. S.	10.7	Clear	208	568	0.37	76	201
A. H.	10.4	Clear	141	464	0.31	58	285
P. R.	15.5	Lactescent	324	480	0.67	53	414
R. R.	14.0	Lactescent	288	580	0.50	486	532
L. D.	14.0	Lactescent	270	330	0.82	220	600

FC/EC, free cholesterol/esterified cholesterol; d, density.

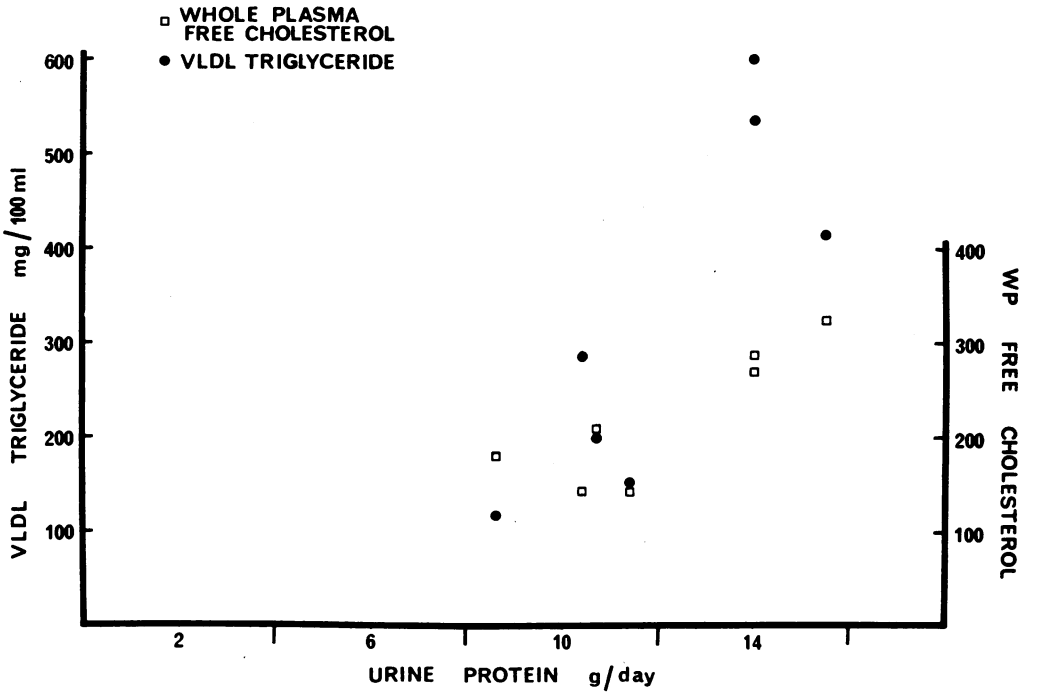

FIGURE 2 Data from seven nephrotic subjects showing the relationship between very low density lipoprotein (*VLDL*) triglyceride (●) and whole plasma (*WP*) free cholesterol (□) and the daily urinary protein excretion.

among the three markedly hypertriglyceridemic subjects, although the serum albumin concentration was similar in the seven subjects. There was a significant direct relationship between protein loss and the plasma triglyceride level ($P < 0.01$) (Fig. 2). Such a relationship was also found between protein loss and plasma free cholesterol but not esterified cholesterol. Since the proportion of free to esterified cholesterol rises as lipoprotein density falls (24) it seems likely that the protein loss was directly related to the concentration of larger lipoproteins of very low density rather than to the concentration of lipids in whole plasma.

Triglyceride turnover. The specific activity of

TABLE III
Triglyceride Studies in Nephrotic and Control Subjects

Nephrotic					Control				
Subject	Concentration	Half-time	Fractional turnover rate	Turnover rate	Subject	Concentration	Half-time	Fractional turnover rate	Turnover rate
	mg/100 ml	*hr*	*hr⁻¹*	*mg/kg per hr*		*mg/100 ml*	*hr*	*hr⁻¹*	*mg/kg per hr*
E. G.	117	10	0.069	4.3	A	33	4	0.17	2.8
V. H.	150	11	0.063	4.7	B	54	5.5	0.13	3.5
A. S.	201	7.5	0.093	9.3	C	47	4.5	0.15	3.5
A. H.	285	7.5	0.093	12.9	D	44	4	0.17	3.7
P. R.	414	12	0.058	11.3	E	52	3	0.23	6.0
R. R.	532	20	0.035	9.2	F	77	4	0.17	6.5
L. D.	600	10	0.069	19.9					
Mean	328	11.1	0.069	10.2 ± 2.00*		51	4.1	0.17	4.3 ± 0.62*

* Standard error of mean.

plasma very low density lipoprotein triglyceride reached a peak in the first or second sample of plasma collected 2 and 4 hr after the injection of glycerol-^{14}C. The fall in specific activity was then exponential for 30–60 hr. The concentrations, half-times of disappearance of ^{14}C, the observed fractional turnover rates, and the calculated turnover rates are shown in Table III. With the exception of subject R.R. the fractional turnover rates in the nephrotic subjects varied from 0.058 to 0.093/hr, although the range of the triglyceride concentrations was greater, and on the whole the fractional turnover rates were unrelated to concentration. However, the turnover rates (in mg/kg per hr) were highest among the more hypertriglyceridemic subjects, so that a significant relationship could be demonstrated between triglyceride concentration and turnover rate in the nephrotic subjects (Fig. 3) ($P = < 0.01$). Moreover, turnover rates were significantly higher in the nephrotic than in the control group ($P = < 0.02$). Increased triglyceride turnover in nephrosis was also evident in subject E.G., whose triglyceride turnover was greater when she was nephrotic than when she was in remission (Table VI). The exception to this general trend was subject R.R. who suffered from significant renal impairment. The fractional turnover rate in R.R. was only 0.035/hr.

Esterified cholesterol turnover. The free cholesterol specific activity in whole plasma reached a peak at about 5 hr after the injection of mevalonic acid. Equilibration between the free and esterified cholesterol specific activities occurred between 30 and 75 hr in all subjects except R.R., in whom it was delayed to 100 hr.

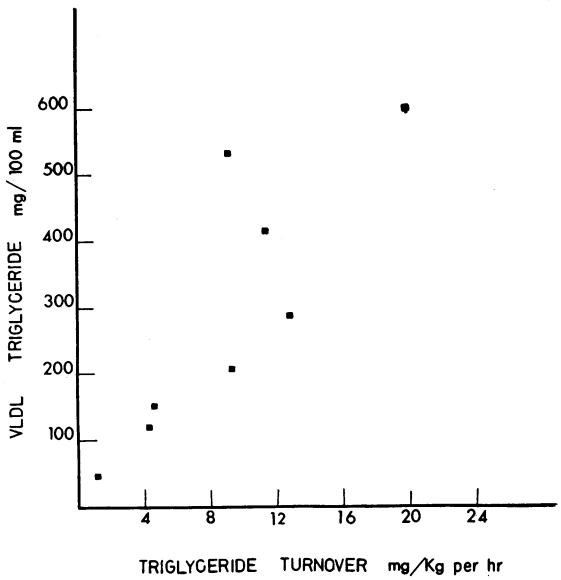

FIGURE 3 Relationship between turnover rate and concentration of triglycerides in very low density lipoprotein (*VLDL*) in seven nephrotic subjects. (Subject E. G. was studied twice.)

The concentration, turnover time, fractional turnover rate, and turnover rate of whole plasma esterified cholesterol are shown in Table IV. The fractional turnover rates were on the average less among the nephrotic subjects than among the seven normocholesterolemic men who served as controls. However, since the pool sizes were very

TABLE IV

Whole Plasma Esterified Cholesterol Studies in Nephrotic and Control Subjects

Nephrotic					Control				
Subject	Concentration	Turnover time	Fractional turnover rate	Turnover rate	Subject	Concentration	Turnover time	Fractional turnover rate	Turnover rate
	mg/100 ml	*hr*	*hr^{-1}*	*mg/kg per hr*		*mg/100 ml*	*hr*	*hr^{-1}*	*mg/kg per hr*
E. G.	450	62	0.016	3.7	1	175	46	0.022	2.0
V. H.	371	71	0.014	2.7	2	140	34	0.030	2.1
A. S.	568	67	0.015	4.3	3	165	66	0.015	1.3
A. H.	464	53	0.019	4.3	4	130	41	0.024	1.6
P. R.	480	77	0.013	2.9	5	155	100	0.010	0.8
R. R.	580	105	0.0095	2.8	6	180	43	0.023	2.0
L. D.	330	56	0.018	2.9	7	155	45	0.022	2.0
Mean	463	70	0.015	3.37 ± 0.27*		160	54	0.021	1.68 ± 0.19*

* Standard error of mean.

TABLE V

Very Low Density Lipoprotein Esterified Cholesterol Studies in Nephrotic and Normal Subjects

Subjects	Concentration	Fractional turnover rate	Turnover rate	VLDL* mg / WP‡ mg	VLDL turnover rate / WP turnover rate
	mg/100 ml	*hr*$^{-1}$	*mg/kg per hr*		
Nephrotics					
E. G.	97	0.015	0.74	0.22	0.20
V. H.	54	0.013	0.36	0.15	0.13
A. S.	76	0.013	0.50	0.13	0.11
A. H.	58	0.012	0.33	0.12	0.08
P. R.	53	0.005	0.14	0.11	0.05
R. R.	486	0.005	1.25	0.84	0.45
L. D.	220	0.018	1.86	0.67	0.64
Controls					
8	20	0.033	0.33	0.13	0.15
9	25	0.032	0.37	0.14	0.15

* Very low density lipoprotein.

‡ Whole plasma.

much greater in the nephrotic patients, the turnover rates were significantly higher among them ($P < 0.001$).

The data for esterified cholesterol in very low density lipoproteins are shown in Table V. The turnover rates have been compared with those of two normocholesterolemic healthy subjects. Cholesterol ester turnover in very low density lipoproteins was clearly increased in the two subjects in whom most of the plasma esterified cholesterol was carried in this lipoprotein fraction. When the contribution by very low density lipoprotein cholesterol ester turnover to total plasma cholesterol ester turnover is related to the relative mass of esterified cholesterol carried in the very low density lipoproteins and whole plasma, it seems clear that in most subjects, turnover in very low density lipoproteins was proportional to mass. In other words, in the group as a whole, there was neither a strikingly disproportionate increase nor decrease in cholesterol ester turnover in very low density lipoproteins relative to that occurring in whole plasma. However in subjects P.R. and R.R., both of whom were also hypertriglyceridemic, the fractional turnover rate of esterified cholesterol was very low, indicating a relative reduction in clearance from plasma.

TABLE VI

The Findings in Subject E. G. Who Was Studied When Nephrotic and after Clinical Remission

	Nephrotic	Remission
Plasma cholesterol ester		
Concentration, *mg/100 ml*	450	112
Turnover rate, *mg/kg per hr*	3.7	2.0
VLDL cholesterol ester		
Concentration, *mg/100 ml*	97	24
Turnover rate, *mg/kg per hr*	0.74	0.23
VLDL triglyceride		
Concentration, *mg/100 ml*	117	45
FTR/hr	0.069	0.050
Turnover rate, *mg/kg per hr*	4.3	1.0
Urine protein, *g/day*	8.6	0.1

VLDL, very low density lipoprotein; FTR, fractional turnover rate.

The studies in subject E.G. have been summarized in Table VI. The concentrations and the turnover rates of the plasma lipids were considerably greater when the patient was nephrotic than after she had recovered.

DISCUSSION

The triglyceride and cholesterol levels were raised in the plasmas of all seven nephrotic subjects. In subjects E.G., V.H., A.S., and A.H. the plasmas were clear, and at least three-quarters of the cholesterol was carried in lipoproteins other than density < 1.006. In these subjects the predominant increase was therefore probably in the low density lipoproteins, with a lesser rise in the very

low density lipoproteins. The remaining three subjects had lactescent plasmas and, although the nature of the particles responsible for the lipidemia was not determined, the high concentration of esterified cholesterol in the density < 1.006 lipoprotein fractions suggests that they were mainly of endogenous origin (25). However, more detailed studies were not carried out to determine the exact nature of the particles responsible for the lactescence.

The interpretation of the turnover data must therefore take into account the variable distribution of the lipids, especially of esterified cholesterol among the different lipoproteins. The turnover of esterified cholesterol is not homogeneous in the different lipoproteins in subjects with nonlipidemic plasma (26, 22). Although data on lipidemic plasma are not available, the turnover of esterified cholesterol in whole plasma does appear to reflect the average of the different turnovers in the various lipoproteins (22). Similarly, there does not appear to be any evidence to show that the turnover of triglyceride in very low density lipoproteins of patients with nephrosis is the same as that in other particles of endogenous origin which float at a density of 1.006, although it seems likely that they both originate in the liver.

The studies show that the hypertriglyceridemia in nephrosis does not appear to stem solely from overproduction or diminished removal of triglyceride. Both mechanisms seem to be involved. The evidence for some degree of overproduction derives from several observations: (*a*) the fractional turnover rates among the seven patients varied much less than the concentrations of triglyceride in the very low density lipoproteins; (*b*) the calculated turnover rates were directly related to triglyceride, so that the highest turover rates were observed in patients with higher triglyceride levels; (*c*) triglyceride turnover rates were on the average less among healthy normotriglyceridemic subjects; (*d*) remission in one subject was associated with a fall in triglyceride concentration and turnover rate. This interpretation assumes that the relationship between the proportions of triglyceride in plasma very low density lipoproteins and that present in the liver, which constitutes a major pool in which the isotope is distributed, was of the same order in the different subjects.

There appears to be also some evidence for decreased removal of triglyceride from plasma. A comparison of the fractional turnover rates and turnover rates in the nephrotic subjects and in other nonnephrotic subjects with similar degrees of hypertriglyceridemia reveals a slower turnover of triglyceride in nephrosis. Thus in the seven nephrotic subjects the mean fractional turnover rate was 0.069/hr and the mean turnover rate 10.2 mg/kg per hr, whereas the values reported by Nestel (27) for three subjects with coronary heart disease with very low density lipoprotein triglyceride levels of 186–220 mg/100 ml were at least twice as high, but in these studies labeled palmitic acid was used as precursor.

Reaven, Hill, Gross, Farquhar, and Brown (28) have also found somewhat higher fractional turnover rates among subjects with carbohydrate-accentuated hypertriglyceridemia. Using a technique similar to the present one they reported a number of studies in subjects in whom triglyceride synthesis was being stimulated by diets rich in carbohydrate. In five studies the triglyceride concentrations in the density < 1.006 lipoprotein fraction were reported as 367, 578, 602, 613, and 707 mg/100 ml which are of the order seen in our nephrotics. The corresponding fractional turnover rates (average 0.092/hr) and turnover rates (average 22.7 mg/kg per hr) were significantly higher than those found among the nephrotics ($P = < 0.01$). Unless the distribution of triglyceride between plasma and liver differs significantly in different hypertriglyceridemic states, the turnover of triglyceride is less in the hypertriglyceridemia associated with nephrosis. It is possible that at high turnover rates, the capacity of the removal mechanisms becomes overloaded, and a limit to the rate at which triglyceride can be cleared from the plasma has been demonstrated in man (29).

The presence of hyperlipidemia has recently been reported in renal disorders other than nephrosis and has been attributed to renal failure (30). Hyperlipidemia was prone to develop especially in subjects who were being kept alive by dialysis, and evidence for diminished glyceride removal was suggested by the finding of low levels of lipoprotein lipase. It is interesting that the lowest triglyceride fractional turnover rate was obtained in subject R.R., the only patient with a significant disturbance of renal function.

The studies of esterified cholesterol turnover carried out in the hypercholesterolemic nephrotic subjects and the normocholesterolemic control subjects showed that the turnover rate was significantly greater in the former ($P < 0.001$). In subject E.G. who was studied twice, remission of the nephrotic state resulted in a fall in the turnover rate from 3.7 to 2.0 mg/kg per hr. These findings are consistent with an increase in synthesis of esterified cholesterol.

The technique used in these studies is interpreted as measuring the rate at which plasma esterified cholesterol is formed from free cholesterol and subsequently hydrolyzed. Although esterification of free cholesterol occurs in several sites, including the liver and intestine(31), it is thought that plasma may be the major site in man, since similar values have been obtained when the rate of esterification has been measured in vivo by the present technique (22) and in vitro by the method of Glomset, Parker, Tjaden, and Williams (32). The calculation would however remain valid even if the plasma cholesterol esters were derived from free cholesterol in the liver before being secreted into the plasma, since free cholesterol in liver and plasma probably reach isotopic equilibrium rapidly. It is unlikely however that in man plasma cholesterol esters are derived to a major extent from a pool of cholesterol esters in the liver (17). Hydrolysis of cholesterol esters, on the other hand, does not appear to occur in plasma, and the removal and breakdown of plasma esterified cholesterol seems to take place predominantly in the liver (31). The calculated turnover rate of plasma cholesterol esters is therefore envisaged as the transport of cholesterol esters from their origin in plasma and possibly to a lesser extent outside plasma, to sites of hydrolysis in tissues, especially the liver. An increase in turnover rate such as we have found in nephrosis can therefore provide information only about plasma esterified cholesterol. However, since esterified cholesterol is derived from free cholesterol, the increased rate of formation of cholesterol esters suggests an increase in the turnover also of free cholesterol, or at least of that pool of free cholesterol which gives rise to esterified cholesterol. The functional significance, if any, of this increase in esterified cholesterol turnover cannot be determined in view of the uncertainty of the role of plasma esterified cholesterol in cholesterol transport (31).

The findings with respect to esterified cholesterol in very low density lipoproteins are similar to those in whole plasma. In most subjects the proportion of turnover to mass was similar in very low density lipoproteins and whole plasma. This finding would indicate a general rise in esterified cholesterol turnover in very low density lipoproteins, and this assumption appears to be supported by the comparison with the findings in the two normal subjects. However, in at least two nephrotic subjects (P.R. and R.R.) the fractional turnover rate of esterified cholesterol was very low.

In nephrotic rats, increased hepatic synthesis of cholesterol has been demonstrated by isotopic techniques (5, 6) although this appears to depend on the duration of the nephrosis. Increased, normal, and decreased cholesterol synthesis have been reported at 2, 3, and 6 wk respectively after induction of the nephrotic state (6).

The mechanisms underlying the increase in the synthesis of lipid in the nephrotic syndrome are unknown. It is clear that an early disturbance in nephrosis is the loss of protein; when this can be prevented hyperlipidemia does not develop (33). Simultaneous disturbances in the concentrations of plasma protein and lipid leading to a fall in the former and a rise in the latter also occur after plasmapheresis (34) and bleeding (35). The loss of protein in urine leads to a rise in both protein and lipoprotein formation (4, 7, 36, 37).

The correlation found in this study between the serum triglyceride concentration and the loss of protein in the urine is in accord with these findings. Baxter, Goodman, and Havel have reported the association of low serum albumin and high serum triglyceride concentrations (1). The additional correlation between protein loss and plasma free cholesterol, and the rising free to esterified cholesterol ratio at higher triglyceride levels, suggest that the increasing loss of protein is associated with the appearance of larger lipoproteins of lower density.

A rise in the rate of synthesis of lipoproteins accompanying the general increase in protein synthesis but without any appreciable loss of lipoprotein would be expected to lead to a rise in the plasma levels. The increase in lipid turnover is

therefore envisaged as a secondary manifestation of raised lipoprotein turnover. The increase in triglyceride turnover would be expected to occur in the liver, but the increase in esterified cholesterol turnover may take place both in liver and plasma. Recent evidence suggests that in man the esterification of free cholesterol in plasma may be quantitatively more important than that occurring in liver (17, 32). Subject R.R. provides additional interest in that he required renal transplantation at a later date. This resulted in the cessation of proteinuria and the return of plasma lipids to normal (although it is appreciated that the use of various drugs after transplantation complicates interpretation).

The hyperlipidemia of nephrosis has been reversed by albumin infusion. This may have resulted from the provision of additional fatty acid acceptor which is required during triglyceride lipolysis. However, the possibility that changes in plasma volume and osmotic pressure may also influence the rate of albumin and possibly lipoprotein synthesis must be considered. Such a mechanism would be consistent with the lowering of plasma lipids in nephrosis by dextran and other macromolecular substances (38) which also produce a decrease in the rate of synthesis of albumin (39).

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