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P. J. Nestel, E. A. Monger

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Research Article

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The turnover rate of plasma esterified cholesterol was highest among the overweight hypercholesterolemic subjects and least among the subjects with familial hypercholesterolemia. Hypercholesterolemic subjects of normal weight had turnover rates similar to those found in normocholesterolemic men.

Significant correlations were found between body surface or body weight and the turnover rate of plasma esterified cholesterol.

We conclude that the factors which determine the development of hypercholesterolemia are not identical in all hypercholesterolemic subjects and that when hypercholesterolemia is associated with overweight there is an increased formation of at least plasma esterified cholesterol.

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Turnover of Plasma Esterified Cholesterol in Normocholesterolemic and Hypercholesterolemic Subjects and Its Relation to Body Build *

P. J. NESTEL † AND E. A. MONGER

(From the University of Melbourne Department of Medicine, Victoria, Australia)

Summary. **The turnover rate of plasma total esterified cholesterol was measured after the intravenous injection of tritiated mevalonic acid in 16 men with coronary heart disease. Four subjects were normocholesterolemic and 12 were hypercholesterolemic; among the latter, 3 suffered from familial hypercholesterolemia and 6 were overweight.**

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Introduction

High concentrations of cholesterol are commonly found in the plasma of men with coronary heart disease. Although the reason for this is not clear, a proportion of such subjects suffer from well-defined disorders of lipid metabolism such as familial hypercholesterolemia (type II familial hyperbetalipoproteinemia)[1] or carbohydrate-induced hyperlipidemia (2) (type III hyperbetalipoproteinemia).[1] The majority of hypercholesterolemic men with coronary heart disease do not readily fit into either of these categories although a careful study of a large number of such subjects remains to be done.

Subjects with coronary heart disease are also frequently overweight (3–5), and a number of studies have demonstrated significant correlations between the serum cholesterol concentration and body fatness. Hypercholesterolemia may therefore be partly attributable to the development of obesity in some patients particularly since it has been shown that a gain in weight is associated with a rise in the plasma cholesterol level (6) and loss of weight with a fall in the cholesterol concentration (7).

There are then a number of definable circumstances in which hypercholesterolemia may be found in association with coronary heart disease, although the mechanisms responsible for the hypercholesterolemia are poorly understood. Crider, Bradford, Alaupovic, and Furman have described an increased conversion of acetate to cholesterol within very low density lipoproteins in response to dietary carbohydrate in 2 subjects with carbohydrate-induced hyperlipidemia (8). Decreased catabolism of cholesterol has been described in 2

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† Address requests for reprints to Dr. P. J. Nestel, Dept. of Clinical Science, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

[1] As defined by Fredrickson and Lees (1).

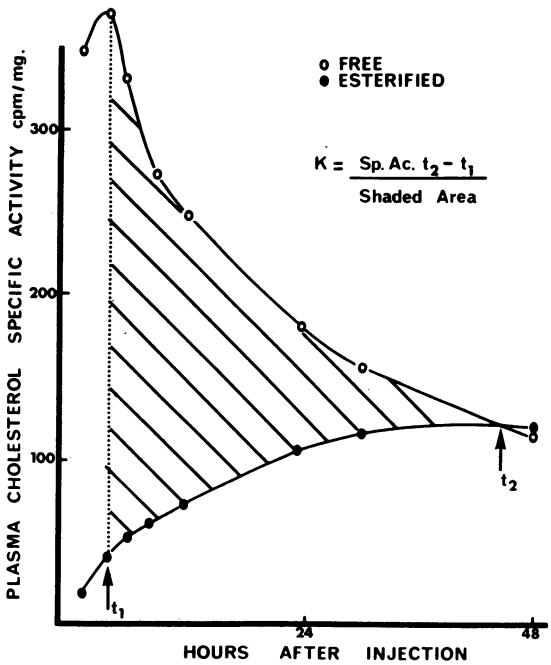

FIG. 1. CHANGES IN SPECIFIC ACTIVITY OF PLASMA FREE AND ESTERIFIED CHOLESTEROL AFTER INTRAVENOUS INJECTION OF MEVALONIC ACID-2-^{3}H IN A REPRESENTATIVE STUDY. The method of calculating the fractional turnover rate (K) is shown.

subjects with familial hypercholesterolemia although the results may have been influenced by the use of liquid formula diets (9). A number of studies have been concerned with the measurement of turnover rates of total exchangeable cholesterol although these investigations have in general not been specifically directed to the causation of hypercholesterolemia. Chobanian, Burrows, and Hollander found similar rates of cholesterol turnover among 4 hypercholesterolemic and 5 normocholesterolemic subjects (10), whereas Nestel, Hirsch, and Couzens reported a significant direct relationship between the serum cholesterol concentration and the fractional turnover rate of the exchangeable pool of cholesterol in 10 men with coronary heart disease (11).

Measurements of total exchangeable cholesterol turnover require many weeks. Furthermore the measurement of turnover within this pool may obscure the kinetics within a smaller more rapidly turning over pool such as that of plasma cholesterol ester. The present paper reports the measurements of turnover rates of plasma esterified cholesterol in 16 subjects with coronary heart disease. The major emphasis has been placed on the comparison of subjects with and without familial hypercholesterolemia and on the relationship between body weight and build and the turnover of plasma esterified cholesterol.

Methods

The 16 men selected for study had all suffered a myocardial infarction not less than 9 months before the studies. Although they were not receiving drugs, a number had modified their diets. Two of the 3 subjects (No. 8 and 9) with familial hypercholesterolemia had been eating a low animal fat, high vegetable oil diet for at least a year, although this had led to only small reductions in cholesterol concentration. Some of the other subjects had also made minor dietary changes. Records of body weight during the preceding year showed no changes that exceeded 2 kg.

The serum cholesterol concentrations, body weights, calculated surface areas, and ages of the 16 subjects are shown in Tables I and III. Four subjects were normocholesterolemic; of the 12 hypercholesterolemic subjects, 6 were overweight and 3 were clearly suffering from familial hypercholesterolemia.

DL-Mevalonic acid-2-^{3}H lactone [2] was prepared for intravenous injection by splitting the lactone ring during an hour's incubation in sodium bicarbonate solution at pH 8.7 at 37° C. Varying doses averaging about 100 μc were administered to each subject. Samples of blood were then obtained 3, 5, 7, and 10 hours later on the first day and at least twice daily for 3 days. The plasma was immediately separated and extracted with 20 vol of chloroform: methanol 2:1 (vol:vol). Separation of esterified cholesterol from free cholesterol was carried out on silicic acid columns by eluting with 1% diethyl ether in heptane and with chloroform, respectively. Separation was greater than 99%. Radioactivity was measured in a liquid scintillation spectrometer, and the concentrations of the free and esterified cholesterol were determined by the method of Sperry and Webb (12).

The decline in the specific activities of free and esterified cholesterol was plotted on cartesian graph paper, and the fractional turnover rate of the esterified cholesterol was calculated graphically (Figure 1). The model used in the calculations was one in which the algebraic expression for the specific activity of the precursor need not be known (13), since the fall in free cholesterol specific activity during the period of the study was complex. However, the specific activity–time curves of the precursor and product (esterified cholesterol) were known and crossed at the point of maximal specific activity of the product. The equation for the calculation of the fractional turnover rate of the product has been defined by Zilversmit (13). K = (specific activity of product at time$_2$ − specific activity at time$_1$) / shaded area. This model is compatible with plasma esterified choles-

[2] Radiochemical Centre, Amersham, England.

terol being derived from free cholesterol either in the plasma or in the liver, since plasma and hepatic free cholesterol reach isotopic equilibrium rapidly (14, 15). The possibility that esterified cholesterol in the liver is a major precursor of plasma esterified cholesterol appears unlikely in the light of our previous finding of slightly higher initial specific activities for plasma than hepatic esterified cholesterol in man (15). This finding did not, however, exclude the possibility of plasma esterified cholesterol being derived from one of the several pools within the liver (15).

The turnover rate of plasma esterified cholesterol was calculated by multiplying the fractional turnover rate by the plasma content of esterified cholesterol. This ignores the pool of esterified cholesterol within the liver, which may be partly in equilibrium with that in the plasma. However, this does not appear to be an unreasonable omission in view of the relatively low concentration of esterified cholesterol in the liver (15),[3] provided that the distribution of esterified cholesterol between plasma and liver is not altered in hypercholesterolemic states.

This method of calculation treats the cholesterol esters of plasma as a single homogeneous group. It has, however, been shown that the turnover of cholesterol esters within different classes of lipoproteins is heterogeneous (16, 17). The fractional turnover rate of the cholesterol esters within high density lipoproteins is greater than that of cholesterol esters within very low density lipoproteins and those within low density lipoproteins, the last having the slowest fractional turnover rate. We therefore studied, in a single healthy subject, the fractional turnover rates of cholesterol esters in these 3 classes of lipoproteins as well as in whole plasma, in order to determine *a*) whether the fractional turnover rate of the cholesterol esters in whole plasma reflected the average for cholesterol esters in the different lipoproteins, and *b*) whether the calculated turnover rate of the cholesterol esters in whole plasma equaled the sum of the turnover rates of cholesterol esters of all the lipoproteins. The methods were similar to those already described except that, in addition, the plasma was separated into lipoproteins of density less than 1.006, density between 1.006 and 1.063, and density greater than 1.063 as in previous studies (17).

Statistical methods were calculated according to Snedecor (18).

Results

The plasma free cholesterol specific activity reached a peak in either the first or second sample of plasma collected 3 and 5 hours after the injection of mevalonic acid. Equilibration between the free and esterified cholesterol specific activities occurred between 30 and 72 hours in 13 subjects but was delayed beyond 90 hours in the 3 men with familial hypercholesterolemia.

Plasma esterified cholesterol turnover. The concentration, pool size, fractional turnover rate, turnover time, and turnover rate of plasma esterified cholesterol are shown in Table I. The observed fractional turnover rates were greatest in the overweight hypercholesterolemic subjects and least in the 3 subjects with familial hypercholesterolemia. The fractional turnover rates in the remaining hypercholesterolemic subjects were of the same order as those found among the 4 normocholesterolemic subjects. The greatest contrast in fractional turnover rates was between the 3 familial hypercholesterolemic subjects and those overweight subjects with comparable concentrations and pool sizes of plasma esterified cholesterol. The fractional turnover rates of the former were only about one-third of those found among the latter.

Similar conclusions could be drawn from a comparison of the calculated turnover rates. The highest turnover rates were found among the heavier hypercholesterolemic subjects. The turnover rates were similar among hypercholesterolemic men of normal weight and normocholesterolemic subjects. The 3 subjects with familial hypercholesterolemia had the lowest turnover rates.

The relationship between turnover rate and body surface area, shown in Figure 2, was highly significant ($p = < 0.001$, $r = 0.76$). The relationship between turnover rate and body weight reached a similar degree of significance.

The data for esterified cholesterol turnover in whole plasma and in the separated lipoproteins are shown in Table II. The fractional turnover rate was highest for cholesterol esters in high density lipoproteins and least for those in low density lipoproteins and was in agreement with previous observations (16, 17). The average fractional turnover rate was calculated for the cholesterol esters in the 3 classes of lipoproteins taking into consideration the relative mass of cholesterol esters within each lipoprotein. The average fractional turnover rate was 0.029 per hour, which was in good agreement with the fractional turnover rate of the cholesterol esters of whole plasma, which

[3] Assume that *1*) concentrations of esterified cholesterol = 160 mg per 100 ml in plasma and 0.40 mg per g in liver; *2*) weight of liver = 1,500 g; plasma volume = 3.2 L. Then total hepatic esterified cholesterol = 0.6 g and total plasma esterified cholesterol = 5.1 g.

TABLE I

Experimental data on plasma esterified cholesterol

Subject	Age	Body weight	% outside desirable weight*	Surface area	Plasma esterified cholesterol data				
					Concentration	Pool size	Fractional turnover rate	Turnover time†	Turnover rate
	years	*kg*		m^2	*mg/100 ml*	*g‡*	*/hour*	*hours*	*g/hour*
1	47	76	+5	1.93	175	6.75	0.022	46	0.15
2	41	63	+6	1.68	140	4.41	0.030	34	0.13
3	47	70	−1	1.85	165	5.78	0.015	66	0.09
4	45	84	+17	2.02	130	5.46	0.024	41	0.13
5	38	58	+2	1.63	250	7.25	0.014	71	0.10
6	55	54	−5	1.58	210	5.67	0.027	38	0.15
7	50	73	+1	1.88	214	7.81	0.017	59	0.13
8§	44	61	−5	1.72	325	10.20	0.010	100	0.10
9§	49	56	−5	1.61	285	7.98	0.007	140	0.06
10§	49	58	−2	1.58	285	8.25	0.011	91	0.09
11	48	82	+22	1.93	210	8.61	0.026	39	0.22
12	39	83	+17	1.98	190	7.88	0.027	37	0.21
13	38	83	+15	2.00	270	11.20	0.024	41	0.27
14	44	88	+17	2.08	220	9.68	0.027	37	0.26
15	58	87	+21	2.05	190	8.26	0.025	40	0.21
16	48	90	+17	2.12	240	10.80	0.032	31	0.34

* Weights of insured persons in the United States associated with lowest mortality.
† Reciprocal of fractional turnover rate (13).
‡ Concentration × plasma volume (5% body weight).
§ Hypercholesterolemic subjects with tendinous xanthomata (familial hypercholesterolemia).

was 0.031 per hour. The sum of the turnover rates for cholesterol esters in the different lipoproteins was 0.158 g per hour, which was within 6% of the turnover rate obtained for whole plasma (0.168 g per hour). The method used for calculating the turnover of cholesterol esters in whole plasma therefore reflects the turnover of the cholesterol esters in the individual lipoprotein classes.

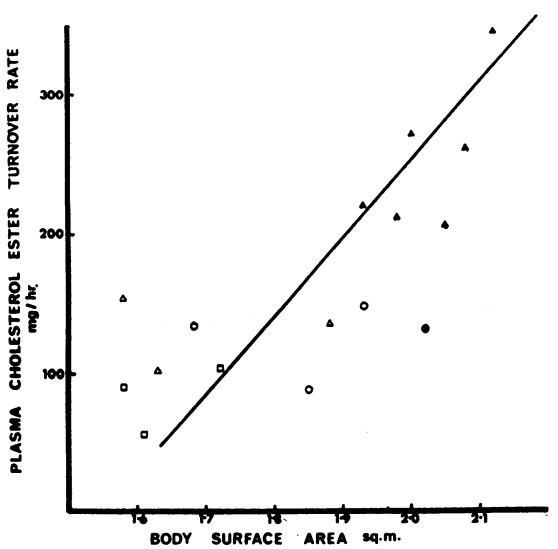

FIG. 2. THE RELATIONSHIP BETWEEN BODY SURFACE AREA AND THE TURNOVER RATE OF PLASMA ESTERIFIED CHOLESTEROL. ○ = normocholesterolemic subjects of normal weight; ● = normocholesterolemic, overweight subject; △ = hypercholesterolemic subjects of normal weight; ▲ = hypercholesterolemic, overweight subjects; □ = subjects with familial hypercholesterolemia.

Radioactivity in plasma free cholesterol. The proportion of injected radioactive biologically active mevalonic acid that was found in plasma free cholesterol at its peak specific activity, i.e., in either the 3- or 5-hour sample, is shown in Table III. From 4.2 to 20.8% has been converted to free cho-

TABLE II

Fractional turnover rates and turnover rates for individual lipoprotein classes and whole plasma in one subject

Lipoprotein density	Esterified cholesterol		Fractional turnover rate	Turnover rate
	Concentration	Pool size		
	mg/100 ml	*g*	*/hour*	*g/hour*
<1.006	20	0.70	0.033	0.023
1.006–1.063	92	3.22	0.027	0.087
>1.063	37	1.30	0.037	0.048
Total	149	5.22	0.029*	0.158
Whole plasma	155	5.42	0.031	0.168

* Average fractional turnover rate obtained by adding the values derived for each lipoprotein from the equation: (fractional turnover rate × concentration)/total lipoprotein concentration.

lesterol and transferred into the plasma at this stage. In general, the amount of label found in plasma free cholesterol was proportional to the size of the pool (Figure 3). Subjects with familial and other varieties of hypercholesterolemia were found on the average to have similar proportions of injected label in plasma free cholesterol.

Since the most rapid fall in the free cholesterol specific activity occurred during the first 24 hours, the ratio of the 24-hour to the peak specific activity was calculated (Table III). The values fell within the range of 0.33 to 0.64, and there was no evidence for an unusually slow or rapid rate of fall in specific activity in any group of subjects.

Discussion

These studies demonstrate that the turnover rate of plasma total esterified cholesterol varies considerably among subjects with coronary heart disease and hypercholesterolemia. Men who were overweight and hypercholesterolemic had turnover rates greater than those found among men who were either hypercholesterolemic but not overweight or who were normocholesterolemic. In particular, the lowest turnover rates were found in 3 subjects with familial hypercholesterolemia who were characterized by tendinous xanthomata and a striking familial incidence of hypercholesterolemia and coronary heart disease. The remaining 9 hypercholesterolemic subjects could not be classified as suffering from a specific disorder of lipid metabolism.

TABLE III

Experimental data on plasma free cholesterol (FC)

Subject	Plasma FC concentration	% injected dose in plasma FC at peak SA*	Ratio 24-hour/4-hour FC SA
	mg/100 ml		
1	71	9.0	0.43
2	70	4.2	0.45
3	79	4.4	0.59
4	53	8.4	0.38
5	101	16.0	0.41
6	95	5.0	0.43
7	86	15.4	0.46
8	140	13.2	0.46
9	125	10.2	0.49
10	127	8.4	0.64
11	90	9.4	0.44
12	85	6.4	0.49
13	130	16.0	0.46
14	95	15.6	0.47
15	80	20.8	0.33
16	118	18.4	0.42

* Biologically active mevalonic acid only.

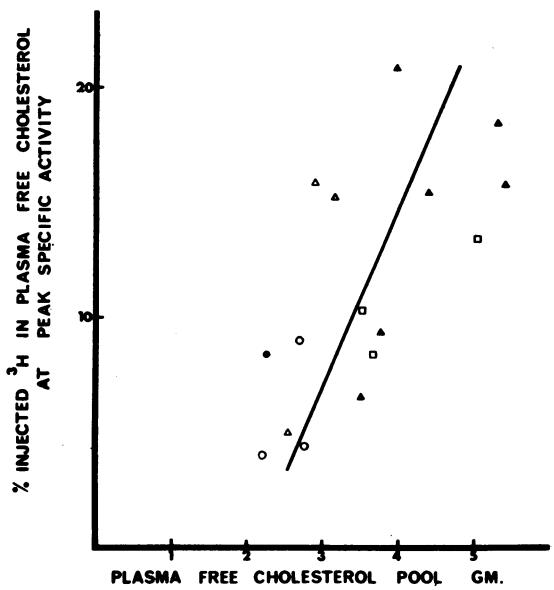

FIG. 3. THE RELATIONSHIP BETWEEN THE POOL SIZE OF PLASMA FREE CHOLESTEROL AND THE PROPORTION OF LABELED BIOLOGICALLY ACTIVE MEVALONIC ACID FOUND IN PLASMA FREE CHOLESTEROL. Symbols as in Figure 2.

Although in this study an estimate has been made of plasma total esterified cholesterol turnover, we appreciate that the turnover of cholesterol esters within the different lipoproteins is not homogeneous. It has been shown that the cholesterol esters of low density lipoproteins have a fractional turnover rate which is less than that of cholesterol esters within high density and very low density lipoproteins (16, 17). The study in which the turnover of cholesterol esters was measured in whole plasma as well as in the separated lipoproteins shows that the turnover in whole plasma reflects the turnover in the different classes of lipoproteins (Table II). The fractional turnover rate of cholesterol esters in whole plasma was very close to the average for the fractional turnover rates of cholesterol esters in the 3 classes of lipoproteins. It is apparent therefore that the fractional turnover rate in whole plasma would be affected by a change in the distribution of cholesterol ester mass among the different lipoproteins although it would still reflect the average value. The turnover rate of cholesterol esters in whole plasma represents the sum of the turnover rates of cholesterol esters in the different lipoproteins and therefore provides

only an over–all measure of a heterogeneous system of turnover rates.

The bulk of cholesterol esters is carried within the low density lipoproteins especially in hypercholesterolemic states (1, 19–21). A notable exception is hypercholesterolemia accompanied by marked hypertriglyceridemia when a substantial portion of cholesterol esters may be carried in very low density lipoproteins (20). We therefore did not study any subjects whose triglyceride level was excessive: the triglyceride concentrations varied from 63 to 194 mg per 100 ml. Hypercholesterolemia may also be associated with a reduction in the concentration of high density lipoproteins (21). The hypercholesterolemic subjects in this study may therefore have differed from the normocholesterolemic subjects in having high concentrations of slowly turning over low density lipoproteins and lower concentrations of the more rapidly turning over high density lipoproteins. Despite this, the heavier hypercholesterolemic subjects had higher turnover rates of total esterified cholesterol than the normocholesterolemic subjects. However, the possible differences in the proportions of low and high density lipoproteins may have led to the subjects with familial hypercholesterolemia having apparently lower turnover rates than the normocholesterolemic subjects. The striking difference between the overweight hypercholesterolemic subjects and those with familial hypercholesterolemia cannot, however, be attributed merely to a difference in the proportions of the different lipoproteins.

An additional finding was the association between body surface area or body weight and the turnover of plasma esterified cholesterol (Figure 2). This suggests that increasing body weight leads to a rise in the formation of esterified cholesterol and is consistent with observations that plasma cholesterol rises with weight gain (6) and that the fall in plasma cholesterol which accompanies weight loss cannot be accounted for solely by qualitative changes in the diet (7).

A significant relationship between body weight and body fatness and the plasma cholesterol concentration has been reported in a number of studies (22, 23) although not in others (24, 25). Body fatness was not directly measured in the present study, and higher body weights need not be directly equated with obesity. Our investigations suggest that hypercholesterolemia reflects an increased rate of formation of at least one pool of body cholesterol when it is associated with overweight. When hypercholesterolemia is found in men with normal weight, factors other than an increased rate of turnover of esterified cholesterol appear to be involved.

These findings therefore suggest that the factors responsible for the development of hypercholesterolemia are not identical in all hypercholesterolemic subjects. However, these conclusions are limited to plasma esterified cholesterol, which represents a relatively small part of the total exchangeable body cholesterol pool. It is possible, for instance, that the low turnover rate in the 3 subjects with familial hypercholesterolemia reflects the presence of a larger pool of esterified cholesterol in the liver and possibly intestine, with which the plasma pool is in equilibrium.

There are no comparable studies in which the turnover of plasma esterified cholesterol has been measured. An increased formation of cholesterol in very low density lipoproteins has been described in carbohydrate–induced hyperlipidemia (8), but none of our subjects suffered from this disorder. Although the finding by Lindstedt and Ahrens (9) of a reduced turnover of cholic acid in 2 subjects with familial hypercholesterolemia was not conclusive because of the nature of the diet, it is consistent with the low turnover rates of esterified cholesterol found among similar subjects in our study.

The mechanisms responsible for the turnover of plasma cholesterol esters, not yet fully established, have been reviewed recently by Goodman (26). Esterification of cholesterol may occur in a number of sites including liver and plasma. It is uncertain, however, whether cholesterol esters are formed mainly in the liver or by the transesterification of free cholesterol in the plasma (27). The higher initial specific activity of plasma than of hepatic cholesterol esters when labeled cholesterol was injected into man points towards the predominance of the plasma esterifying system (15), although the multiplicity of cholesterol ester pools found in human liver may yet reveal the presence of a major precursor pool in the liver (15). The present findings are consistent with the plasma being a major site of cholesterol esterification. The turnover rate of plasma esteri-

fied cholesterol was of the order of 130 mg per hour in our normocholesterolemic subjects. This is in close agreement with the *in vitro* initial rate of cholesterol esterification in human plasma (27).

The subsequent degradation of cholesterol esters appears to occur within the liver and probably involves hydrolysis of the esters with the release of free cholesterol. An exchange of intact cholesterol esters during the circulation of plasma lipoproteins through the liver has also been suggested (26). Whatever the mechanisms are, it seems likely that in man they operate equally on the individual cholesterol esters within a class of lipoproteins (16, 17) although at different rates for different lipoproteins.

The data relating to free cholesterol are difficult to interpret because of the rapid equilibration of free cholesterol pools in liver, plasma, and erythrocytes (14). The significant relationship between the size of the plasma free cholesterol pool and the proportion of label transferred into this pool (Figure 3) is consistent with the rapid rate of equilibration between hepatic and plasma free cholesterol and has been reported by others (28). Minor differences in the formation of hepatic free cholesterol cannot be estimated from such studies without a knowledge of the size and turnover of cholesterol in the liver. There was no evidence that the removal of label from the plasma free cholesterol during the first 24 hours differed substantially among the patients (Table III). Hennes, Moore, and Masters (28) have reported reduced rates of fall in the specific activity of free cholesterol during the first day in subjects with familial hyperlipidemia. The significance of their finding is uncertain in view of the many factors that contribute to the fall in specific activity: the esterification and catabolism of free cholesterol and the equilibration between free cholesterol in the plasma and that in erythrocytes and other tissues.

Plasma volumes were not measured directly, and it is possible that these may have been less than shown in the calculations based on body weight. Such errors might have been largest among the overweight, but they certainly could not have been great enough to explain the differences in the esterified cholesterol turnover rates between those who were overweight and those who were not. Moreover the overweight subjects had fractional turnover rates that were also greater than those found among most of the other hypercholesterolemic subjects.

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