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## Kinetics of Triglyceride Turnover of Very Low Density Lipoproteins of Human Plasma \*

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Ahrens and co-workers (1) have collated and extended prior observations (2-7) that high carbohydrate, low fat diets may cause hypertriglyceridemia in certain subjects. They termed this phenomenon "carbohydrate-induced lipemia" (1). Despite uncertainties concerning the degree or duration of plasma triglyceride rise needed to merit this appellation, this general response to a high carbohydrate diet is frequently observed in patients with evidence for accelerated atherogenesis (1, 8-10). Furthermore, elevated plasma triglycerides are common in patients with "premature" arteriosclerotic heart disease on ad libitum diets (11-16). Consequently, it becomes important to define the factors controlling plasma triglyceride concentration in man.

It can be assumed that changes in concentration of water soluble macromolecules confined to the plasma space, such as lipoproteins, result from variations in rates of entry into and removal from plasma, or both. Hypertriglyceridemia in patients with "fat-induced lipemia," another and a relatively uncommon syndrome (1,

17), is associated with decreases in plasma post-heparin lipolytic activity (17, 18). This latter finding suggests that the primary cause of the hypertriglyceridemia in fat-induced lipemia is defective removal of newly absorbed dietary chylomicrons due to deficiency in lipoprotein lipase or other tissue lipases. Although normal plasma postheparin lipolytic activity has been described in patients with carbohydrate-induced hypertriglyceridemia (1, 17), it does not necessarily follow that the plasma lipid change must be due to endogenous overproduction. Indeed, it has recently been suggested that the cause of hypertriglyceridemia in this syndrome is due to a defect in removal of endogenous plasma triglyceride (8).

To determine if a rise in plasma lipoprotein concentration is caused primarily by increased production or by decreased removal, it is necessary to compare turnover rate with concentration through a wide range of these variables in a defined human population. At present, we find no data in normal or abnormal populations that permit such an analysis. However, we have recently devised and validated a model for triglyceride turnover in man using isotopically labeled glycerol as a precursor for plasma triglyceride (19). We report here our use of these techniques in a study of the relationship between concentration and turnover rate of plasma triglyceride in a group of human subjects. The results demonstrate a close dependency of these two variables under a wide variety of experimental conditions.

### Methods

Subjects selected for study were either assumed to have no abnormality of lipid metabolism or were expected to have wide variations of triglyceride concentrations in response to weeks of ingestion of either high or low fat diets. Relevant clinical features of the 12 patients are

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TABLE I  
General description of patients studied

Initials	Age	Sex	Height <i>cm</i>	Weight <i>kg</i>	Clinical diagnoses*
G. B.	52	M	170	66.5	ASHD
E. C.	41	M	164	89.8	ASHD, xanthoma tuberosum and planum
F. C.	71	M	165	60.0	Carcinoma of lung
P. G.	52	M	178	74.0	ASO
C. H.	33	M	180	85.1	Subsiding jaundice secondary to chlorpromazine
R. L.	52	M	185	77.0	ASHD
L. McD.	51	M	173	77.1	ASHD
R. N.	44	M	170	73.2	ASHD
J. P.	48	M	174	74.5	ASHD, ASO, familial hypercholesterolemia, xanthoma tendinosum
C. P.	38	M	188	103.1	Obesity
G. S.	21	M	170	60.4	Bronchiectasis and membranous glomerulonephritis
G. T.	57	F	152	40.0	ASHD

\* Description of terms: ASHD = arteriosclerotic heart disease; ASO = arteriosclerosis obliterans.

given in Table I. All but one (F.C.) were fully ambulatory. Nine patients were fed isocaloric liquid formula diets as the sole source of calories for 3 to 8 weeks before measurement of triglyceride turnover. Techniques of formula feeding have been previously described (20); compositions as percentage of daily calories from proteins, fats, and carbohydrates were as follows: high fat, 14:69:17, high carbohydrate, 14:<1:85. Formula ingredients were derived from corn oil, dried skimmed milk powder, and a partially polymerized dextrose product containing dextrans and small amounts of maltose.<sup>1</sup> In three patients the antecedent diet was an ad libitum, regular hospital diet. In six patients an attempt was made to produce variations in triglyceride concentration by dietary means, and triglyceride turnover was measured during both a high fat and a high carbohydrate dietary period. In three patients, 10 additional studies were done on diets either high or low in fat, both with and without the daily administration of 125 mg of chlorpropamide. Body weights of subjects were stable for at least 1 week before each study.

**Experimental techniques.** Details of experimental methods were previously described (19) and with one exception are unchanged. A general description of the studies follows: Isotopically labeled glycerol was given intravenously after an overnight fast. Fasting was continued and blood was removed at frequent intervals during the subsequent 8 hours. This blood was used to obtain  $S_T > 20$  lipoproteins by ultracentrifugation at 14° C. A more complete and less variable recovery of  $S_T > 20$  triglyceride was obtained by use of the following modification of the technique previously reported (19): Polycarbonate tubes<sup>2</sup> of 11-ml capacity were filled with only 7 ml of plasma.  $S_T > 20$  lipoproteins were removed by aspiration of the top 3 ml after ultracentrifugation (40 rotor, Spinco model L)<sup>3</sup> for 22 hours. Lipids were then

extracted from these lipoproteins (19), and the triglycerides were isolated by thin layer chromatography, their concentration and radioactivity were determined, and specific activities were calculated.

**Calculations.** A previous report from this laboratory has validated a two-compartment, nonrecycling model of hepatic and plasma  $S_T > 20$  triglyceride turnover in man (19). Turnover rates are calculated from the slope of the specific activity disappearance curve of endogenously labeled  $S_T > 20$  plasma triglyceride after the intravenous injection of isotopically labeled glycerol. The validity of this calculation is based upon our previous discovery of the rate-determining role in the two pool system of plasma  $S_T > 20$  triglyceride fractional turnover rates (19).

The calculations are as follows: 1) Plasma volume is estimated as 4.5% of body weight (21). 2) Pool size = (milligrams  $S_T > 20$  triglyceride per milliliter plasma) × (plasma volume in milliliters). 3)  $t_{1/2}$  = half-time of disappearance of  $S_T > 20$  triglyceride from plasma. 4) Fractional turnover rate =  $0.693 \div t_{1/2}$ . 5) Turnover rate = (fractional turnover rate in hour<sup>-1</sup>) × (pool size in milligrams) ÷ kilogram body weight.

These studies were performed while plasma triglyceride concentrations were constant. Therefore, turnover rate of plasma triglyceride is equal to plasma triglyceride flux from liver into plasma. These data then allow one to define the mathematical relationship of plasma triglyceride concentration and turnover rate that exists in the population under study.

## Results

The relationship between plasma  $S_T > 20$  triglyceride concentration and turnover rate for each patient studied is tabulated in Table II, and portrayed in Figure 1. It can be seen that plasma triglyceride concentration in nine to eleven de-

<sup>1</sup> Dexin, Burroughs, Wellcome and Co., Tuckahoe, N. Y.

<sup>2</sup> "Autoclear" tubes and sealing caps, International Equipment Co., Needham Heights, Mass.

<sup>3</sup> Beckman Instruments, Palo Alto, Calif.

TABLE II  
*Relationship between plasma  $S_t > 20$  triglyceride concentration and turnover rate*

Patient	Condition*	Plasma volume†	$S_t > 20$ TG		$t_1$	k	Turnover rate $S_t > 20$ TG
			Mean $\pm$ CV‡	Pool size§			
		ml	mg per 100 ml	g	hours	hours <sup>-1</sup>	mg per kg per hour
G. B.	HC	2,993	104 $\pm$ 5	3.11	2.8	0.248	11.7
E. C.	HC	4,041	367 $\pm$ 7	14.83	5.0	0.139	23.0
	HC + chlor.	4,041	473 $\pm$ 4	19.11	8.4	0.083	17.7
F. C.	Ad lib.	2,700	23 $\pm$ 17	0.62	3.7	0.187	2.0
P. G.	HF	3,300	441 $\pm$ 12	14.52	6.6	0.105	20.6
	HC	3,300	1,590 $\pm$ 9	52.47	20.6	0.034	23.8
C. H.	Ad lib.	3,820	112 $\pm$ 8	4.31	3.0	0.231	10.0
R. L.	HC	3,465	578	20.03	8.5	0.081	21.0
	HC	3,465	707	24.50	9.2	0.075	24.9
L. McD.	HF	3,470	65 $\pm$ 25	2.26	1.8	0.385	11.3
	HF + chlor.	3,470	50 $\pm$ 30	1.74	1.9	0.365	8.3
	HC	3,470	156 $\pm$ 23	5.41	2.7	0.257	18.1
	HC + chlor.	3,470	134 $\pm$ 10	4.65	3.9	0.178	10.8
R. N.	HF	3,294	110 $\pm$ 10	3.62	3.4	0.204	10.0
	HC	3,294	602 $\pm$ 9	19.83	8.6	0.081	22.0
J. P.	HF	3,352	81 $\pm$ 26	2.72	2.7	0.257	9.4
	HF	3,352	93 $\pm$ 17	3.12	2.7	0.257	10.7
	HF + chlor.	3,352	70 $\pm$ 13	2.35	3.7	0.187	5.9
	HC	3,352	141 $\pm$ 25	4.73	5.8	0.119	7.6
	HC + chlor.	3,352	68 $\pm$ 40	2.28	3.7	0.187	5.8
C. P.	HF	4,950	340 $\pm$ 11	16.83	4.1	0.169	24.8
	HC	4,950	613 $\pm$ 13	30.34	8.3	0.083	22.9
G. S.	Ad lib.	2,718	23 $\pm$ 17	0.63	1.3	0.533	5.6
G. T.	HF	1,800	61 $\pm$ 43	1.10	1.7	0.408	11.3
	HC	1,800	120 $\pm$ 8	2.16	2.4	0.289	15.5

\* Conditions: HF = high fat diet; HC = high carbohydrate diet; chlor. = chlorpropamide; ad lib. = ad libitum diet.

† Plasma volume is assumed as 4.5% of body weight.

‡ CV = Coefficient of variation = (standard deviation  $\div$  mean) 100. Values for the mean were obtained from nine to eleven plasma samples drawn during 6 to 8 hours of the experiment.

§ See Methods for techniques for calculation of pool size, fractional turnover rate (k),  $t_1$ , and turnover rates.

|| Fractional turnover rate; see Methods for technique of calculation.

terminations was relatively constant during the 6 to 8 hours that triglyceride turnover rate was determined (Table II). The results indicate that at each higher plasma triglyceride concentration there were generally associated increases in triglyceride turnover rate. This relationship between plasma triglyceride concentration and turnover rate was analyzed by Spearman's rank correlation coefficient (22), and the hypothesis that these two variables are unrelated can be rejected at the level of less than 0.0001.

It can also be seen that concentration and turnover rate were not linearly related throughout, and that concentration rises steeply after

turnover rates begin to exceed 15 mg  $S_t > 20$  triglyceride per kg body weight per hour. The shape of the curve suggests that the removal mechanisms for plasma  $S_t > 20$  triglycerides follow the kinetics of a saturable system, and that the plot is not compatible with simple diffusion as the mechanism of transport out of the system (23). To more precisely define these relationships, a mathematical model was formulated based on the following assumptions: 1) A steady rate of hepatic secretion of  $S_t > 20$  lipoproteins maintains a steady concentration of plasma  $S_t > 20$  triglycerides. 2) There is some large number of "removal sites" through which

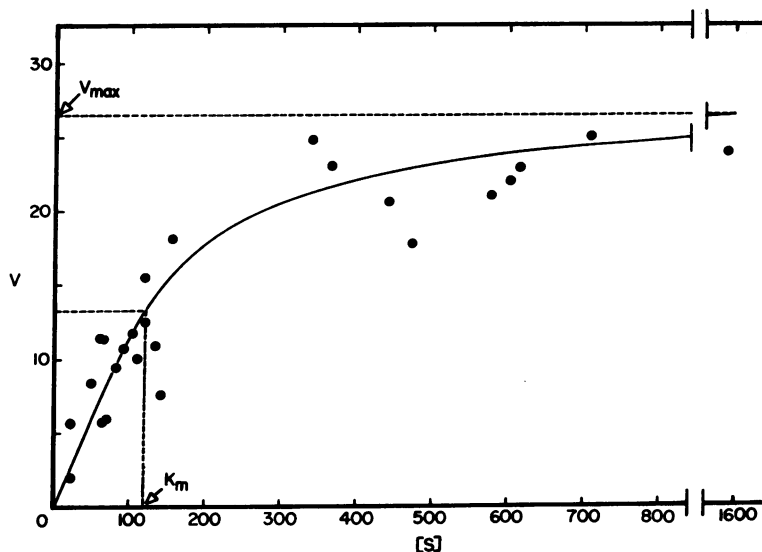
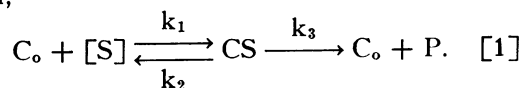


FIG. 1. RELATIONSHIP BETWEEN  $S_T > 20$  TRIGLYCERIDE TURNOVER RATE ( $V$ ) AND CONCENTRATION  $[S]$ . These values are plotted from each of the 25 studies listed in Table II. Units:  $V$  (velocity) =  $S_T > 20$  triglyceride turnover per kilogram body weight per hour;  $[S]$  = milligrams  $S_T > 20$  triglyceride per 100 ml plasma;  $V_{max}$  = maximal estimated velocity;  $K_m$  =  $[S]$  at  $\frac{1}{2}$  maximal velocity.

$S_T > 20$  triglycerides may leave the plasma. 3) If a removal site is "occupied," it is unavailable for use by another triglyceride until the "removal process" has been completed. 4) The rate of total removal of triglyceride from the system is directly proportional to the number of removal sites occupied. 5) The rate at which triglycerides arrive at the removal sites is directly proportional to the plasma triglyceride concentration.

Terms and formulas to express these assumptions in the kinetics of a saturable system follow. Because of its common use, terminology analogous to the Michaelis-Menten formulation (24) was used.  $[S]$  = concentration of  $S_T > 20$  plasma triglycerides in milligrams per 100 ml = concentration of substrate.  $C_o$  = concentration of removal sites in sites per 100 ml plasma.  $k$  = fractional turnover rate.  $CS$  = concentration of occupied removal sites in sites per 100 ml plasma.  $V$  = input rate of  $S_T > 20$  triglycerides from liver to plasma = removal rate of  $S_T > 20$  triglycerides from plasma = velocity of the removal reaction = milligrams  $S_T > 20$  triglycerides per 100 ml plasma per hour.  $P$  =  $S_T > 20$  triglycerides removed from plasma = products.

Then,



Then, the equations governing this system are:

$$d[S]/dt = -k_1[S](C_o - CS) + k_2 CS + V. \quad [2]$$

$$dCS/dt = k_1[S](C_o - CS) - (k_2 + k_3) CS. \quad [3]$$

And, in the steady state condition, one has

$$d[S]/dt = dCS/dt = 0. \quad [4]$$

Solving these equations for  $V$ , we obtain:

$$V = \frac{[S]V_{max}}{K_m + [S]}, \text{ where } V_{max} = k_3 C_o, \quad [5]$$

$$\text{and } K_m = \frac{k_2 + k_3}{k_1}.$$

( $V_{max}$  = maximal turnover rate allowed in this system, and  $K_m$  = substrate concentration,  $[S]$ , at  $\frac{1}{2} V_{max}$ .)

Figure 1 illustrates the results of these experiments when  $[S]$  (concentration of plasma  $S_T > 20$  triglycerides) is plotted against  $V$  (turnover rate). The turnover rates of Figures 1 and 2 are calculated per unit body weight rather than

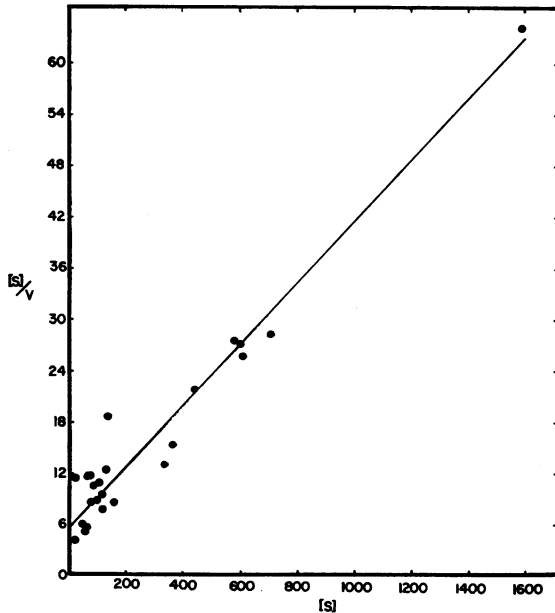


FIG. 2. LINEAR TRANSFORM OF THE DATA OF FIGURE 1 ( $1/[S]$  AGAINST  $V$ ) TO ACHIEVE A MORE ACCURATE ESTIMATE OF  $K_m$  AND  $V_{max}$  THAN IS POSSIBLE FROM FIGURE 1. Slope obtained as the best fit line through the data points.  $V_{max} = 1/\text{slope}$ , and  $K_m = (\text{intercept of slope with ordinate}) (V_{max})$ .

unit plasma volume (see Equation 5 of calculations.) As  $V_{max}$  is approached, the rate of removal appears to be independent of substrate concentration, and the reaction approaches zero order kinetics. At lower levels of substrate concentration, removal rate appears to be directly proportional to substrate concentration, and the relationship seems to be first order.

To calculate  $V_{max}$  and  $K_m$  more accurately than possible from the curve of Figure 1, a linear transform of the Michaelis-Menten formulation of  $1/[S]$  against  $V$  was constructed (Figure 2). The slope of the best fit line through these data (Figure 2) was 0.0095. Since in this type of plot,  $V_{max} = 1/\text{slope}$  (24), then  $V_{max} = 26.5$  mg  $S_f > 20$  triglyceride per kg body weight per hour. Also in this system,  $K_m$  equals the product of the intercept on the vertical axis times  $V_{max}$ . Therefore,  $K_m = 118$  mg  $S_f > 20$  triglyceride per 100 ml plasma.

Five paired studies were designed to investigate the effect of chlorpropamide on triglyceride turnover. Results showed that turnover rate, when measured after several weeks of chlorpropa-

mid treatment, was always lower than in previous studies on the same diet (Table II). It was not possible to discern any systematic deviation of these data from the general shape of the curve of Figure 1.

Also, there were six comparisons of high and low fat diets (Table II). In one subject (J.P.), little difference in  $S_f > 20$  triglyceride concentration or turnover was noted. In the remaining five comparisons, lower concentrations were present in the high fat diet; all pairs but one (C.P.) resulted in distinctly lower turnover rates on the high fat diet.

### Discussion

The concentration of any solute in plasma will tend to rise and fall as its flux in or out of plasma is varied. A rise in plasma concentration to a new steady state level can only result from either increased entry rate or decreased removal rate of the solute in question. During nonsteady state conditions, as the solute concentration is rising, its removal rate from the plasma must be less than its entry rate. Eventually, efflux will again equal influx, and solute concentration will now be stable at its higher plasma level. Consequently, whenever solute concentration has increased, removal mechanisms have failed to maintain homeostasis. However, it does not follow that a primary defect in solute removal is responsible for the rise in concentration. To establish the presence of such removal defects it is necessary to find a plasma concentration of  $S_f > 20$  triglyceride associated with a lower turnover rate than is normally present for that concentration. Our results (Figures 1 and 2) argue against the existence of primary removal defects because they deny the presence of two or more distinct population groups differing only in plasma triglyceride removal rate. In fact, the homogeneity of the fit of the experimental data to a single curve (Figures 1 and 2) indicates that the population studied was remarkably homogeneous in triglyceride removal efficiency. In these studies, higher plasma  $S_f > 20$  triglycerides were generally accompanied by higher turnover rates of this substance (Table II, Figure 1). At each increment in plasma concentration more triglycerides were being made and secreted into plasma

and more triglycerides were being removed from plasma. Production and removal were equal since each experimental point represented a steady state in concentration. Higher plasma concentration occurred because removal mechanisms did not keep pace with increased production, but increased production was the primary cause for the rise in plasma  $S_T > 20$  triglyceride concentration.

This does not mean that certain individuals will not demonstrate important variations from the relationship that has been described, and in these instances hypertriglyceridemia may well result from primary removal defects. For example, it seems quite likely that patients with "fat-induced hypertriglyceridemia" would demonstrate an elevated level of plasma triglyceride in association with low turnover rates. Furthermore, the recent report of decreased plasma postheparin lipolytic activity in patients with myxedema (25) suggests that hypertriglyceridemia in this disorder may also result from lessened removal efficiency. However, in contrast to these and other unusual disorders, the patients we studied should be more typical of the general population. All of our subjects were consuming constant diets, their body weights were stable, and individuals with fasting hyperglycemia or with fat-induced hypertriglyceridemia were excluded. Within this group of patients varying from clearly normal to abnormal in plasma triglyceride concentration, turnover rates were determined at different triglyceride levels that were varied by dietary alterations or by administration of chlorpropamide. By these means some of the group were raised or lowered in  $S_T > 20$  triglyceride concentration and in turnover rate to levels that bracketed the triglyceride concentrations of others of the group. This interlocking of responses through the wide range of observed concentrations suggests that a larger sample of the general population would follow the observed relationship between plasma triglyceride concentration and turnover rate (Figures 1 and 2).

The data relating plasma triglyceride concentration to turnover rate were presented in the nomenclature of the Michaelis-Menten formulation, not because the precise enzymatic nature of clearing has been identified, but because the experimental data are consistent with such kinetics. The function relating concentration to turnover

rate in these studies is clearly explicable by a saturable system, perhaps an enzyme dependent mechanism, and cannot, by its nonlinearity, be attributed to simple diffusion. Moreover, there is much evidence in numerous mammalian species that an enzymatic process is responsible for clearing triglyceride of either chylomicrons (26) or of low density lipoproteins (27). Although other enzymes have not been excluded (17), the major enzyme in this reaction is very likely lipoprotein lipase, an enzyme first isolated and characterized by Korn (28). This enzyme is found in many tissues, but it is in highest concentration in adipose tissue (29, 30) or heart muscle (29) of many animal species. Although the precise anatomic site of clearing is unknown, Robinson and Harris have presented evidence for an endothelial location of lipoprotein lipase (31). This suggests an action within capillary beds of the principal consumers of plasma triglyceride, striated muscle and adipose tissue.

Regardless of the exact nature of the enzymes concerned with removal from plasma of the lipid moieties of low density lipoproteins, it seems clear that knowledge of turnover rates is essential in order to understand the relationship between hepatic triglyceride secretion and abnormalities of lipid metabolism in man. Our data (Figures 1 and 2) clearly show that sole reliance upon change in concentration may mislead one in attempts to relate these changes with the more important issue of how much triglyceride is being made and secreted by the liver. For example, an increase in secretion (turnover) rate of from 10 to 15 mg triglyceride per hour per kg body weight is associated with a rise of only 40 mg  $S_T > 20$  triglyceride per 100 ml plasma. In contrast, a similar increment in turnover from 20 to 25 mg triglyceride per hour per kg body weight would result in an increase of 600 mg  $S_T > 20$  triglyceride per 100 ml plasma. However, it should be noted that this degree of "saturability" may not occur in all species. Our studies in the dog, for example, indicate that  $S_T > 20$  triglyceride concentration and turnover rate are more linearly related and that dog turnover rates exceed three-fold those of man at the  $K_m$  of man (32). These relationships, considering the low  $K_m$  of man (118 mg  $S_T > 20$  triglyceride per 100 ml plasma), allow one to predict greater lipemia in man than

in the dog for comparable stimulation of hepatic triglyceride synthesis.

Finally, our observation that chlorpropamide decreased  $S_f > 20$  triglyceride turnover rates in the three subjects studied is intriguing in view of recent observations that patients with either carbohydrate-induced hypertriglyceridemia (8–10, 33) or “essential” hypertriglyceridemia (34) appear to have a very mild form of maturity onset diabetes. Studies are in progress to confirm this effect of chlorpropamide and to relate it to our current hypothesis that plasma insulin levels are an important determinant of the rate of hepatic triglyceride synthesis and secretion that can be induced in man by high carbohydrate diets (10).

### Summary

These studies establish the relationship between the concentration and turnover rate of plasma  $S_f > 20$  triglyceride in a defined human population. The results demonstrate that triglyceride concentration and turnover rate are highly correlated in this group, and that higher concentrations are associated with higher turnover rates. Furthermore, the function relating these two variables is consistent with a saturable, and possibly enzyme dependent, mechanism for removal of this lipoprotein triglyceride from plasma. Since the clearing efficiency within this group seems remarkably homogeneous, increased production rather than a triglyceride removal defect is therefore responsible for rising plasma  $S_f > 20$  triglyceride concentration. Finally, the low substrate concentration at  $\frac{1}{2}$  maximal turnover rate of this system (118 mg  $S_f > 20$  triglyceride per 100 ml plasma) attests to the ready saturability of triglyceride removal mechanisms in man.

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