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Philip J. Barter, Paul J. Nestel

Research Article

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PHILIP J. BARTER and PAUL J. NESTEL

From the Department of Clinical Science, The John Curtin School of Medical Research, the Australian National University, Canberra, Australia

A B S T R A C T The process of removal of triglyceride from the plasma may involve a sequential conversion of larger to smaller glyceride-rich lipoproteins. This has been studied within the species of lipoproteins comprising the very low density lipoproteins (VLDL) which transport the bulk of endogenously formed triglyceride. Palmitic acid-^{14}C which was used to label the plasma glycerides was administered either as a prolonged constant infusion or as a pulse label. The specific activity-time curves of triglyceride fatty acids (TGFA) were analyzed both in total VLDL and in two subfractions of VLDL. The nature of the curves for total VLDL that were observed during the constant infusions were consistent with slow isotopic equilibration of precursors of VLDL-TGFA or with the presence of a precursor-product relationship between different components of VLDL-TGFA. The curves did not indicate any detectable differences in (fractional) turnover rates of independently metabolized pools of VLDL-TGFA. Differences in the specific activity-time curves of TGFA in two subfractions of VLDL (Sf > 100 and Sf 20–100) were consistent with a precursor-product relationship between TGFA in the two subfractions; again there was no indication of significant differences in (fractional) turnover rates. The specific activity-time curves of TGFA in the two subfractions of VLDL that were obtained with single injections of radio-palmitate showed a consistent difference in the rates at which TGFA became labeled in the two subfractions, being slower in the Sf 20–100 fraction. The findings from all experiments when considered together, were compatible with a precursor-product relationship that suggested that larger VLDL were converted to progressively smaller species as triglyceride was being removed.

INTRODUCTION

Triglyceride that is newly synthesized in the liver is secreted into the plasma predominantly in the very low density lipoproteins (VLDL)[1] (1). The VLDL comprise a wide morphological spectrum with the lipid to protein ratio increasing with size (2–4). It is not clear whether all the triglyceride transported in VLDL represents a kinetically homogeneous compartment. A recent report from our laboratory showed consistent differences in the specific activity of triglyceride fatty acid (TGFA) transported in VLDL of different sizes during a constant infusion of palmitic acid-[14]C (5). We suggested that larger VLDL might be degraded to smaller one during the process of triglyceride removal, although differences in the (fractional) turnover rates[2] of TGFA in the various subclasses of VLDL could not be excluded.

In the present studies, the specific activity of TGFA in total VLDL and in two subfractions of VLDL has been measured during prolonged constant infusions and after single injections of palmitic acid-[14]C. We have attempted to determine whether the observed differences between the subfractions are the result of differing (fractional) turnover rates of independently metabolized pools or whether they indicate that TGFA transported in smaller VLDL is initially secreted into the plasma in larger lipoproteins. Our findings support the latter suggestion.

METHODS

Experimental procedures

15 subjects were studied. There were eight male and one female normal volunteers[3] aged 19–42, two males aged 43

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[1] *Abbreviations used in this paper:* TGFA, triglyceride fatty acids; VLDL, very low density lipoproteins.

[2] (Fractional) turnover rate = fraction of pool turning over per unit time.

[3] The nature of the studies was fully explained to all subjects and signed constant was obtained from the normal volunteers.

and 52 with hepatic cirrhosis (4 and 11), two males aged 39 and 43 (1 and 13), and two females aged 58 and 61 (2 and 12) with type IV hyperlipoproteinemia (6). All subjects had been eating their habitual diets and studies were commenced in the morning after an overnight fast. Two kinds of study were carried out in which palmitic acid-1-^{14}C was infused either at a constant rate or as a pulse injection.

Constant infusions. A venous catheter was inserted in each arm and kept patent by a slow infusion of isotonic saline. 45 min later an infusion of palmitic acid-1-^{14}C (The Radiochemical Centre, Amersham, England) complexed as the sodium salt to human albumin (7) was commenced via one catheter. The infusion was continued at a rate of 0.2 ml/min (using a Harvard constant infusion pump Harvard Apparatus Co., Millis, Mass.) for 7–15 hr. A total of 25–75 μCi was given to each subject. 10 ml of blood was collected into chilled tubes containing 10 mg of dipotassium EDTA from the catheter in the opposite arm at 30–60 min intervals after the first 1½ hr of palmitate infusion. Plasma was separated at 4°C within 1 hr of blood collection.

Single injections. A single injection of palmitic acid-1-^{14}C was administered intravenously and blood was collected from an indwelling catheter in the opposite forearm at 15–30 min intervals for 4½–6 hr.

Analytical procedures

The VLDL were separated from portions of plasma by preparative ultracentrifugation (8). In seven studies, two subclasses of VLDL (Sf > 100 and Sf 20–100) were isolated from 3 ml portions of plasma by the method described by Gustafson, Alaupovic, and Tierman (4). No further attempt was made to purify these subclasses by repeated ultracentrifugation since the aim was simply to obtain two fractions of VLDL containing predominantly larger or smaller lipoproteins.

The total VLDL and the subclasses were extracted in the solution described by Dole (9). Portions of the lipid extract were assayed for triglyceride content (10) and radioactivity. The latter was determined with an efficiency of 80% in a liquid scintillation system using 0.3% PPO in toluene as scintillator. Separation of the VLDL lipids by thin-layer silicic acid chromatography (hexane-ether-methanol-acetic acid, 180 : 40 : 6 : 4, solvent) had shown that > 96% of the radioactivity was in the triglyceride fraction. There was less than 7% variation in triglyceride concentration during any experiment.

Free fatty acid (FFA) specific radioactivity was measured in whole plasma that had been extracted in Dole's solution (9). Acidic and neutral lipids were separated according to Borgström (11) with a recovery of 96%. During constant infusions of radiopalmitate the "steady-state" FFA specific radioactivity varied by less than 15%.

THEORETICAL CONSIDERATIONS

Previous experiments had shown consistent differences in the specific activity of TGFA transported in VLDL of different sizes during a 2½ hr constant infusion of palmitic acid-^{14}C (5). The findings were consistent with either of two possibilities (or a combination of the two possibilities). (*a*) That TGFA in VLDL of different sizes exist as independently metabolized pools in which the (fractional) turnover rate of TGFA varies as a function of VLDL particle size, or (*b*) that TGFA trans-

ported in smaller VLDL were initially secreted into the plasma in larger lipoproteins. The present experiments attempt to differentiate between the two propositions.

Plasma FFA are a major precursor of VLDL-TGFA in the fasting state (1). When tracer amounts of labeled FFA are infused at a constant rate isotopic equilibration of intermediates between plasma FFA and VLDL-TGFA occurs sequentially until ultimately the VLDL-TGFA attain a constant specific radioactivity, C, equivalent to that of the immediate precursor. Hence the equilibrated specific activity of the immediate precursor can be deduced without the necessity of defining a precise compartment.

If during a constant infusion of labeled precursor it is assumed that the specific activity of the immediate precursor of VLDL-TGFA is constant at level C from zero time, and if the TGFA in VLDL is assumed to represent a single pool, then (reference 12)

$$S_{TG} = C(1 - e^{-kt}) \qquad (1)$$

where k is the (fractional) turnover rate of VLDL-TGFA and S_{TG} denotes the VLDL-TGFA specific activity. Converting equation 1 to logarithms,

$$-\log\left(1 - \frac{S_{TG}}{C}\right) = kt \qquad (2)$$

so that the plot of $-\log(1 - [S_{TG}/C])$ against time will be linear with a gradient equal to k (Fig. 1*a*).

If TGFA in VLDL exist as independently metabolized pools possessing differing (fractional) turnover rates, the plot will not be linear, but rather will possess a gradient decreasing with time; as faster pools reach isotopic equilibrium the curve will reflect the (fractional) turnover rates of the slower pools (Fig. 1*b*).
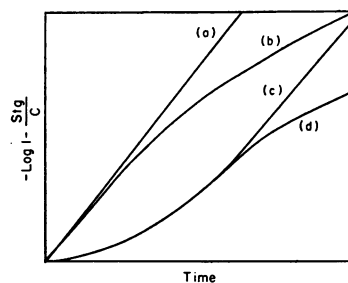

FIGURE 1 Theoretical curves of $-\log (1 - [S_{TG}/C])$ (see text) plotted against time during the constant infusion of a labeled precursor. (*a*) A homogeneous VLDL triglyceride pool with instantaneous isotopic equilibration of all precursors. (*b*) More than one kinetically distinct pool (differing turnover rates) of VLDL triglyceride with instantaneous isotopic equilibration of all precursors. (*c*) A homogeneous VLDL triglyceride pool with precursors equilibrating slowly. (*d*) More than one kinetically distinct pool of VLDL triglyceride with precursors equilibrating slowly.
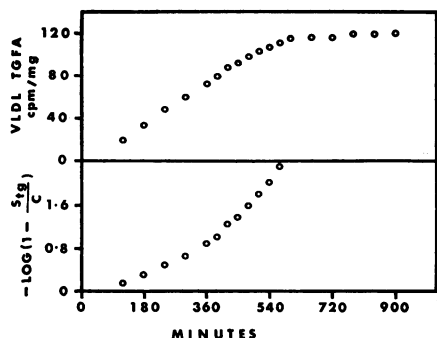
FIGURE 2 Subject 1, upper curve. Specific activity-time plot of VLDL TGFA during a constant infusion of palmitic acid-1-^{14}C. Lower curve, the VLDL TGFA specific activity expressed as $-\log(1 - [S_{TG}/C])$ (see text) and plotted against time.

If isotopic equilibration of the immediate precursor of VLDL-TGFA is not instantaneous, S_{TG} and hence the value of $-\log(1 - [S_{TG}/C])$ at any given time will be lower than that computed from equation 1. However, as the specific activity of the immediate precursor approaches C, the gradient of the curve plotted by $-\log(1 - [S_{TG}/C])$ against time will increase towards that of the theoretical curve until it achieves linearity with a gradient of k (Fig. 1c). Such a curve would also describe the situation in which TGFA transported in smaller VLDL are derived from larger VLDL. The TGFA in the larger VLDL could be regarded as the immediate precursor of that in the smaller VLDL. Consequently the slope of the curve of $-\log(1 - [S_{TG}/C])$ against time for total VLDL-TGFA would continue to increase until the TGFA in all but the smallest VLDL have achieved isotopic equilibration with their respective immediate precursors (Fig. 1c).

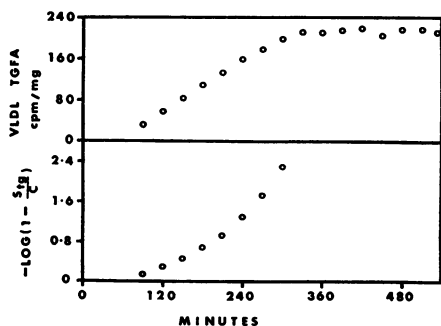Fig. 1d shows a curve which may result from a combination of the situations producing curves b and c; this curve will reflect whichever of the two situations predominates: i.e. it could show an increasing gradient, a decreasing gradient or could even be linear.

Any comparison between experimental curves and those shown in Fig. 1 can only be at a qualitative level. A decreasing slope will suggest the existance of independently metabolized pools of VLDL-TGFA possessing distinct (fractional) turnover rates, while an increasing slope suggest incomplete isotopic equilibration of precursors of VLDL-TGFA or the presence in the plasma of a precursor-product relationship between different components of VLDL-TGFA.

RESULTS

Constant infusions. Figs. 2 and 3 show the TGFA specific activity-time curves in total VLDL during constant infusions of palmitic acid-1-^{14}C in two subjects. The results from all eight experiments are presented in Table I. There was a wide variation in the time required

TABLE I

Specific Activities of VLDL Triglyceride Fatty Acids during Constant Infusions of Palmitic Acid-1-^{14}C

	VLDL Triglyceride specific activity							
Subject.....1	2	3	4	5	6	7	8
VLDL TG concentration*...270	130	108	160	59	73	89	63
Time‡								
				cpm/mg				
90	—	—	28	51	44	33	31	68
120	19	12	49	75	75	53	56	107
150	—	—	70	103	98	67	82	136
180	33	23	89	122	121	84	108	175
210	—	30	111	138	133	96	132	202
240	48	34	127	168	145	109	158	233
270	—	39	140	183	176	117	178	247
300	59	45	159	186	184	126	197	256
330	—	50	174	203	203	137	212	260
360	72	56	183	202	212	140	211	263
390	79	58	188	207	227	150	216	256
420	88	62	191	211	233	152	219	260
450	92	63	195	208	234	154	205	
480	98	67	199	210	236	149	218	
510	103	70	200	208	236	152	218	
540	107	76	200	212	230	153	211	
570	111	79	198					
600	115	84	202					
660	120	83						
720	120	83						
780	123							
840	123							
900	124							

FIGURE 3 Subject 7, upper curve. Specific activity-time plot of VLDL TGFA during constant infusion of palmitic acid-1-^{14}C. Lower curve. Plot of $-\log(1 - [S_{TG}/C])$ against time.

* The VLDL triglyceride concentration is expressed in mg/100 ml.
‡ Minutes after commencement of infusion.

for complete isotopic equilibration of VLDL-TGFA (5–11 hr), being generally shorter when the TGFA pools were small. There was, however, a marked similarity in the pattern of the VLDL-TGFA specific activity (S_{TG})-time curves in all studies. The curves are dominated by a long linear segment followed by an abrupt flattening to a constant level (Figs. 2 and 3). A more critical assessment of the curves can be made by plotting $-\log$ (1 − [S_{TG}/equilibrated S_{TG}]) against time (see theoretical considerations). The lower segments of Figs. 2 and 3 show two such plots from 90 min until S_{TG} had reached 90% of the equilibrated level.[4] Table II presents the values from all studies. In every case the gradient of $-\log$ (1 − [S_{TG}/equilibrated S_{TG}]) against time increased progressively with time and could not in any single case be described in terms of a single exponential function. The existence of differing (fractional) turnover rates of TGFA in independently metabolized pools of VLDL would have resulted in a decreasing gradient; consequently there is no support for such a suggestion. The findings were consistent with

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[4] As complete equilibration is approached the accuracy of values 1 − [S_{TG}/equilibrated S_{TG}] becomes increasingly suspect. We have not computed values beyond 90%.

TABLE II
Values for $-\log(1 - [S_{TG}*/\text{equilibrated } S_{TG}])$ during Constant Infusion of Palmitic Acid-1-^{14}C

			−log(1 − [S_{TG}/equilibrated S_{TG}])					
Subject....1	2	3	4	5	6	7	8
Time‡							
90			0.15	0.28	0.21	0.24	0.15	0.30
120	0.17	0.16	0.28	0.44	0.38	0.43	0.30	0.53
150			0.43	0.68	0.54	0.58	0.47	0.74
180	0.31	0.32	0.59	0.87	0.72	0.81	0.69	1.12
210		0.45	0.81	1.07	0.83	1.00	0.93	1.51
240	0.49	0.52	1.01	1.61	0.96	1.26	1.29	2.27
270		0.63	1.20	2.06	1.38	1.47	1.70	
300	0.65	0.78	1.58	2.18	1.52	1.77	2.35	
330		0.92	2.04		1.99	2.31		
360	0.88	1.11	2.47		2.31			
390	1.02	1.19						
420	1.25	1.36						
450	1.37	1.41						
480	1.58	1.63						
510	1.80	1.83						
540	2.02	2.43						
570	2.30							

* S_{TG}, specific activity of VLDL TGFA (cpm/mg).
‡ Minutes after commencement of infusion.

slow isotopic equilibration of precursors of VLDL-TGFA or with the presence in the plasma of a pre-

TABLE III
Specific Activities of Triglyceride Fatty Acids in Two Subfractions of VLDL during a Constant Infusion of Palmitic Acid-1-^{14}C

	Triglyceride fatty acid specific activity					
Subject........	9		10		11	
	Sf > 100	Sf 20–100	Sf > 100	Sf 20–100	Sf > 100	Sf 20–100
Triglyceride concentration*........20	21	19	29	57	42	
Time‡						
			cpm/mg			
60	93	57	48	29		
90	169	118	83	71		
120	221	185	161	122	244	185
150	264	235	210	203	344	256
180	307	279	265	243	385	358
210	365	336	350	310	453	456
240	411	393	378	353	489	453
270	402	433	425	388	622	580
300	440	426	465	425	656	610
330	432	462	432	454	717	639
360	438	448	485	460	782	769
390			476	480	808	709
420					821	792
450					855	754
480					781	829
510					846	821
540					805	857

* Triglyceride concentrations are expressed as mg/100 ml.
‡ Minutes after commencement of palmitic acid-1-^{14}C infusion.

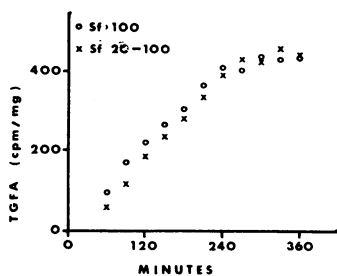*Precursor-Product Relationship between Pools of VLDL Triglyceride*   177

FIGURE 4 Subject 9, specific activity-time plots for TGFA in two subclasses of VLDL (Sf > 100 and Sf 20–100) during a constant infusion of palmitic acid-1-^{14}C.
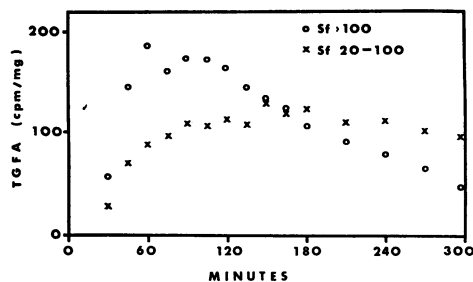

FIGURE 5 Subject 13, specific activity-time plots for TGFA in two subclasses of VLDL (Sf > 100 and Sf 20–100) after a single intravenous injection of palmitic acid-1-^{14}C.

cursor-product relationship between different components of VLDL-TGFA. Because none of the curves of $-\log$ ($[S_{TG}/\text{equilibrated } S_{TG}]$) against time became linear it was not possible to estimate the (fractional) turnover rates.

TGFA specific activities were also measured in two VLDL subfractions during constant infusions of palmitic acid-1-^{14}C in subjects 9–11 (Table III). The specific activity of TGFA in the Sf > 100 fraction was higher at each time point than in the Sf 20–100 fraction until equilibration was complete (Fig. 4). After the first hour of infusion and until shortly before isotopic equilibration

was complete, the two curves in all three subjects were approximately parallel. There was therefore no suggestion that the TGFA in the two subfractions had differing (fractional) turnover rates. The results would, however, be consistent with a precursor-product relationship between TGFA in larger and smaller VLDL.

Single injection. The TGFA specific activities in two subfractions of VLDL (Sf > 100 and Sf 20–100) were also measured after a single intravenous injection of palmitic acid-1-^{14}C. One such experiment is shown in Fig. 5 and Table IV presents the findings in the four subjects 12–15. There was a striking and consistent

TABLE IV
Specific Activities of Triglyceride Fatty Acids in Subclasses of VLDL after a
Single Injection of Palmitic Acid-1-^{14}C

	Triglyceride fatty acid specific activity							
Subject.......	12		13		14		15	
	Sf > 100	Sf 20–100	Sf > 100	Sf 20–100	Sf > 100	Sf 20–100	Sf > 100	Sf 20–100
Triglyceride concentration*.......Time‡	358	155	89	51	54	69	17	23
				cpm/mg				
30	23	16	58	28	29	24	161	103
45	51	31	143	71	65	56	383	242
60	72	38	187	88	90	70	443	329
75	81	49	160	96	98	82	449	421
90	93	53	174	108	114	84	562	473
105	76	60	171	106	128	105	487	431
120	90	62	165	113	117	109	393	432
135	86	57	145	108	110	112	342	408
150	83	61	134	130	107	97	328	391
165	84	60	125	119	—	—	—	—
180	74	62	106	122	92	101	254	317
210	68	58	91	110	82	99	198	323
240	63	60	77	111	76	102	153	221
270	59	62	65	101	64	82	121	211
300	53	61	48	97	—	—	94	168
330	55	62	46	78	—	—	83	142

* Triglyceride concentrations are expressed as mg/100 ml.
‡ Minutes after injection of palmitic acid-1-^{14}C.

difference between the two subfractions in all studies. The TGFA specific activity in Sf > 100 VLDL rose more quickly and reached a higher peak sooner than TGFA in the Sf 20–100 fraction. The specific-activity time curves of the two subfractions crossed in a manner that further suggested a precursor-product relationship. These curves, however, were also consistent with differences in the (fractional) turnover rates of independently metabolized pools of TGFA in VLDL of different sizes.

DISCUSSION

Constant infusions. Considering the studies with total VLDL first, the results were compatible with slow isotopic equilibration anywhere between plasma FFA and TGFA in the final smallest species of VLDL, assuming that progressive conversion of larger to smaller lipoproteins does occur. There was no evidence for independently metabolized pools of TGFA possessing differing (fractional) turnover rates.

The studies in which two subfractions of VLDL (Sf > 100 and Sf 20–100) were analyzed separately also failed to show that TGFA in VLDL of different sizes are independently metabolized at different (fractional) turnover rates. Rather, the results were consistent with TGFA in the larger VLDL being a precursor of that in the smaller VLDL. Delayed isotopic equilibration of precursors of VLDL-TGFA, a possibility suggested with studies of total VLDL, could not alone account for the differences between the two subfractions.

Single injections. These studies resulted in specific activity-time curves that were compatible with classical precursor-product relationships between TGFA in Sf > 100 and Sf 20–100 VLDL, although the possibility could not be excluded that the TGFA in the two subfractions represented two independently metabolized pools.

In summary, the results of all experiments when considered together, are most readily compatible with the concept of a precursor-product relationship between TGFA in larger and smaller VLDL.

The possible conversion of very low density lipoproteins to lipoproteins of higher density has been strongly implied in several studies (5, 13–15). A precursor-product relationship between the pools of TGFA transported in VLDL and low density lipoproteins has been suggested on the basis of specific activity-time curves for TGFA transported in the two lipoprotein classes after an injection of palmitic acid-1-^{14}C (1, 16). In this study we have found a similar relationship between TGFA transported in larger and smaller VLDL (Fig. 5). All our experiments are compatible with the suggestion that a proportion of the TGFA transported

in smaller VLDL had initially been secreted into the plasma in larger ones. Two possibilities exist for such a precursor-product relationship between the TGFA in larger and smaller VLDL. There could be a direct transfer of TGFA molecules from larger to smaller VLDL, or perhaps more likely on the basis of findings in vitro (15), larger VLDL become progressively smaller as triglyceride is removed. The formation of LDL may represent the final step in this process.

While stepwise degradation of lipoproteins has been clearly shown in vitro (15), some published in vivo studies (5, 13, 14) could also be interpreted to show that TGFA in larger lipoproteins are cleared more efficiently than smaller species. When lipoproteins of different size have been injected intravenously in humans, TGFA transported in chylomicrons have been found to have a higher (fractional) turnover rate than those in VLDL (17–19). Furthermore, Quarfordt and Goodman (20), who injected different species of chylomicrons into rats, reported that the rate at which chylomicron TGFA was cleared was proportional to particle size. However, it is possible that reinjected lipoproteins do not represent accurately the turnover of endogenously produced species, possibly because of partial sequestration in the liver (21). Certainly an analysis of the specific-activity-time curves for TGFA in total VLDL (Figs. 2 and 3) and in subfractions of VLDL (Fig. 4) during constant infusions of radiopalmitate gave no indication of the existence of independently metabolized pools of VLDL-TGFA with differing (fractional) turnover rates. Although our results therefore do not support the concept that the rate at which TGFA is removed from plasma is a function of VLDL size, this cannot be altogether excluded since the isotope kinetics might have been dominated by the precursor-product relationship between components of VLDL-TGFA to an extent that masked all other influences.

Finally, it should be emphasized that regardless of the mechanism, the observed differences found between the VLDL subfractions clearly show that in terms of isotope kinetics the VLDL-TGFA cannot be described as a single pool.

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