

# Hydrolysis of rat chylomicron acylglycerols: a kinetic model

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**Abstract** A quantitative model describing the kinetics of hydrolysis of rat chylomicron acylglycerols by bovine milk lipoprotein lipase has been developed using data from studies on rat lymph chylomicrons containing doubly labeled acylglycerols. The detailed analysis indicates that, in addition to hydrolysis from tri- to di-, di- to mono-, and monoacylglycerol to glycerol, an apparently direct hydrolysis pathway of tri- to monoacylglycerol is also present. This accounts for the transient accumulation of monoacylglycerol seen in some of the experiments. For most hydrolysis steps, a Michaelis-Menten mechanism adequately describes the rate of hydrolysis as a function of lipoprotein lipase concentration. A higher order, more complex mechanism, however, is necessary for the apparent tri- to monoacylglycerol hydrolysis pathway. A mathematical function that describes the way free fatty acid released can control the rates of hydrolysis, and how the presence of the binding sites for free fatty acid on albumin in the incubation medium can modulate this, is included. The model simultaneously satisfies the kinetics of hydrolysis for tri-, di-, and monoacylglycerol together with the kinetics of the glycerol and fatty acid moieties for a wide range of albumin and lipoprotein lipase concentrations.—**Foster, D. M., and M. Berman.** Hydrolysis of rat chylomicron acylglycerols: a kinetic model. *J. Lipid Res.* 1981. **22**: 506–513.

**Supplementary key words** lipoprotein lipase · albumin · triacylglycerol · diacylglycerol · monoacylglycerol

Studies of hydrolysis of chylomicron acylglycerols (tri (TAG), di (DAG), and mono (MAG)) in vitro indicate that TAG is hydrolyzed by lipoprotein lipase (LPL) to free fatty acids (FFA) when incubated in a medium containing albumin [1–7]. The action of LPL depends in part on the presence of a cofactor protein [8, 9], presumably apolipoprotein C-II, a constituent of the surface film of the chylomicron. Albumin is required as a receptor for the FFA released; virtually all FFA is bound to albumin. When insufficient albumin is present in the medium, hydrolysis slows down markedly [7].

Scow and Olivecrona [10] have studied the effects of albumin in the incubation medium on the products formed by the action of highly purified bovine milk

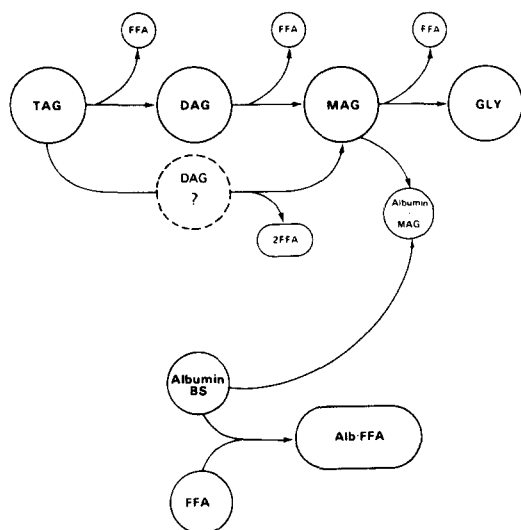
LPL on rat chylomicron TAG. Their studies led to the following proposed mechanism for hydrolysis: TAG is taken from the core of the chylomicron and immediately hydrolyzed in the surface film by LPL to 2-MAG; the 2-MAG moves from the enzyme until it is isomerized to 1(3)-MAG and then it returns to the enzyme to be hydrolyzed to glycerol and FFA; the FFA formed are immediately removed from the surface film by albumin making space available for other acylglycerols. When the binding sites on albumin for FFA are saturated, the FFA accumulates in the surface film impeding entry of TAG into the film.

In this study, we develop a formal model to explain the kinetic data given in [10]. The model presents an integrated picture of hydrolysis of rat lymph chylomicron acylglycerols that allows us to examine some of the conclusions reached by Scow and Olivecrona [10], as well as providing additional insights into the mechanisms involved.

## METHODS

A description of the methods and materials used in the experiments has been given by Scow and Olivecrona elsewhere [10]. The original data for these experiments were made available to us by one of the authors. In brief, chylomicron containing doubly labeled TAG ( $^{14}\text{C}$ oleic acid and  $^3\text{H}$ glycerol) were isolated from rat thoracic duct lymph and incubated at various concentrations of LPL and albumin. The kinetics of the delipidation products, TAG, DAG, MAG, FFA, and glycerol, were followed for 60 min. The data were analyzed using non-linear ordinary differential equation (compartmental) models. All

Abbreviations: TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; LPL, lipoprotein lipase; FFA, free fatty acid. References are in brackets [ ] instead of in parentheses ( ) in order to avoid confusion with rate constants.



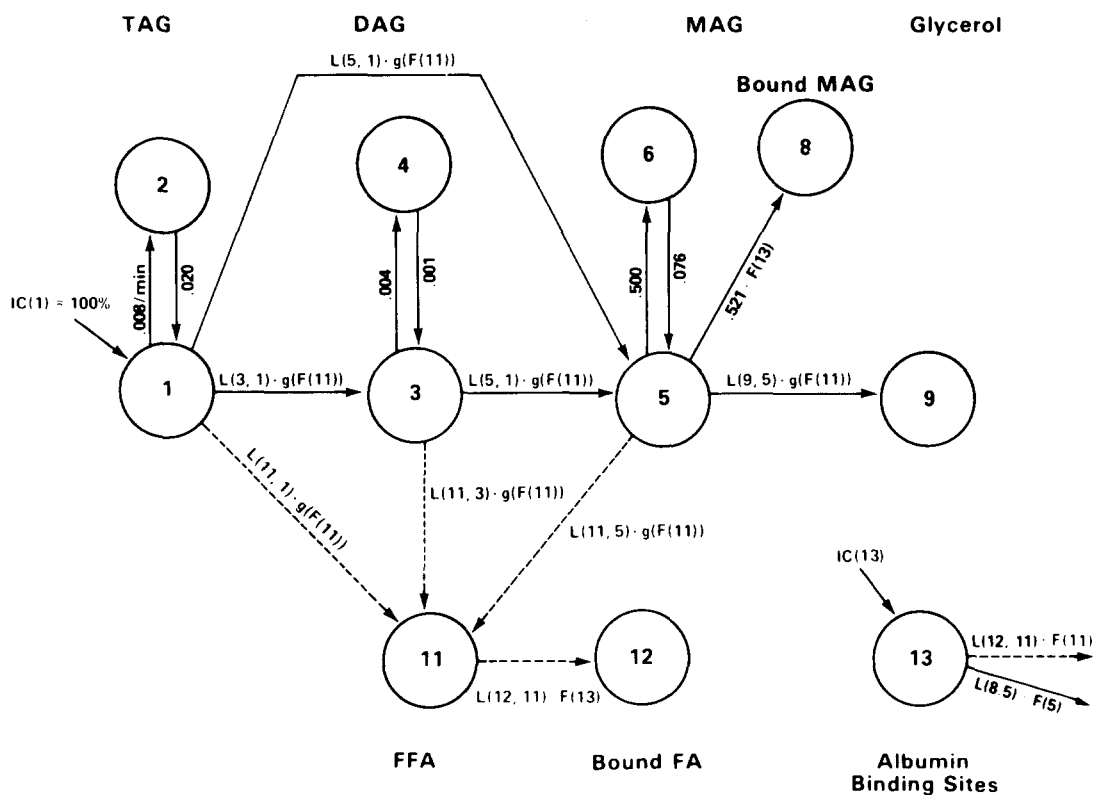
**Fig. 1.** Schematic model indicating the steps in chylomicron acylglycerol hydrolysis. The direct pathway from TAG to MAG is shown as possibly passing through DAG as an intermediate. The schematic indicates the release of FFA; the FFA is collected in an FFA pool shown in the lower part of the schematic. This is the model for the binding of FFA to albumin. Binding of MAG to albumin is also shown.

simulations and least-squares data fitting were carried out using the SAAM computer program [11]. The least squares fitting procedure estimates parameter values and automatically generates estimates for the uncertainties (standard deviations) of the parameter values [11] based in part on the scatter in the fitted data [15]. The modeling methodology employed is similar to that described previously [11, 15, 16].

## MODEL DEVELOPMENT AND RESULTS

The kinetic model was developed and tested in several stages. The overall structure follows what is known about the biochemistry of acylglycerol hydrolysis, whereas the finer structure was dictated by the data and required certain physiologically reasonable assumptions. A detailed description for the derivation of the kinetic model is given in the Appendix.

The general equation describing the dissociation of chylomicron acylglycerols can be written as:



**Fig. 2.** Detailed kinetic model for acylglycerol hydrolysis. The solid lines indicate the acylglycerol submodel while the dotted lines indicate the fatty acid submodel. All rate constants are in units of  $\text{min}^{-1}$ . The unit of measure in compartment 1, denoted  $F(1)$ , is the percent of the initial amount of either acylglycerol, fatty acid, or binding sites.  $L(11,1) = (L(3,1) + 2L(5,1))/3$ ,  $L(11,3) = L(5,3)/3$ , and  $L(11,5) = L(9,5)/3$ . The function  $g(x)$  is described in the text, where here  $x = F(11)$ .

$$\frac{d[C]}{dt} - L_c[C][LPL^*]g(x) \quad 1)$$

where [C] is the concentration of acylglycerol, [LPL\*] is the concentration of *activated* LPL,  $L_c$  is the dissociation constant, and  $g(x)$  is a function describing the effect of FFA on the hydrolysis of C. In solving equation 1), it was assumed that once LPL is mixed with the chylomicron suspension, binding and activation is instantaneous, and [LPL\*] does not change for the duration of the experiment. It was also assumed that in the presence of excess albumin,  $g(x)$  is close to 1. This means that when excess binding sites are available, FFA binds rapidly to albumin and has no affect on hydrolysis; it is only when the sites are depleted that an effect is seen. These assumptions together imply that hydrolysis in the presence of excess albumin obeys first order kinetics.

A schematic indicating the steps involved in hydrolyzing chylomicron acylglycerols is given in Fig. 1. The detailed kinetic model is given in Fig. 2. Each of the parameters L(3,1), L(5,3), L(9,5), and L(5,1) involved in the hydrolysis steps represents a product of the form  $L_c [LPL^*]$ , and is in units  $\text{min}^{-1}$ .

Hydrolysis is indicated as a sequence of reactions TAG to DAG, DAG to MAG, and MAG to glycerol with a FFA being released at each step. In order to fit all the data, however, we had to postulate a direct pathway from TAG to MAG with the concomitant release of two FFA. We cannot tell from these data whether these TAG molecules are hydrolyzed directly to MAG, i.e., if two FFA are cleaved

simultaneously, or whether the conversion is first to DAG but too rapid for us to resolve. This is shown in Fig. 1 as the dotted compartment for DAG. The binding of FFA to albumin is indicated in the lower part of the schematic in Fig. 1. When the binding sites on albumin are fully occupied, FFA accumulate (on the chylomicrons) and, as seen in equation 1), affect the rate of hydrolysis through  $g(x)$ . Some MAG was found to bind to albumin; this binding could be described as directly proportional to the number of free binding sites (BS) on albumin.

A summary of the rate constants for each hydrolysis step derived in fitting the data is given in Table 1. The values indicate that as LPL/TAG<sub>0</sub> increases, (TAG<sub>0</sub> is the initial triacylglycerol concentration) L(3,1), L(5,3), and L(9,5) rise to a plateau. L(5,1) however is zero for LPL/TAG<sub>0</sub> below 0.33. Above this value, it continues to rise through LPL/TAG<sub>0</sub> equal to 9.0.

There were three experiments where LPL/TAG<sub>0</sub> = 0.46 with insufficient albumin in the incubation medium to bind all potential FFA ( $BS_0/FA_0 < 1$ ). In fitting the data for these cases, the following expression was derived for  $g(x)$ , the control function describing the effect of excess FFA on hydrolysis:

$$g(x) = 0.14e^{-900x} + 0.58e^{-300x} + 0.28e^{-90x} + 0.001 \quad 2)$$

where "x" represents the excess free fatty acid in the surface layer expressed as a fraction of FA<sub>0</sub> (compartment 11 in the model shown in Fig. 2). The con-

TABLE 1. Summary of rate constants

LPL/TAG <sub>0</sub>	BS <sub>0</sub> /FA <sub>0</sub>	TAG → DAG L(3,1)	TAG → MAG L(5,1)	DAG → MAG L(5,3)	MAG → GLY L(9,5)
		(min <sup>-1</sup> )	(min <sup>-1</sup> )	(min <sup>-1</sup> )	(min <sup>-1</sup> )
.079	2.0	.015 ± .001	0.0 <sup>a</sup>	.257 ± .023	.399 ± .028
.165	2.0	.032 ± .002	0.0 <sup>a</sup>	.614 ± .043	.480 ± .042
.33	2.0	.075 ± .021	.008 ± .021	.761 ± .223	.460 ± .033
.46	0.0	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
.46	0.16	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
.46	0.53	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
.46	1.5	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
.46	2.3	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
.46	4.9	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
.46	10.4	.075 ± .011	.116 ± .011	.876 ± .118	.779 ± .097
1.03	1.6	.075 ± .016	.144 ± .022	.724 ± .141	1.52 ± .14
2.2	1.7	.075 ± .016	.334 ± .027	.756 ± .153	2.00 ± .11
4.9	3.8	.075 ± .016	.461 ± .029	.984 ± .164	1.05 ± .38
9.0	3.5	.075 ± .016	.650 ± .033	.993 ± .165	1.14 ± .41

<sup>a</sup> Fixed parameter value.

Rate constants (min) ± standard deviations for the model shown in Fig. 1 for experiments with given LPL/TAG<sub>0</sub> and BS<sub>0</sub>/FA<sub>0</sub>. A detailed explanation is given in the Appendix.

For all of the above conditions, the following applies: MAG → bound MAG L(8,5) = 0.152 ± 0.006 min<sup>-1</sup> and FFA → bound FA L(12,11) = 0.25 min<sup>-1</sup>.

stant term 0.001 indicates that over the 60 min of the experiment some hydrolysis independent of FFA is occurring.

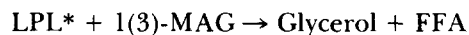
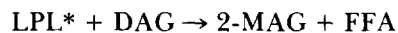
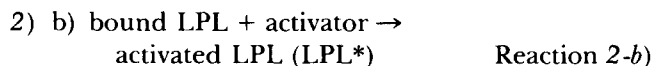
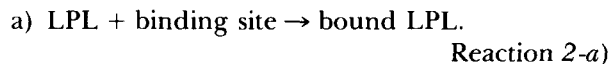
The detailed kinetic model indicates that the sub-models for the TAG, DAG, and MAG subsystems contain two exchanging compartments. These are required to fit the data, and are explained in the Appendix.

The model accounts for all kinetic data in the experiments studied. As a result, it permits a detailed analysis of the effect of LPL and albumin on the hydrolysis of chylomicron acylglycerols.

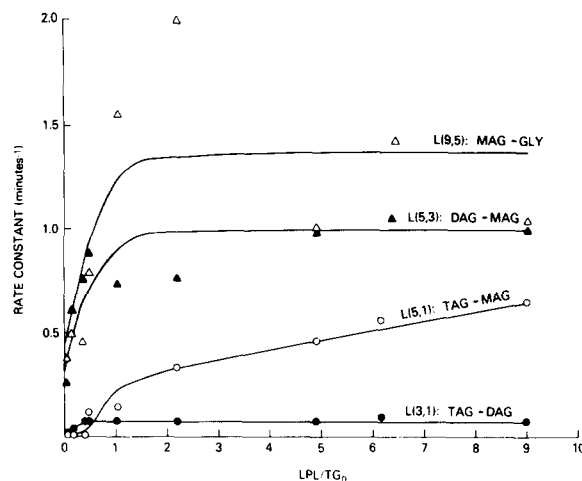
## DISCUSSION

A quantitative model describing the kinetics of hydrolysis of rat chylomicron acylglycerols by bovine milk LPL, and the effect of albumin binding sites on the products formed, has been derived and tested using data from studies on rat lymph chylomicrons containing doubly labeled acylglycerols. These studies were carried out for various initial concentration ratios  $LPL/TAG_0$  and  $ALB_0/FA_0$  in the incubation medium and thus permit a detailed investigation of the hydrolysis process in vitro. Our results confirm the general conclusions reached by Scow and Olivecrona [10]. There is a stepwise delipidation from tri- to di- to monoacylglycerol to glycerol. In addition, there is also a small but very rapid transition from tri- to monoacylglycerol, too rapid to detect a diacylglycerol intermediate. Michaelis-Menten mechanisms at the various steps are adequate to account for all the kinetics at the various concentrations of triacylglycerol, LPL, and albumin.

The kinetic analysis presents a clearer picture of how the hydrolysis process takes place. To interpret the results, let us consider the processes implied by Equation 1):



In deriving the model, we have assumed that for each experiment  $[LPL^*]$  does not change much during the experiment. However for each experiment,  $[LPL^*]$  depends upon the initial  $LPL/TAG_0$ . Thus



**Fig. 3.** The rate constants  $L(3,1)$ ,  $L(5,1)$ ,  $L(5,3)$ , and  $L(9,5)$  plotted as a function of  $LPL/TAG_0$ . The values are taken from Tables 1 and 1A, and are the model-derived values for the various conditions studied.

$[LPL^*]$  is governed both by the initial  $LPL/TAG_0$  and the binding and activation reactions 2-a) and 2-b). In discussing our results, we will therefore assume  $LPL/TAG_0$  is a representative measure of  $[LPL^*]$  for purposes of comparing the rate constants.

In **Fig. 3**, we have plotted  $L(3,1)$ ,  $L(5,1)$ ,  $L(5,3)$ , and  $L(9,5)$  as a function of  $LPL/TAG_0$ . All but  $L(5,1)$  appear to obey simple Michaelis-Menten kinetics which indicates that the binding and activation of sites for  $LPL^*$  saturates for these steps. One might ask why are these individual values  $L(3,1)$ ,  $L(5,3)$ , and  $L(9,5)$  different for each experiment, i.e., why are  $L(9,5)$  and  $L(5,3)$  distinctly greater than  $L(3,1)$ ? The simplest explanation is that *each* of these rate constants has incorporated in it the process of the acylglycerol coming into contact with an activated lipase. Since TAG must come from the core of the chylomicron to the surface whereas DAG and MAG are presumably in or near the surface, we would expect  $L(3,1)$  to be less, and  $L(5,3)$  and  $L(9,5)$  to be approximately equal, as is the case. Moreover, this is consistent with the observation that, under appropriate conditions, hydrolysis of MAG is more rapid than TAG [12].

As seen in **Fig. 3**,  $L(5,1)$  does not saturate for the values of  $LPL/TAG_0$ , but in fact displays three phases. The first phase is a delay, i.e.,  $L(5,1)$  is zero or close to zero for  $LPL/TAG_0$  less than 0.33. Next  $L(5,1)$  rises rapidly until  $LPL/TAG_0$  is about 1.5 to 2. For values of  $LPL/TAG_0$  between 2 and 9,  $L(5,1)$  rises slowly and linearly.

To interpret these results we propose the following



mechanism. First, for low values of  $LPL/TAG_0$ ,  $L(5,1)$  is not seen because only a few sites are occupied with activated LPL per chylomicron, and these hydrolyze TAG to DAG, DAG to MAG, or MAG to glycerol. As  $LPL/TAG_0$  increases above a certain critical level (about 0.4), a specific multivalent binding occurs with a concomitant activation that yields a site capable of cleaving fatty acid from the 1 and 3 positions of TAG. It is not clear if this is done simultaneously or if there is a brief time during which the acylglycerol is DAG; from these kinetic data, it is treated as "simultaneous." This binding appears to saturate at a  $LPL/TAG_0$  of about 1.0, and is responsible for the rapid rise seen in Fig. 3. Finally the linear portion of the curve is probably due to a non-specific receptor for LPL in the surface of the chylomicron.

The rate constants  $L(5,3)$  and  $L(9,5)$  describe the hydrolysis of the DAG and MAG molecules, respectively, that have access to the active enzyme sites, while the sum of  $L(3,1)$  and  $L(5,1)$  describes the hydrolysis of TAG. From Fig. 3 we see that the former two constants and the latter sum of constants increase most rapidly to  $LPL/TAG_0$ , equal to about 1.5. Assuming 0.9 unit of lipolytic activity per  $\mu\text{g}$  of protein whose monomeric molecular weight is 50,000 (see [10]) this corresponds to 43 molecules of activated LPL per chylomicron. Moreover the gradual rise seen above  $LPL/TAG_0$  of 1.5 in [10] is apparently due to the direct hydrolysis of TAG to MAG,  $L(5,1)$ , as seen in Fig. 3, which we believe may be due to the non-specific binding of LPL.

Thus the analysis of these curves reveals many of the complex details of hydrolysis. It would be of interest to know if the sites of the apparent multivalent binding are a relatively fewer number of low affinity sites for LPL relative to those responsible for  $L(3,1)$ ,  $L(5,3)$ , and  $L(9,5)$ , or if there are dimers being formed at the binding sites that are simply dependent on the amount of LPL in the medium. The model tends to support the former since, if dimers were formed, we would expect these rate constants to decrease as  $LPL/TAG_0$  increases above a certain critical level. Finally it would be of interest to study experimentally if nonspecific binding and activation of LPL can occur as the model indicates.

Scow and Olivecrona [10] speculate that the TAG is hydrolyzed to 2-MAG that leaves from the enzyme until it is isomerized, and then returns to the enzyme to be hydrolyzed to glycerol and FFA. If indeed this is the case and is responsible for the transient accumulation seen in MAG activities reported in [10], then we would expect to have two separate com-

partments in series for MAG, one representing 2-MAG and the other 1-MAG. We could not resolve such a configuration from our data, mainly because the glycerol activity curve immediately and rapidly rises in all experiments. Thus we do not distinguish between 1-MAG and 2-MAG in the model. Isomerization, therefore, must occur very rapidly and does not control the kinetics.

There are two ways that albumin affects the hydrolysis of chylomicron acylglycerols. One is through the binding of the FFA released,  $L(12,11)$ , and the other is through the binding of MAG,  $L(8,5)$  [13, 14]. We found that  $L(12,11)$  was substantially larger than  $L(8,5)$  (see the Appendix). These data indicate the possibility that when the binding sites are limited, FFA binds much faster than MAG, and it is not until  $BS_0/FA_0$  is large that the effect of bound MAG is seen. Thus in the model, FFA and MAG compete for binding sites on albumin. We cannot distinguish with these data this possibility versus noncompetitive binding.

Each of the submodels for TAG, DAG, and MAG is comprised of two exchanging compartments. For TAG, it would be tempting to speculate that compartment 2 represents core TAG while compartment 1 represents TAG which has ready access to LPL. This would mean the initial amount of labeled TAG is distributed in some ratio between these compartments. We were unable to resolve this, largely because TAG is so rapidly hydrolyzed. Thus we believe that the TAG has, within the resolution of these data, ready access to LPL and that a small amount, represented by compartment 2, is held up for some reason from being hydrolyzed. The rate constants  $L(3,1)$  and  $L(5,1)$  are determined by the movement of TAG in the core of the chylomicron and hydrolysis.

It is difficult to interpret the exchange compartment 4 for DAG. This is required to fit the final two data in the DAG radioactivity curve both of which have very low values. It could represent a very small fraction of DAG which is held up from being hydrolyzed, but it could also be that the radioactivity at 60 min is just a background "noise".

The exchange compartment, compartment 6, in the MAG subsystem is more substantial, and probably represents MAG which is formed and which does not have ready access to LPL. This would be consistent with the mechanisms described by Scow and Olivecrona [10]. Here, to accommodate the excess MAG, the surface layer of the chylomicron is extended. The MAG in the extended area is beyond reach of the LPL and so there is a delay. ■■

## APPENDIX

Fig. 2 shows the detailed structure of the model which we derived to fit the data from the experiments both separately and, in specific groups indicated below, simultaneously. Fourteen individual experiments reported in [10] were used in the derivation and testing of this model. In this Appendix we will indicate how a model for each subsystem was derived and combined to form the system model. Fig. A-1 shows a typical fit of the data.

### TAG subsystem

For the 14 experiments studied, the TAG radioactivity decay curve showed two components indicating a two-compartment model (compartments 1 and 2, Fig. 2). Such a model can be described either 1) as two distinct compartments decaying at different rates, or 2) as two compartments that exchange. The former implies two distinct moieties of TAG which is difficult to justify from the known biochemistry of the system. Thus we chose the latter. All the initial radioactivity in the system is in compartment 1. The exchange rate constants for this subsystem shown in Fig. 2 were derived by fitting the series of experiments in which  $LPL/TAG_0$  is 0.46 and  $BS_0/FA_0$  are 1.5, 2.3, 4, 9, and 10.4. Since  $LPL/TAG_0$  is the same for these four experiments and there is an excess of binding sites for FFA available, we could, by fitting these data individually and simultaneously, determine if there were differences in the exchange rate constants  $L(2,1)$  and  $L(1,2)$ . As indicated in Table A-1, no substantial differences were found. Next we did a similar study on the TAG decay curves from the remaining experiments where  $BS_0/FA_0$  was greater than 1. The results are summarized in Table A-2. In the

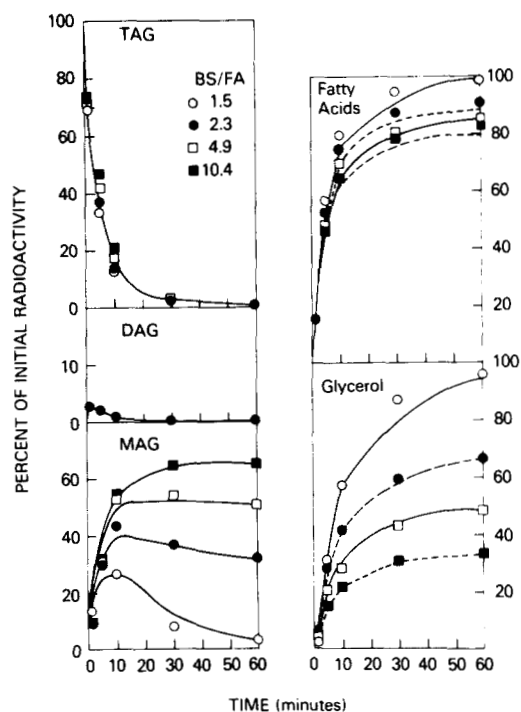


Fig. A-1. Model predictions for a simultaneous fit (continuous and dashed lines) versus experimental observations for TAG, DAG, MAG, glycerol, and fatty acid when  $LPL/TAG_0$  is 0.46 for various  $BS/FA$  ratios.

latter study  $L(2,1)$  and  $L(1,2)$  were set equal to 0.008/min and 0.020/min, respectively, the values determined for the former, because no substantial difference in these constants was seen. These are shown in Fig. 2.

### DAG subsystem

To derive the model for DAG, we fitted the TAG and DAG data both individually and jointly for the

TABLE A-1. Rate constants ( $\text{min}^{-1}$ )  $\pm$  standard deviations for model when  $LPL/TAG_0$  is 0.46

$\frac{BS_0}{FA_0}$	L(2,1)	L(1,2)	L(3,1)	L(5,1)	L(5,3)	L(6,5)	L(5,6)	L(9,5)
Individual fit								
1.5	.017 $\pm$ .010	.044 $\pm$ .026	.108 $\pm$ .017	.129 $\pm$ .026	.911 $\pm$ .164	.256 $\pm$ .097	.102 $\pm$ .041	.433 $\pm$ .056
2.3	.009 $\pm$ .002	.025 $\pm$ .006	.127 $\pm$ .053	.071 $\pm$ .055	1.49 $\pm$ .63	.256 <sup>a</sup>	.102 <sup>a</sup>	1.05 $\pm$ .23
4.9	.007 $\pm$ .002	.017 $\pm$ .006	.088 $\pm$ .028	.095 $\pm$ .030	1.01 $\pm$ .31	.256 <sup>a</sup>	.102 <sup>a</sup>	1.15 $\pm$ .44
10.4	.007 $\pm$ .002	.014 $\pm$ .006	.138 $\pm$ .073	.023 $\pm$ .073	1.74 $\pm$ .92	.256 <sup>a</sup>	.102 <sup>a</sup>	1.48 $\pm$ .83
Simultaneous fit								
1.5	.008 $\pm$ .001	.020 $\pm$ .003	.075 $\pm$ .011	.116 $\pm$ .011	.876 $\pm$ .118	.500 $\pm$ .150	.076 $\pm$ .028	.779 $\pm$ .097
2.3	.008 $\pm$ .001	.020 $\pm$ .003	.075 $\pm$ .011	.116 $\pm$ .011	.876 $\pm$ .118	.500 $\pm$ .150	.076 $\pm$ .028	.779 $\pm$ .097
4.9	.008 $\pm$ .001	.020 $\pm$ .003	.075 $\pm$ .011	.116 $\pm$ .011	.876 $\pm$ .118	.500 $\pm$ .150	.076 $\pm$ .028	.779 $\pm$ .097
10.4	.008 $\pm$ .001	.020 $\pm$ .003	.075 $\pm$ .011	.116 $\pm$ .011	.876 $\pm$ .118	.500 $\pm$ .150	.076 $\pm$ .028	.779 $\pm$ .097

<sup>a</sup> Fixed equal to  $L(6,5)$  and  $L(5,6)$  for  $BS_0/FA_0$  equal to 1.5 case.

For all of the above conditions, the following applies:  $L(8,5) = 0.152 \pm 0.006 \text{ min}^{-1}$  and  $L(12,11) = 0.25 \text{ min}^{-1}$ .

TABLE A-2. Rate constants ( $\text{min}^{-1}$ )  $\pm$  standard deviations for experiments with excess albumin

LPL TAG <sub>0</sub>	BS <sub>0</sub> FA <sub>0</sub>	L(3,1)	L(5,1)	L(5,3)	L(9,5)
Individual fit					
.079	2.0	.015 $\pm$ .005	0 $\pm$ .004	.26 $\pm$ .086	.40 $\pm$ .02
.165	2.0	.032 $\pm$ .003	0 $\pm$ .03	.61 $\pm$ .512	.48 $\pm$ .034
.33	2.0	.085 $\pm$ .032	0 $\pm$ .03	.85 $\pm$ .383	.45 $\pm$ .032
1.03	1.6	.048 $\pm$ .020	.17 $\pm$ .024	.49 $\pm$ .181	1.5 $\pm$ .14
2.2	1.7	.066 $\pm$ .035	.34 $\pm$ .151	.71 $\pm$ .320	2.0 $\pm$ .18
4.9	3.8	.066 $\pm$ .018	.70 $\pm$ .024	.91 $\pm$ .173	2.9 $\pm$ 1.6
9.0	3.5	.075 $\pm$ .025	.65 $\pm$ .046	1.0 $\pm$ .22	2.5 $\pm$ 1.1
Simultaneous fit					
.079	2.2	.015 $\pm$ .001	0.0 <sup>a</sup>	.257 $\pm$ .023	.399 $\pm$ .036
.165	2.0	.032 $\pm$ .002	0.0 <sup>a</sup>	.614 $\pm$ .043	.480 $\pm$ .041
.33	2.0	.075 $\pm$ .021	.008 $\pm$ .021	.761 $\pm$ .223	.460 $\pm$ .032
1.03	1.6	.075 $\pm$ .016	.144 $\pm$ .022	.724 $\pm$ .141	1.52 $\pm$ .13
2.2	1.7	.075 $\pm$ .016	.334 $\pm$ .027	.756 $\pm$ .153	2.00 $\pm$ .11
4.9	3.8	.075 $\pm$ .016	.461 $\pm$ .029	.984 $\pm$ .164	1.05 $\pm$ .38
9.0	3.5	.075 $\pm$ .016	.660 $\pm$ .033	.993 $\pm$ .165	1.14 $\pm$ .41

<sup>a</sup> Fixed parameter value.

Exchange rate constants L(2,1), L(1,2), L(4,3), L(3,4), L(6,5), and L(5,6) are set equal to those in Table A-1 for the simultaneous fit.

For all of the above conditions, the following applies: L(8,5) = 0.152  $\pm$  0.006.

experiments where LPL/TAG<sub>0</sub> was 0.46 and BS<sub>0</sub>/FA<sub>0</sub> were 1.5, 2.3, 4.9, and 10.4. First we found that apparently not all TAG went through DAG, i.e., TAG was not a sole precursor for the DAG radioactivity measured in these studies. The portion of TAG that apparently did not pass through DAG accounted for the additional pathway L(5,1) as described below. Moreover, to fit the final two points of the DAG radioactivity curve, a slowly turning over compartment in the DAG subsystem was required. Hence, compartment 4 was introduced. A summary of the results is given in Table A-1. The remaining studies were analyzed similarly. A summary of the results is given in Table A-2.

### MAG subsystem

In developing the MAG subsystem it was observed that when BS/FA exceeded 2.3, some MAG apparently bound to the albumin and hence was not hydrolyzed. No binding was apparent when BS/FA was below 1.5. Therefore we first considered the experiment where LPL/TAG<sub>0</sub> was 0.46 and BS<sub>0</sub>/FA<sub>0</sub> was 1.5. Using the TAG and DAG subsystems previously developed, we found a rapid pathway into compartment 5 of the MAG subsystem directly from TAG, compartment 1, was required in order to satisfy the early portion of the MAG activity curve, and that this pathway accounted fully for the portion of the TAG decay not seen in DAG. The MAG subsystem, like the previous two, required a second exchange

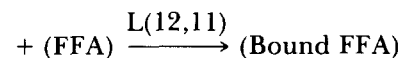
compartment, compartment 6, to account for the final portion of the curve. The parameter values were determined by fitting jointly the MAG and glycerol data. The results are given in Table A-1. The rapid rise of glycerol radioactivity precluded any delay in the MAG subsystem and thus we do not differentiate between 2-MAG and 1(3)-MAG. Isomerization therefore must occur rapidly.

To explain the experiments where LPL/TAG<sub>0</sub> was 0.46 and BS<sub>0</sub>/FA<sub>0</sub> were 2.3, 4.9, and 10.4, we proposed that MAG binding to albumin is proportional to the amount of albumin in the system. The results of the analysis are indicated in Fig. 2 and the tables.

### Binding of FFA to albumin

The binding of FFA to albumin is described by

(A-1) (Free Binding Sites)



We assumed in the derivation of the model that this binding is very rapid so that FFA has no effect on the rates of hydrolysis when there were excess binding sites available. A value of L(12,11) = 10  $\text{min}^{-1}$  was used in the simulations. Since there was not enough information in these data to determine L(12,11) with any degree of precision, a value sufficiently large not to affect the kinetics was chosen. The value of L(8,5) = 0.152  $\pm$  0.006(SD) was de-



terminated from the experiments in which  $BS_0/FA_0 > 1$ . Thus  $L(12,11)$  is substantially larger than  $L(8,5)$ .

### Control of hydrolysis by FFA

The model could account for all kinetic data in the experiments when excess albumin was present in the incubation medium. It remained to investigate the three experiments where  $LPL/TAG_0$  was 0.46 and  $BS_0/FA_0$  was 0.0, 0.16, and 0.53. Here the assumption is that hydrolysis slows down as the FFA accumulates, presumably in the surface layer of the chylomicron. The problem was to derive an expression, depending upon the concentration of this FFA, i.e., compartment 11, that would modulate the rate of hydrolysis. This is given by equation 2) in the text. For these cases the model is non-linear since the rate constants representing hydrolysis are affected by the FFA concentration.

The expression derived is the sum of three exponentials given previously in the text.

The authors thank Mrs. Sue Smith, Mrs. Kitty Marconi, and Miss Christine Nettles for their patience in typing the manuscript.

*Manuscript received 14 April 1980 and in revised form 17 November 1980.*

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