

# Kinetic Studies of Plasma Free Fatty Acid and Triglyceride Metabolism in Man

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**ABSTRACT** Plasma transport of free fatty acids (FFA) and triglyceride fatty acids (TGFA) was studied in seven subjects with normal lipid metabolism, one case of total lipodystrophy, and one case of familial hyperlipemia (Type V). Studies were carried out after intravenous injection of radioactive FFA, of lipoproteins previously labeled in vitro in the triglyceride moiety, or both.

Computer techniques were used to evaluate a series of multicompartamental models, and a general model is proposed that yields optimum fitting of experimental data for both FFA and TGFA. The results show that as much as 20–30% of FFA leaving the plasma compartment in normal subjects is transported to an exchanging extravascular pool and quickly reenters the plasma pool as FFA. The rate of irreversible delivery of FFA from plasma to tissues averaged 358  $\mu\text{Eq}/\text{min}$  in normals. The lipodystrophy patient, despite the virtual absence of adipose tissue (confirmed at autopsy), had a plasma FFA concentration and a total FFA transport, both more than twice normal. Total TGFA transport ranged from 25 to 81  $\mu\text{Eq}/\text{min}$  in four normal controls. The rate constant for TGFA turnover in the patient with Type V hyperlipemia was so small that total transport could not be quantified from the data available; the TGFA half-life was over 500 min.

In two normal subjects given injections of autologous lipoproteins labeled in vitro with triolein- $^{14}\text{C}$  and simultaneously given oleic acid- $^3\text{H}$ , it was shown that the time course for the disappearance of the TGFA in the in vitro labeled samples conformed almost exactly to that of the physiologically labeled lipoprotein TGFA syn-

thesized from injected FFA (as evidenced by the simultaneous fitting of both sets of data using the same multicompartamental model and the same rate constants). Radioactivity appeared in the plasma FFA fraction at a significant rate after injection of plasma labeled in vitro with TGFA. It was estimated that as much as 50% of the total TGFA transported underwent rapid and rather direct conversion to FFA in the two normal subjects studied this way. The kinetic data suggest that such conversion of TGFA to FFA was not preceded by any extensive dilution, such as would result from complete mixing with tissue triglyceride stores.

## INTRODUCTION

Plasma transport of fatty acids in free form (FFA) and in the form of glycerides (TGFA) has been extensively studied in animals and in man. The kinetic behavior of these lipids in plasma is evidently quite complex and there appear to be significant interspecies differences. The early studies of Fredrickson and Gordon (1) and of Dole (2) showed that a significant fraction of FFA leaving the plasma compartment quickly reenters it (as FFA) from a second compartment.<sup>1</sup> Calculation of the maximum net transport of FFA (the maximum available for concurrent utilization) must take account of these fatty acids returning quickly to the plasma FFA compartment. The kinetics of triglyceride synthesis, secretion into plasma in lipoprotein form, and subsequent removal is also complex, and the mechanisms are poorly understood. From human and animal studies it is clear that the liver triglycerides do not constitute a single homogenous compartment (3–5, 7), and it is equally clear, both in experimental animals and in man, that the

<sup>1</sup> This reentry of FFA radioactivity into the plasma compartment after passing through an extravascular compartment (or series of such extravascular compartments) has been referred to as recycling or reflux.

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Received for publication 11 October 1967 and in revised form 16 April 1969.

triglycerides in different classes of plasma lipoproteins are kinetically distinguishable (4, 6-8). In dealing with the kinetics of such a complex system, conclusions must be regarded as tentative, particularly when, as in clinical studies, one is largely limited to data for the plasma compartment.

In most of the present studies, the time course of labeling of the plasma FFA and TGFA fractions in man was determined after pulse labeling by intravenous injection of labeled FFA. A number of multicompartmental models was constructed and their compatibility with the experimental results tested with a computer-based approach (Simulation, Analysis, and Modeling: SAAM) developed by Berman, Weiss, and Shahn (9-11). The scope and limitations of this approach in the analysis of lipid transport (in the rat) has been demonstrated by the work of Baker and Schotz (12).

The data from FFA injection studies were used to derive values for turnover of FFA and TGFA ( $\mu\text{Eq}/\text{min}$ ) in seven subjects without apparent disorder of lipid transport, in one patient with Type V hyperlipemia (13), and in one child with total lipodystrophy (14). An excellent fit of experimental and computer-derived data was obtained using the same model in all subjects.

An attempt was made to determine to what extent plasma TGFA enter the plasma FFA pool before being taken up into tissues for storage in ester form or for oxidation. Quantitative evaluation of such a pathway might shed light on the mechanisms for removal of TGFA from plasma. If labeled triglyceride molecules are taken up intact and mixed with sizeable tissue stores of unlabeled triglyceride, reentry of label into the plasma should be delayed and of small magnitude, which reflects extensive isotopic dilution. On the other hand, hydrolysis of TGFA may occur at or near the vascular endothelium, particularly if lipoprotein lipase is involved, as suggested by the work of Robinson and Harris (15). In this case, kinetic studies might reveal a rapid and quantitatively important pathway from TGFA to FFA without extensive dilution. Indeed, evidence for such a pathway has been presented by Laurell in the rat (16), by Havel and Fredrickson in dogs (17), and by Havel, Felts, and VanDuyne in rabbits (4), but no data have been reported for low density lipoprotein TGFA in man. Data from our FFA-injection studies were indecisive on this point. Consequently it was necessary to turn to experiments in which autologous lipoproteins labeled *in vitro* with TGFA- $^{14}\text{C}$  were injected. Because the FFA fraction was initially unlabeled in these experiments, it became feasible to evaluate the extent of TGFA conversion to FFA. In these experiments FFA- $^3\text{H}$  was simultaneously injected, and all of the data were fitted to a common model for the FFA-TGFA system.

## METHODS

11 studies were carried out in nine subjects. Five subjects were young normal volunteers with no evidence of organic disease and with normal plasma lipid levels. Two subjects, R. C. (age 54) and O. B. (age 56), had been admitted for evaluation of previous thyroid and neurological disturbances, respectively, but both men were found to be euthyroid with normal plasma lipid levels at the time of study. Studies were also done in one subject with Type V familial hyperlipemia (L. W.) (13), and in one child with total lipodystrophy (M. W.) (14). All subjects were maintained for at least 2 wk before the tests on a standard NIH diet with calorie intake 38% from carbohydrate, 45% from fat, and 17% from protein. The subjects were permitted no food after the evening meal, i.e. for about 16 hr before the injection of labeled substrate. Two intravenous catheters were placed, one in each antecubital vein, after which the subject rested comfortably in bed for at least 40 min before the administration of of radioisotope.

After three control blood samples had been drawn from one catheter, the appropriately labeled fatty acid-albumin solution was rapidly injected through the other catheter (within 2 sec). Three subjects received the label over a 30-min period by constant infusion using a Bowman pump, and one subject (W. C.) received both a single rapid injection and, 30 min later, a constant infusion of labeled FFA. The dose administered varied from 6  $\mu\text{C}$  to 15  $\mu\text{C}$  of carbon-14 and represented a true tracer amount (between 0.005 and 0.01 mEq of fatty acid). After rapid injection of FFA- $^{14}\text{C}$ , blood samples were taken at 2-min intervals for 20 min, and then at 10-min intervals for the duration of the 1st hr. Thereafter, samples were taken at 15-min intervals for 2-3 additional hr. Blood samples were collected in dry syringes, transferred to tubes containing heparin, mixed thoroughly, and immediately placed in ice until analytical procedures could be begun.

*Measurement of plasma lipid specific radioactivity.* The samples were centrifuged at 5°C and the serum was extracted by the method of Dole (18) using iso-octane (2,2,4-trimethylpentane) in place of heptane. The fatty acids were isolated from the iso-octane phase by extraction with alkaline-ethanol as previously described (19), which left the esterified fatty acids in the iso-octane phase. The iso-octane was removed by evaporation, and the lipids were then redissolved in chloroform and fractionated by silicic acid column chromatography to isolate glycerides, cholesteryl esters, and phospholipids. The radioactivity in each fraction was determined on an aliquot by liquid scintillation counting in toluene contains 0.5% 2,5-diphenyloxazole. The radioactivity was assayed in a Packard Tri-Carb liquid scintillation spectrometer with appropriate internal standards of tritium or carbon-14 in each vial to correct for quenching and to determine the simultaneous counting efficiency for each isotope. All samples were counted for 10-min periods or 30-min periods to attain a SD of  $\pm 5\%$  for each count rate. The lipid content of each fraction was determined on additional aliquots by direct titration for FFA (18), hydroxamate ester assay for glycerides and cholesteryl esters (20), and by phosphorus measurement for phospholipids (21).

The labeled FFA fraction obtained 45 min after injection of palmitic acid-1- $^{14}\text{C}$  was analyzed to establish that the isolation procedure excluded any labeled complex lipids or labeled short-chain breakdown products. The FFA fraction, isolated by the routine procedure, was fractionated on a silicic acid column. No significant radioactivity was found

in phospholipids or other complex lipids. The FFA from a similar sample were converted to methyl esters and fractionated by gas-liquid chromatography (G-LC) with collection and radioassay of each peak. 90% of the radioactivity injected into the column was recovered, and 100% of the recovered label was coincident with the peak of methyl palmitate.

As a further check on the completeness of separation of TG from FFA, chromatographically pure triolein- $^{14}\text{C}$  (300,000 cpm) was added to plasma and the FFA fraction isolated by the routine procedure. Analysis in duplicate gave recoveries in the TG fraction of 277,450 and 300,250 cpm, respectively; the FFA fraction contained 472 and 420 cpm, respectively. Thus there was less than 0.2% contamination of FFA by TG.

*Preparation of labeled substrates.* The palmitic acid- $^{14}\text{C}$  and oleic acid- $^{14}\text{C}$  were obtained from Nuclear-Chicago Corporation, Des Plaines, Ill., and repurified before use by preparative gas-liquid chromatography. This radioactive material was then complexed with human serum albumin using sterile techniques as previously described (19) and stored frozen in sterile 2-cc injection vials until used. By GLC-radiochromatography, as described above, the radioactivity present in the purified preparations was limited to the palmitic acid peak or the oleic acid peak, respectively. All preparations were checked by culture for sterility and by intravenous injection into rabbits for pyrogenicity.

Lipoproteins labeled with triglyceride- $^{14}\text{C}$  were prepared by a modification of the method of Avigan (22). Triolein- $^{14}\text{C}$  was obtained from Nuclear-Chicago Corporation and purified by thin-layer chromatography (TLC) in a system of petroleum ether:diethyl ether:acetic acid (90:20:1). In the presence of sterile conditions, 10 ml of plasma from blood collected with 50 mg of ethylenediaminetetraacetate (EDTA) was placed in a 30 ml evacuated flask containing 1100 mg of Celite 454 onto which  $10\ \mu\text{C}$  (0.5 mg) of triolein- $^{14}\text{C}$  had been previously adsorbed. After a 6 hr incubation with shaking at  $36^\circ\text{C}$  in a Dubnoff incubator, the plasma was drawn through a  $0.45\ \mu$  Millipore filter and centrifuged in a 40.3 rotor for 30 min at 15,000 rpm in a Model L Spinco ultracentrifuge. Any floating triglyceride or chylomicrons that might have been present were removed by slicing the centrifuge tube  $\frac{1}{2}$  inch below the surface and discarding the top fraction. The infranatant solution was again drawn through a  $0.45\ \mu$  Millipore filter and appropriate aliquots were tested for pyrogenicity and sterility. Labeled plasma was stored at  $5^\circ\text{C}$  for 48 hr while these tests were done. An aliquot was taken to determine distribution of radioactivity, and the sample was reinjected into the donor subject.

It was found that a Celite/triglyceride mass ratio greater than 2000:1 was required to give a maximum uptake in plasma when the Celite/plasma ratio was greater than 25:1 (mg:ml). Evidently, the rate of uptake is strongly dependent on the total surface area of the film of labeled glyceride on the Celite. After 2 hr of incubation at  $36^\circ\text{C}$ , approximately 12% of the adsorbed label was incorporated into plasma lipoproteins, whereas 22% was incorporated after 6 hr of incubation. Increasing the incubation time of 12 hr did not further improve the degree of labeling. Incubation at  $5^\circ\text{C}$  markedly reduced the rate of uptake of label.

To test the stability of the in vitro-labeled lipoproteins, a typical preparation was incubated at  $37^\circ\text{C}$  for 3 hr, which approximated the duration of the in vivo studies. There was no spontaneous release of radioactivity to the FFA fraction under these conditions. The distribution of radioactivity

among the plasma fractions was then determined. A sample of labeled plasma adjusted to density 1.21 was centrifuged overnight at 105,000 *g*. The  $d > 1.21$  fraction contained less than 2% of the total radioactivity. The supernatant solution ( $d < 1.21$ ) was fractionated using the heparin-manganese method (23) to yield the precipitate, which contained beta- and prebeta lipoproteins, and the supernatant solution contained alpha lipoproteins. The alpha lipoprotein fraction contained 14% of the total lipoprotein triglyceride radioactivity, and the remaining 86% was in the low density fraction (beta plus prebeta lipoproteins). The distribution of radioactivity in the labeled plasma was also determined by paper electrophoretic separation of the lipoproteins and strip scanning for radioactivity. As shown by the example in Fig. 1, approximately 80% of the radioactivity migrated with the visible betalipoprotein band, but differentiation between prebeta and beta lipoproteins could not be accurately made. Since only  $10\ \mu\text{l}$  of plasma was required for this rapid electrophoretic lipoprotein analysis, all preparations were evaluated in this fashion before injection, and in all those preparations used at least 80% of the radioactivity was located in a discrete peak corresponding to the visible beta plus prebeta lipoprotein band. In the experiments described below, the total plasma glycerides are isolated together and treated kinetically as if they constituted a single pool. The limitations of this approximation are discussed later.

An attempt was made to determine explicitly the distribution of radioactivity between very low density (VLD) and low density (LD) fraction in human plasma labeled in vitro. Duplicate samples were labeled as described above, stored 48 hr at  $5^\circ\text{C}$ , and then fractionated in the preparative ultracentrifuge (22 hr at  $d = 1.006$  for VLD; 22 hr at  $d = 1.063$  for LD; infranatant taken as high density [HD]). The distribution of TG radioactivity in the two samples was, respectively: VLDL, 40.1% and 39.6%; LDL, 28.5% and 22.8%; HDL, 31.4% and 37.6%. The higher values for percentage of radioactivity in HDL compared with those obtained by paper electrophoresis and heparin-manganese precipitation may reflect alterations in lipoprotein produced by the extended time involved in this subfractionation. In vitro transfer of TG radioactivity from VLDL to lipoproteins of higher density has been previously reported (4, 24); recent studies in this laboratory confirm transfer of VLDL triglyceride to the HDL fraction but the process is very slow at  $5^\circ\text{C}$ . The labeled plasmas as actually used were kept at this low temperature until injected. Thus the value of less than 20% of triolein- $^{14}\text{C}$  in HDL (by paper electrophoresis) more closely reflects what the patients actually received.

*Kinetic analysis.* The data were analyzed and fitted to various hypothetical multicompartamental models using the NIH-SAAM program described by Berman et al. using IBM-360 digital computers (9-11). The program derives values for the parameters of an assumed model to yield a least squares fit of the data. This is accomplished by the generation of theoretical data based on initial estimates of the kinetic parameters for the model, and the calculation of partial derivatives for each theoretical datum with respect to each adjustable parameter in the model. From the partial derivatives, equations of conditions and normal equations are generated, and corrections for the initial estimates of the parameter values are calculated. This procedure is iterated until a least squares fit of the data is obtained, which yields a set of values for the parameters along with estimates of their uncertainties.

## RESULTS

In each subject, over the several hours of plasma sampling, the plasma concentrations of FFA and TGFA remained essentially constant, varying by no more than  $\pm 5\%$ . The kinetic parameters derived apply then to a true steady state. The specific radioactivity of the isolated plasma phospholipids and cholesteryl esters was always less than 3% of that of the TGFA. Data for these esters were not analyzed.

### Notation and nomenclature

We use the following terms and symbols (25):

**Compartment.** A subdivision of a model used for the analysis of the experimental observations.

**Compartment size.** The total amount of material (in  $\mu\text{Eq}$ ,  $\text{mEq}$ , etc.) in a compartment.

**Rate constant ( $\lambda$ ).** The fraction of the amount of material in a compartment that is transported from that compartment to another per unit time. All values in the present study are given in units of  $\text{min}^{-1}$ . Letter subscripts are used, the first indicating the compartment into which transport is occurring and the second, the compartment from which transport is occurring (e.g.,  $\lambda_{B,A}$  indicates fraction of material entering compartment B from compartment A per unit time). When the fraction transported from the compartment leaves the system under consideration irreversibly, the compartment entered is designated "O" (out) (e.g.,  $\lambda_{O,A}$  indicates irreversible transport of material from compartment A into a "sink" from which there is no return to the system). When there are several pathways for transport from a

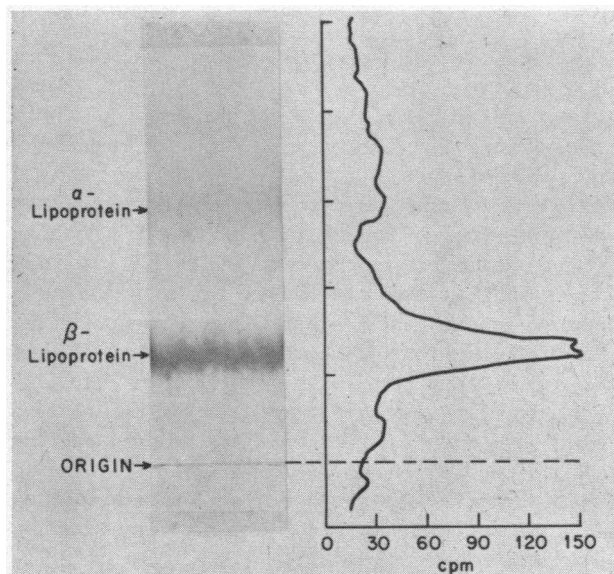


FIGURE 1 Paper electrophoresis of human plasma lipoproteins labeled in vitro with triolein- $^{14}\text{C}$ . Radioactivity assayed using a Packard Radiochromatogram Scanner.

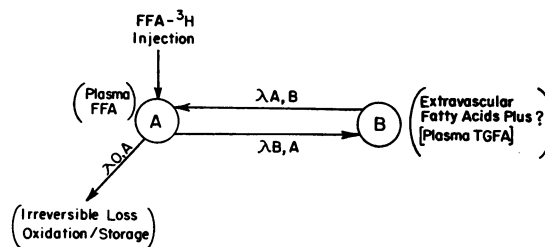


FIGURE 2 Two-compartment model used to describe the metabolism of free fatty acids in man. Compartment A represents the plasma albumin bound FFA pool. Compartment B represents all metabolic pools that receive fatty acids from the plasma FFA pool and subsequently return them to the plasma FFA pool. Any TGFA derived from FFA and returning fatty acids to the FFA pool would be included in Compartment B.

compartment (A), the sum of the rate constants describing this total transport is designated  $\lambda_{A,A}$ .

**Transport.** The amount of material transferred from one compartment to another per unit time (transport =  $\lambda \times$  compartment size).

### Plasma FFA turnover

For each patient studied, the FFA data alone were first fitted to the two compartment model shown in Fig. 2. This model is the simplest compartmental system which can be constructed to describe the observed biphasic decay curve of FFA- $^3\text{H}$  as illustrated in Fig. 3. It differentiates the transport of FFA into two metabolic pathways—irreversible loss from the plasma (rate constant,  $\lambda_{O,A}$ ), and reversible “exchange” with an unknown second compartment (rate constant,  $\lambda_{B,A}$ ). The flow into this second compartment (compartment B) and return to the plasma as FFA undoubtedly represents the sum of many metabolic processes. These would include not only exchange with extravascular FFA or membrane-bound fatty acid, but also any rapid incorporation into and subsequent release from plasma and tissue esterified fatty acids, all processes which potentially make fatty acid available again as FFA to the plasma compartment. The irreversible FFA loss is shown occurring from compartment A ( $\lambda_{O,B} = 0$ ). This is arbitrary since data obtainable by sampling only in compartment A are not adequate to differentiate between a loss from A and a loss from B (26). Similarly, a portion of the unlabeled input, shown here into A only, could as well be into B. The arbitrariness of these assignments, however, does not seem to influence the calculation of the rate of irreversible FFA loss or subsequent interpretations of the TGFA data.

The FFA content of the plasma space, compartment A, was set equal to the product of the calculated total intravascular dilutional volume for albumin (41.3 ml/kg body weight [27]) and the observed FFA concentration

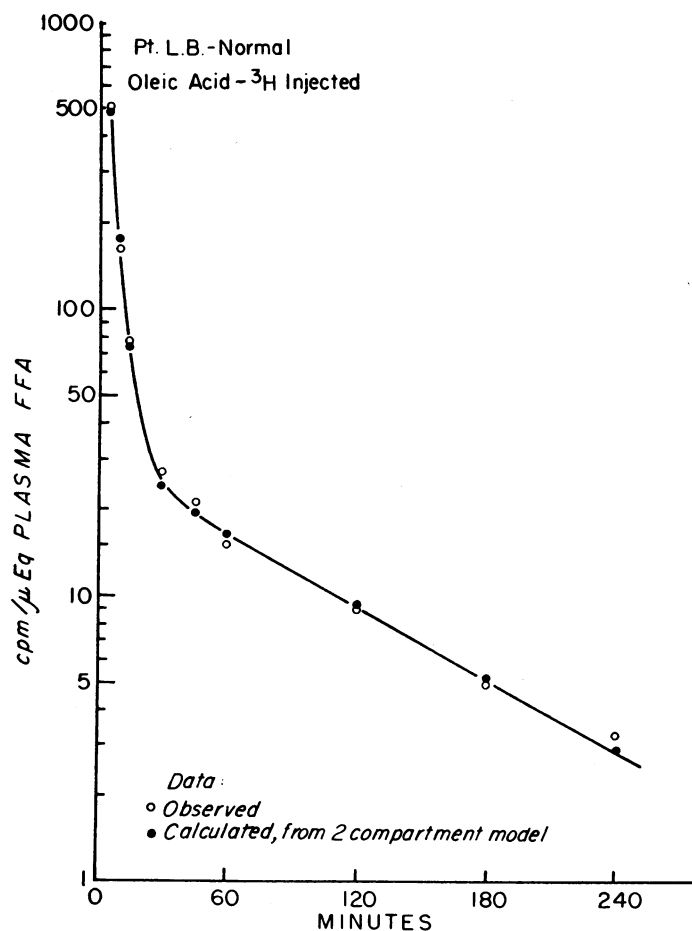


FIGURE 3 Disappearance of intravenously injected palmitic acid-<sup>14</sup>C from plasma in a normal subject. The open circles represent the experimental data; the closed circles represent the data generated from the two-compartment model shown in Fig. 2.

in the plasma. In each study a highly satisfactory fit was obtained for FFA data derived either from a single injection of FFA or from the sequential single injection, constant-infusion experiment (Subject W. C., Table I). Representative results are shown for a single injection study in Fig. 3, and for a combined experiment in Fig. 4. The results obtained using this model to analyze kinetic data are listed in Table I.

In eight studies in control subjects, the sum of the rate constants out of the plasma FFA pool ( $\lambda_{A,A} = \lambda_{B,A} + \lambda_{O,A}$ ) ranged from  $0.175 \text{ min}^{-1}$  to  $0.391 \text{ min}^{-1}$  (half-life from 1.8 min to 3.9 min). It is important to recognize that even though the initial disappearance of FFA follows first-order kinetics described by this sum of rate constants, only the transport through  $\lambda_{O,A}$  is potentially available for tissue oxidation or storage. The values for  $\lambda_{O,A}$  ranged from  $0.151 \text{ min}^{-1}$  to  $0.348 \text{ min}^{-1}$ . The remainder of the transport is involved only in a

reversible exchange with pool B. The rate constant defining this exchange transport ( $\lambda_{B,A}$ ) ranged from  $0.024 \text{ min}^{-1}$  to  $0.103 \text{ min}^{-1}$ . Irreversible transport (oxidation and (or) storage) ranged from 198 to  $622 \mu\text{Eq/min}$ ; exchange transport ranged from 49 to  $126 \mu\text{Eq/min}$ . The exchange transport in the two women in the study appeared to represent a slightly larger fraction of the total FFA turnover (29 and 38% respectively) than was seen in the men in whom it ranged from 11 to 24% of the total FFA turnover. Since these patients also received oleic acid-<sup>3</sup>H while the others received palmitic acid-<sup>14</sup>C, the differences observed might also relate to the difference in the metabolic handling of saturated and unsaturated fatty acids previously reported by Nestel (29).

Patient M. W. was a 10 yr old female with *total lipodystrophy* (14). There was almost a complete absence of subcutaneous fat and a massively enlarged liver.

TABLE I  
Kinetic Parameters for Plasma FFA Transport after the Injection of Labeled FFA  
using Two-Compartment Analysis (Fig. 2)

Subject	Sex	Weight kg	Age yr	Plasma FFA concn $\mu\text{Eq/ml}$	Total plasma FFA pool* $\mu\text{Eq}$	FFA half-life ( $0.693/\lambda_{A,A}$ ) min	Total FFA trans- port* $\mu\text{Eq/min}$	Rate constant, irreversible ( $\lambda_{O,A}$ ) $\text{min}^{-1}$	Rate constant, exchanging ( $\lambda_{B,A}$ ) $\text{min}^{-1}$	Irre- versible trans- port* $\mu\text{Eq/min}$	Exchanging trans- port* $\mu\text{Eq/min}$
Normal lipid metabolism											
R. R.	M	84.9	18	0.70	2020	3.2	438	$0.176 \pm 6\% \ddagger$	$0.041 \pm 12\% \ddagger$	355	83
L. F.	M	79.0	19	0.70	2020	3.9	354	$0.151 \pm 6\%$	$0.024 \pm 12\%$	305	49
W. C.	M	56.1	24	0.40	1160	2.3	341	$0.224 \pm 9\%$	$0.070 \pm 15\%$	260	81
J. W.	M	54.3	22	0.45	1300	1.8	508	$0.348 \pm 3\%$	$0.043 \pm 18\%$	452	56
O. B.	M	72.4	56	0.61	1760	2.6	461	$0.214 \pm 9\%$	$0.048 \pm 12\%$	367	84
R. C.	M	57.8	54	0.70	2020	2.0	707	$0.308 \pm 6\%$	$0.042 \pm 12\%$	622	85
S. A. §	F	58.0	20	0.40	1160	2.5	318	$0.171 \pm 3\%$	$0.103 \pm 12\%$	198	120
L. B. §	F	67.9	20	0.60	1730	2.8	427	$0.174 \pm 3\%$	$0.073 \pm 12\%$	301	126
Abnormal lipid metabolism											
L. W.    (I)	M	70.0	23	0.45	1300	3.2	281	$0.106 \pm 21\%$	$0.110 \pm 18\%$	138	143
L. W. ¶ (II)	M	59.5	23	1.35	3900	7.0	386	$0.048 \pm 18\%$	$0.051 \pm 12\%$	187	199
M. W. **	F	32.2	10	1.44	4170	2.3	1242	$0.179 \pm 24\%$	$0.119 \pm 21\%$	746	496

\* Calculated from estimated total plasma volume (41.3 ml/kg) and normalized to a body weight of 70 kg.

‡ Percent standard deviation.

§ Oleic acid- $^3\text{H}$  injected instead of palmitic acid- $^{14}\text{C}$ .

|| Familial hyperlipemia, type V.

¶ Repeat study during infusion of norepinephrine.

\*\* Total lipodystrophy.

The patient died about a year after completion of this study. Whereas there was no grossly visible fat in most areas, including the omentum, some fat was present in the subclavicular region and microscopically there was chronic inflammatory infiltration and fat necrosis in periclavicular, perirenal, and peri-orbital "fat." The liver showed microdroplets of fat intracellularly. At the time of the present study, she was found to have an elevated plasma FFA concentration ( $1.44 \mu\text{Eq/ml}$ ) with a normal plasma TGFA concentration ( $4.8 \mu\text{Eq/ml}$ ). The marked elevation in FFA concentration was accompanied by a markedly elevated total plasma FFA transport,  $1242 \mu\text{Eq/min}$ . The irreversible fatty acid transport was  $746 \mu\text{Eq/min}$ , so that the amount available for tissue oxidation and/or storage was approximately twice that usually seen in the normal subjects.

Patient L. W. was a subject with a mixed fat- and carbohydrate-inducible hyperlipemia on whom two studies were performed. At the time of the first study (L. W. I), his total TGFA concentration was moderately elevated ( $13 \mu\text{Eq/ml}$ ) and lipoprotein analysis by paper electrophoresis showed a faint chylomicron band and a well defined prebeta band (Type V) (13). Plasma FFA level was within normal limits, as was total plasma FFA transport. The data differed from those in our eight normal subjects in that a larger fraction of the FFA transport, 51% of the total, represented exchange with the extravascular compartment (average normal value from Table I,  $21\% \pm 9\%$  of the total, range 11–37%). In the second study (L. W., II), the patient was given

a constant intravenous infusion of norepinephrine at a rate of  $0.2 \mu\text{g/kg}$  per min for 40 min before the infusion of labeled FFA was begun, and norepinephrine was continued for the duration of the study (about 3 hr). As expected, the norepinephrine infusion elevated plasma FFA levels, from  $0.6 \mu\text{Eq/ml}$  before infusion to  $1.35 \mu\text{Eq/ml}$  at the time the tracer study was begun. The FFA level remained at about  $1.45 \mu\text{Eq/ml}$  during the 1st hr of the study and then fell gradually during the final 2 hr, to  $1.10 \mu\text{Eq/ml}$  at the end. As shown in Table I, under the influence of norepinephrine, the plasma FFA pool was three times as large as it had been in the control study. However, both  $\lambda_{O,A}$  and  $\lambda_{B,A}$  were much smaller. Thus there was only a 37% increase in the total transport of FFA from the plasma. This increase in FFA transport represented only an additional  $56 \mu\text{Eq/min}$  in the exchanging transport and an additional  $49 \mu\text{Eq/min}$  flowing in the irreversible pathways for oxidation and (or) storage. Thus the rapid and marked increase in plasma FFA concentration in this patient had a very minor effect on FFA transport, in contrast to previously reported results in normal subjects, in whom less prolonged norepinephrine infusions of 15–25 min increased both FFA levels and FFA turnover to approximately comparable degrees (i.e. did not importantly affect the rate constant for FFA removal from plasma) (19).

#### Conversion of plasma FFA to plasma TGFA

The conversion of intravenously injected labeled FFA to serum TGFA was measured in seven of the studies

described above and the results were fitted to the model system shown in Fig. 5. The experimentally observed time course for plasma FFA-<sup>14</sup>C (compartment A) was

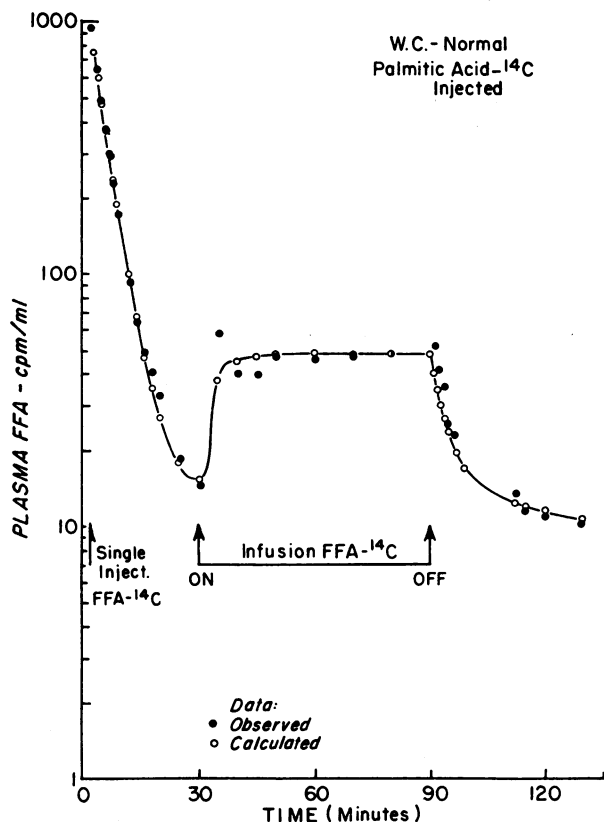


FIGURE 4 Plasma radioactivity as a function of time in a normal subject following the sequential administration of a single-injection and constant infusion of palmitic acid-<sup>14</sup>C. The closed circles represent the experimental data; the open circles represent data generated from the two-compartment model shown in Fig. 2.

simulated by the FFA model. The transport of plasma FFA into the pathway of glyceride synthesis (compartment L) is governed by the rate constant  $\lambda_{L,A}$ . There was a significant time lapse between the administration of labeled FFA and its appearance in significant concentrations as labeled TGFA (Fig. 6). To simulate this time delay with the SAAM program, five sequential compartments were used within the TG-synthesizing system (L-1 through L-5). The use of fewer sequential compartments gave unsatisfactory agreement between experimental and computer-generated data. The rate constants governing transport between successive compartments in this system were arbitrarily set equal to one another ( $\lambda_L$ ) and chosen to be compatible with the observed time delay and the shape of the TGFA radioactivity curve referred to above. Thus, for a time delay of 30 min, the mean transit time per compartment ( $1/\lambda_L$ ) averages 6 min. Since this is a mean transit time, a certain amount of FFA will traverse the five compartments with a transit time much shorter than the average. Thus, some labeled fatty acid was detectable in TGFA within 10 min of injection. The FFA entering this pathway ultimately are discharged as glycerides into a plasma compartment (C) which represents the newly synthesized plasma lipoproteins.

The experimental data for build-up and decay of TGFA radioactivity were compatible with the model shown in Fig. 5 in which plasma TGFA is regarded as a single compartment. A representative example of the closeness of fit in normal subjects is shown in Fig. 7, whereas Figs. 8 and 9 demonstrate the suitability of fit in two pathological states, lipodystrophy and familial hyperlipemia. These data illustrate the capability of the model to generate data which will fit the requirements of both normal and abnormal subjects. The  $\lambda_{C,C}$  values derived in four such studies in normal controls

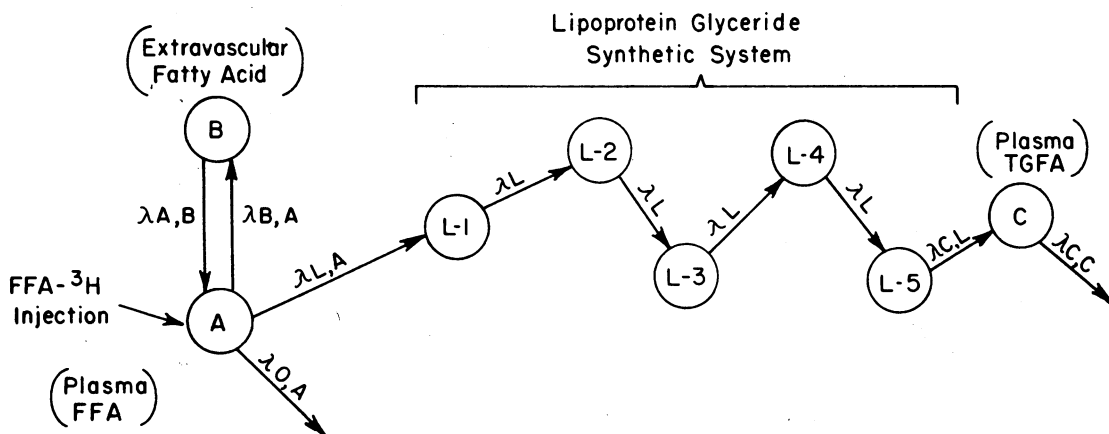


FIGURE 5 Multicompartmental model used to describe the conversion of FFA in the plasma to TGFA in the plasma.

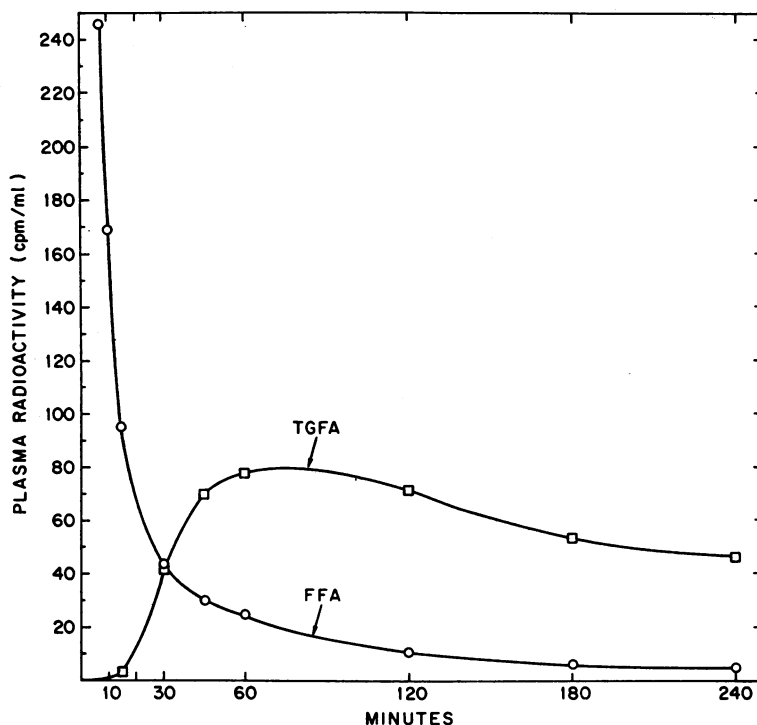


FIGURE 6 Linear plot showing disappearance of intravenously injected radioactive FFA (circles) and appearance, after a lag period, of labeled TGFA (squares) in a normal subject (L. F.).

ranged from  $0.22 \times 10^{-2}$  to  $0.78 \times 10^{-2} \text{ min}^{-1}$  (Table II, column 4). The TGFA pool size was taken to be equal to estimated plasma volume (41.3 ml/kg body wt) times measured TGFA plasma concentration. As discussed in more detail below, the calculation of glyceride transport ( $\mu\text{Eq}/\text{min}$ ) from the computed  $\lambda_{c,c}$  is an approximation because there were actually several kinetically distinct TGFA components contained in the fraction analyzed. The largest fraction of radioactivity is probably associated with the very low density fraction (4, 7, 8) which contains most of the plasma glyceride. However, the different lipoprotein species not only contain differing amounts of glyceride but also demonstrate different turnover rates (7, 8, 28). In the four control subjects estimated TGFA transport ranged from 25 to 81  $\mu\text{Eq}/\text{min}$ . As discussed later, these values are probably somewhat high because the total TGFA was treated as a single pool.

Patient M. W., the 10 yr old girl with lipodystrophy, had a plasma TGFA level in the normal range and the calculated TGFA transport, 26  $\mu\text{Eq}/\text{min}$  is in marked contrast with the grossly elevated FFA transport (1242  $\mu\text{Eq}/\text{min}$ ) (Table I).

In the first study in the hyperlipemic subject (L. W. I), the fractional turnover rate of plasma TGFA was extremely small, too small to allow assignment of a definite

value with any precision from data covering the 4 hr period of observation. The computer-calculated rate constant was  $0.0 \pm 0.592 \times 10^{-3} \text{ min}^{-1}$  (half-life probably greater than 500 min; the negative value has no physical reality). In the second of the two studies (L. W. II), carried out during a constant intravenous infusion of norepinephrine, the plasma TGFA concentration was higher at the beginning of the study (20  $\mu\text{Eq}/\text{ml}$ ) and rose during 4 hr of norepinephrine infusion to a final value of 27  $\mu\text{Eq}/\text{ml}$ . Again the rate constant for TGFA transport was too small to be determined with any precision ( $0.0 \pm 0.765 \times 10^{-3} \text{ min}^{-1}$ ). Nevertheless, these results are adequate to indicate that in the fasting state the half-life for TGFA turnover in this patient is *at least* 3–10 times greater than the normal.

In all of these studies an estimate for  $\lambda_{L,A}$  was obtained, indicating the rate at which FFA enter the TGFA synthetic pathway and emerge in plasma TGFA (Fig. 5). This does not include FFA taken up by the liver but *not* reappearing in plasma lipoprotein TGFA. Multiplied by the size of the plasma FFA pool, it yields a *minimum* value for TGFA output by the liver, not including TGFA formed from nonradioactive precursors. In two normal subjects this value agreed very closely with the value for total TGFA turnover calculated from  $\lambda_{c,c}$  (Table II). This would rule out any significant

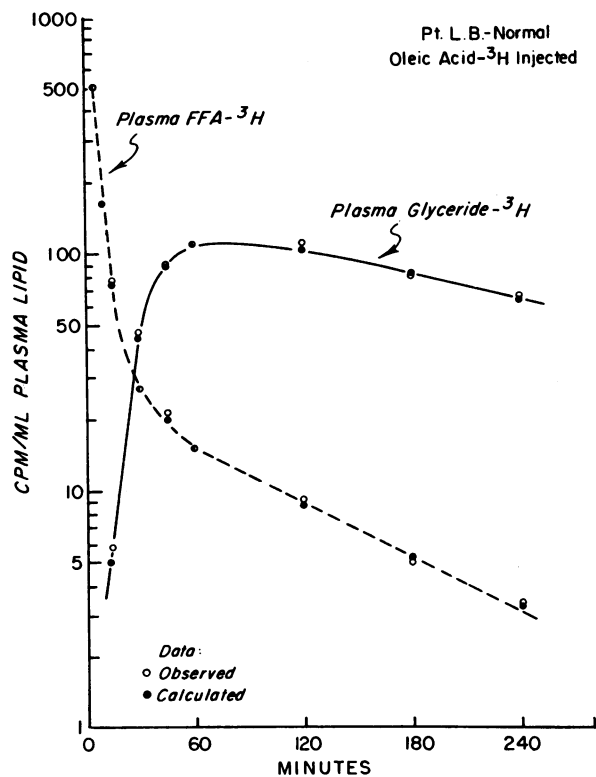


FIGURE 7 Plasma TGFA and FFA radioactivity as a function of time after the intravenous injection of oleic acid- $^3\text{H}$  into a normal subject (L. B.). The open circles represent the experimental values; the closed circles represent the data generated from computer analysis using the multicompartiment model shown in Fig. 5.

source for TGFA in these subjects other than plasma FFA. In the other two subjects, however, minimum TGFA transport calculated from  $\lambda_{L,A}$  was smaller than that calculated from  $\lambda_{C,C}$ , suggesting that these subjects were synthesizing TGFA either from precursors other than plasma FFA, or from slowly labeled FFA pools.

Using this alternative calculation, it was possible to obtain at least a minimum estimate of TGFA turnover in the Type V hyperlipemic subject even though  $\lambda_{C,C}$  was indeterminate. As shown in Table II, the total TGFA turnover in this subject was at least as high as that in control subjects ( $44 \mu\text{Eq}/\text{min}$ ). Under the influence of norepinephrine, this increased dramatically, up to  $90 \mu\text{Eq}/\text{min}$ . Dividing these values by the total plasma TGFA pool allows an estimation of the minimum rate constant for TGFA transport which was not resolvable in model 5. Thus in L. W. I, this was 0.00117 giving a minimum half-life of 592 min, while during the norepinephrine infusion (L. W. II) the rate constant for TGFA turnover was 0.00155 giving a minimum half-life of 462 min for the total TGFA pool.

The mean transit time for fatty acids through the five-compartment synthetic pathway was similar in all subjects, normal and abnormal (Table II); 31–40 min in the normal subjects; 32 min in the subject with lipodystrophy; 48 min in the hyperlipemic subject under comparable conditions, and 32 min in the same subject during infusion of norepinephrine. These values represent the mean value of the transit time for the fatty acids but label can be detected as early as 10 min after FFA- $^3\text{H}$  injection, as shown in Fig. 6.

#### Conversion of plasma glyceride-FA to plasma FA

In the model used for determining transport of TGFA (Fig. 5), all losses from the plasma TGFA (compartment C) were pooled together and described by a single rate constant  $\lambda_{C,C}$ . An attempt was next made to evaluate the extent to which recycling of TGFA to FFA occurred by dividing the outflow from compartment C between an irreversible outflow ( $C \rightarrow O$ ;  $\lambda_{O,C}$ ) and an additional outflow returning TGFA to the plasma FFA pool ( $C \rightarrow A$ ;  $\lambda_{A,C}$ ). However, the results obtained with computer analysis indicated that the data were inadequate to determine this.

In a further attempt to evaluate recycling of TGFA to FFA, the experimental design was altered by introducing lipoproteins labeled in vitro with triolein- $^{14}\text{C}$  subsequently referred to as in vitro-labeled TGFA. With this experimental design, the FFA pool is initially free of radioactivity and recycling of labeled fatty acids to the FFA pool can be more sensitively evaluated. At the same time, this method provided an independent measure of the values for TGFA turnover.

The serum lipoproteins of two normal subjects were labeled in vitro with triolein- $^{14}\text{C}$  as described under Methods. Approximately  $1.2 \mu\text{c}$  of lipoprotein-bound triolein- $^{14}\text{C}$  was injected into each subject and the radioactivity in the plasma glycerides and FFA was analyzed at intervals for 240 min.

The decay curve for in vitro-labeled TGFA remained first-order for 4 hr in one patient and showed a biphasic disappearance in the other (Figs. 10 and 11, bottom frames). In both patients the initial volume of isotope dilution calculated from the ordinate intercept and injected dose was equal to the plasma volume. In both studies radioactivity first appeared in the plasma FFA pool after about 10 min and a well defined curve of accumulation and decay was obtained. As discussed under Methods, control studies showed that contamination of the FFA fraction by radioactive TGFA was less than 0.2% with the techniques used. Thus, the data indicate the presence of a pathway between the plasma TGFA and the plasma FFA. A kinetic model was developed to include such a pathway.

The kinetic parameters governing the behavior of the FFA were independently determined in these same experiments by simultaneously injecting  $^3\text{H}$ -labeled oleic acid along with the  $^{14}\text{C}$ -labeled, triolein-containing lipoproteins. Knowing the kinetic parameters governing the FFA fraction determined concurrently in this way, it was possible to analyze the data for  $^{14}\text{C}$ -labeled FFA generated from the injected triolein- $^{14}\text{C}$  and estimate the magnitude of this pathway.

Adding a single exchanging pool equilibrating with plasma TGFA allowed a satisfactory fit of the biphasic decay of the in vitro-labeled TGFA. However, the characteristics of the curve for the appearance of  $^{14}\text{C}$ -labeled FFA in the plasma could only be matched if the in vitro-labeled TGFA were considered to be made up of at least two independent components, not exchanging with each other, and both contributing to the plasma FFA. Furthermore, to allow for the observed 10 min time lapse before the first appearance of  $^{14}\text{C}$ -labeled FFA in the plasma, a two-compartment delay line had to be

introduced. A model including these features, shown in Fig. 12, provided a satisfactory fit of the data from both normal subjects (see Figs. 10 and 11), except that in subject L. B. the calculated values for build-up of FFA- $^{14}\text{C}$  during the first 15 min of the experiment exceeded the observed values by about 15%. This small inconsistency does not affect significantly the fit of the remainder of the data.

One of the two TGFA compartments called for by the model (C-II in Fig. 12) had a very short half-life, 7.5 min in one subject (S. A.) and 23.6 min in the other (L. B.), and contained only 15% of the total injected radioactivity present in the in vitro-labeled TGFA. This component in the model is difficult to relate to any defined normal lipoprotein fraction in the plasma. In the preparation of the in vitro-labeled TGFA, any labeled chylomicrons were removed by ultracentrifugation before injection of the labeled plasma into the patients. Since studies in man have indicated that the half-lives of the remaining lipoproteins are in excess of 30 min and

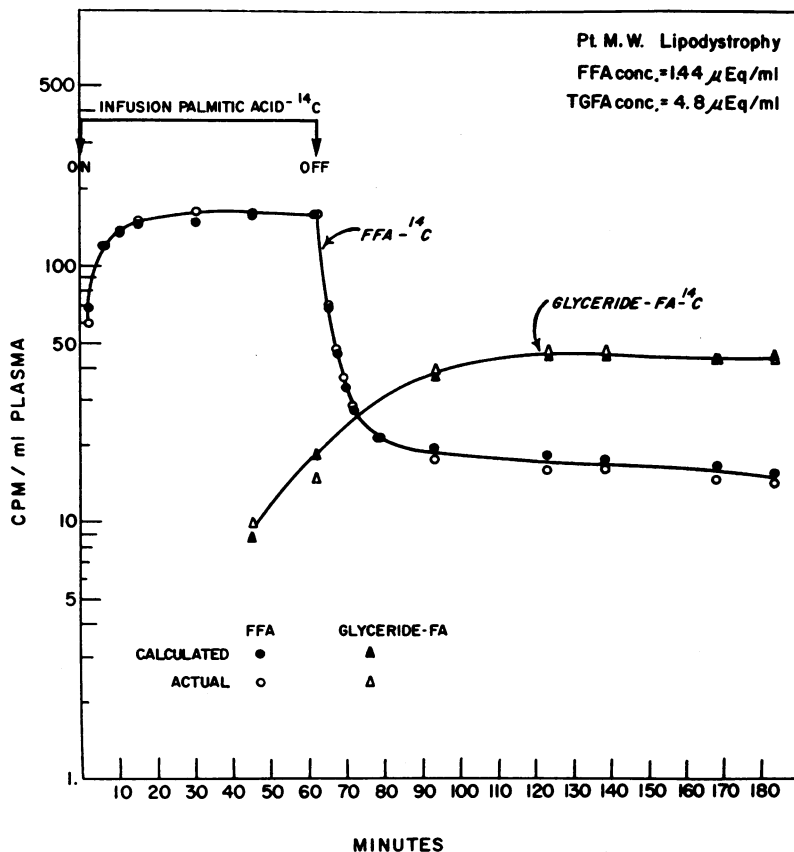


FIGURE 8 Plasma TGFA and FFA radioactivity as a function of time following the intravenous infusion of palmitic acid- $^{14}\text{C}$  into a patient (M. W.) with lipodystrophy. The open symbols represent the experimental values; the closed symbols represent the data generated from the computer analysis using the multicompartamental model shown in Fig. 5.

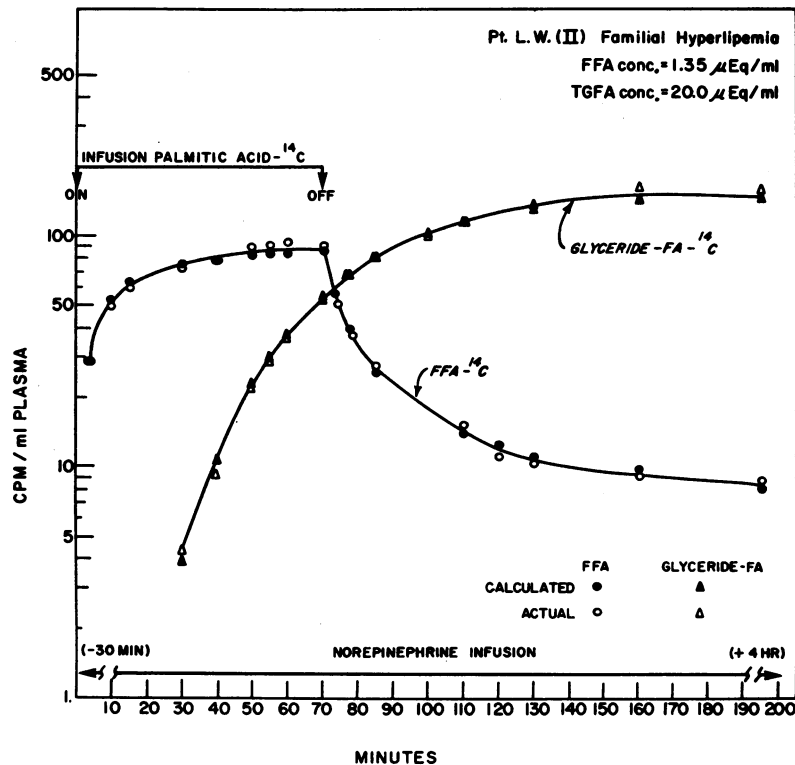


FIGURE 9 Plasma TGFA and FFA radioactivity as a function of time following the intravenous infusion of palmitic acid- $^{14}\text{C}$  into a patient with familial hyperlipemia during an infusion of norepinephrine (L. W. 11-see text). The open symbols represent the experimental values; the closed symbols represent the data generated from the computer analysis using the multicompartmental model shown in Fig. 5.

may in fact exceed 200 min, component C-II does not appear to correspond to any of these lipoproteins (7, 8). The possibility that component C-II reflects an artifact introduced by the preparative procedure must be considered. A likely possibility, in view of the recognized instability of the plasma lipoproteins, would be labeled triolein bound to a partially denatured lipoprotein. This conclusion seems consistent with the resolution of the model obtained by using the additional data derived from the concomitant injection of FFA- $^3\text{H}$  in these patients. In neither patient was component C-II required in the model to obtain a satisfactory fit of the data describing synthesis of TGFA and its degradation back to plasma FFA. When the computer solution was constrained to insist upon the inclusion of component C-II, the results indicated that in neither patient could this pool correspond to more than 6% of the total stable TGFA pool. Therefore, it was concluded that component C-II could justifiably be considered only as a portion of the in vitro-labeled TGFA; that it should receive no stable or labeled material in the model from the plasma FFA pool; and that it should contribute no stable fatty acid to the degradation pathway returning fatty acid

to the plasma FFA pool. In this way the inclusion of component C-II into the model allows us to correct the experiment for a potential labeling artifact, so that the conversion of plasma glyceride-FA to plasma free-FA can be evaluated.

With the assumption that component C-I might adequately account for the kinetics of the total chemically determined plasma TGFA pool and that component C-II is a portion of in vitro-labeled TGFA alone, the models used for describing the kinetics of injected in vitro-labeled TGFA (Fig. 12) and injected labeled FFA (Fig. 5) were combined as shown in Fig. 13. The final step in the pathway from plasma FFA to component I of the plasma TGFA is thus represented by a solid arrow (L-5  $\rightarrow$  C-I), and the potential pathway to component C-II (L-5  $\dashrightarrow$  C-II) is dotted to indicate that this pathway is not used in these final calculations. This over-all model was then used to determine how well it could account for all four sets of tracer data: (a) FFA- $^3\text{H}$ ; (b) TGFA- $^3\text{H}$  derived from FFA- $^3\text{H}$ ; (c) TGFA- $^{14}\text{C}$ ; and (d) FFA- $^{14}\text{C}$  derived from TGFA- $^{14}\text{C}$ . The experimental data and the model-generated data are compared graphically in Figs. 10 and 11. The kinetic param-

TABLE II  
Kinetic Parameters for Plasma TGFA Transport after the Injection of Labeled FFA  
using Multicompartment Analysis (Fig. 5)

Subject*	Plasma TGFA concn	Total plasma TGFA pool*	Rate constant for TGFA transport ( $\lambda_{c,c}$ )	Half-life	Total TGFA transport* ( $\lambda_{cc} \times$ total plasma TGFA pool)	Rate constant for FFA going to TGFA synthesis ( $\lambda_{L,A}$ )	TGFA transport* from plasma FFA ( $\lambda_{LA} \times$ plasma FFA pool)	Mean delay during TGFA synthesis
	$\mu\text{Eq/ml}$	$\mu\text{Eq}$	$\text{min}^{-1} \pm \text{SD}$	$\text{min}$	$\mu\text{Eq/min}$	$\text{min}^{-1}$	$\mu\text{Eq/min}$	$\text{min}$
Normal lipid metabolism								
R. R.	4.1	11,860	$0.401 \times 10^{-2} \pm 25\%$	169	48	$0.0296 \pm 7\%$	59	$36 \pm 6\%$
L. F.	3.8	11,000	$0.216 \times 10^{-2} \pm 11\%$	321	25	$0.0141 \pm 11\%$	28	$40 \pm 6\%$
S. A.†	3.4	9,850	$0.779 \times 10^{-2} \pm 13\%$	89	77	$0.014 \pm 18\%$	16	$31 \pm 10\%$
L. B.‡	5.0	14,400	$0.558 \times 10^{-2} \pm 7\%$	126	81	$0.023 \pm 12\%$	41	$33 \pm 7\%$
Abnormal lipid metabolism								
L. W. (I)	13.0	37,600	$0.0 \pm 0.592 \times 10^{-3}$	>500	not resolvable	$0.034 \pm 5\%$	44	$48 \pm 3\%$
L. W. (II)	20.0	57,800	$0.0 \pm 0.765 \times 10^{-3}$	>500	not resolvable	$0.023 \pm 6\%$	90	$32 \pm 6\%$
M. W.	4.8	13,900	$0.190 \times 10^{-2} \pm 75\%$	365	26	$0.0049 \pm 11\%$	20	$32 \pm 10\%$

\* See footnotes to Table I.

† Oleic acid- $\text{H}^3$  injected instead of palmitic acid- $\text{C}^{14}$ .

eters used to generate the theoretical results shown in these figures are listed for TGFA metabolism in Table III and for FFA metabolism in Table IV. Stable input into compartment A must balance loss from the system defined by the measured plasma pool sizes of FFA and TGFA and their rate constants as determined from the isotopic data alone. In other words, a solution was required to satisfy both the tracer data and the steady-state concentrations of both FFA and TGFA.

Subject L. B. was found to have a plasma concentration of TGFA 47% greater than that of subject S. A. (plasma pool 14,400  $\mu\text{Eq}$  in the former; 9250  $\mu\text{Eq}$  in the latter). In spite of this difference, the estimated total TGFA transport was 56  $\mu\text{Eq/min}$  in both patients, with half-lives of 177 min and 121 min, respectively.

The computer-derived rate constant for total TGFA transport was in both subjects smaller when calculated from the combined oleic acid- $\text{H}^3$  and triolein- $\text{C}^{14}$  data and the uncertainty in the values was greater. Thus the  $\lambda_{c,c}$  for subject L. B. derived from the oleic- $\text{H}^3$  acid data was  $0.0056 \pm 7\%$  (Table II); calculated from the combined data it was  $0.0039 \pm 20\%$  (Table III). The fact that in both subjects the rate constant was smaller when calculated from the combined data is compatible with the possibility that a larger fraction of the triolein- $\text{C}^{14}$  added in vitro was taken up in the more slowly metabolized beta and alpha lipoprotein fractions. As discussed under Methods, the HDL fraction (alpha lipoprotein on paper electrophoresis) did contain at least 14–20% of

the total triolein radioactivity taken up in vitro, more than is found in HDL during the early phases of an FFA injection study (7).

The calculated values for transport of fatty acid from TGFA to the plasma FFA compartment (C-I via  $D_1$  and  $D_2$  to A) were 35 and 38  $\mu\text{Eq/min}$  in these patients, i.e., over 60% of total transport. The values for irreversible transport from plasma TGFA (tissue oxidation and/or storage) were 21 and 18  $\mu\text{Eq/min}$ .

The parameters for FFA metabolism determined from the same general model are shown in Table IV. Subject L. B. had an FFA concentration 50% larger than that in subject S. A. In both subjects the FFA half-life was about 2.8 min. The transport of FFA into the lipoprotein-glyceride synthetic system of 40  $\mu\text{Eq/min}$  in subject L. B. balances reasonably well with the total TGFA transport of 56  $\mu\text{Eq/min}$  (Table III), which suggests that in this case plasma FFA could account for most of the precursor fatty acid used in lipoprotein triglyceride synthesis. In subject S. A. the calculated FFA input into the glyceride synthetic system (16  $\mu\text{Eq/min}$ ) was less than one-half the total TGFA transport (56  $\mu\text{Eq/min}$ ) (Table III). However, it should be noted that the latter figure was only known to within  $\pm 30\%$ , i.e., there was a 2 out of 3 possibility that the true value might be as low as 39  $\mu\text{Eq/min}$ . Still in both cases total TGFA transport was larger than FFA input, suggesting the input of additional precursor sources.

## DISCUSSION

The present studies were undertaken to explore the possibility of evaluating free fatty acid and triglyceride transport in man by the use of small doses of precursor FFA- $^{14}\text{C}$  and by sampling only the plasma compartment. Use of computer techniques made it feasible to simulate the observed data with multicompartamental models, the

criteria for appropriateness being their agreement with available information about fatty acid metabolism and transport, on the one hand, and the closeness of fit of experimental and computer-generated data, on the other. Closeness of fit, as in any kinetic study of this type, establishes only that the proposed model is compatible with the observed results. The fact that the same general

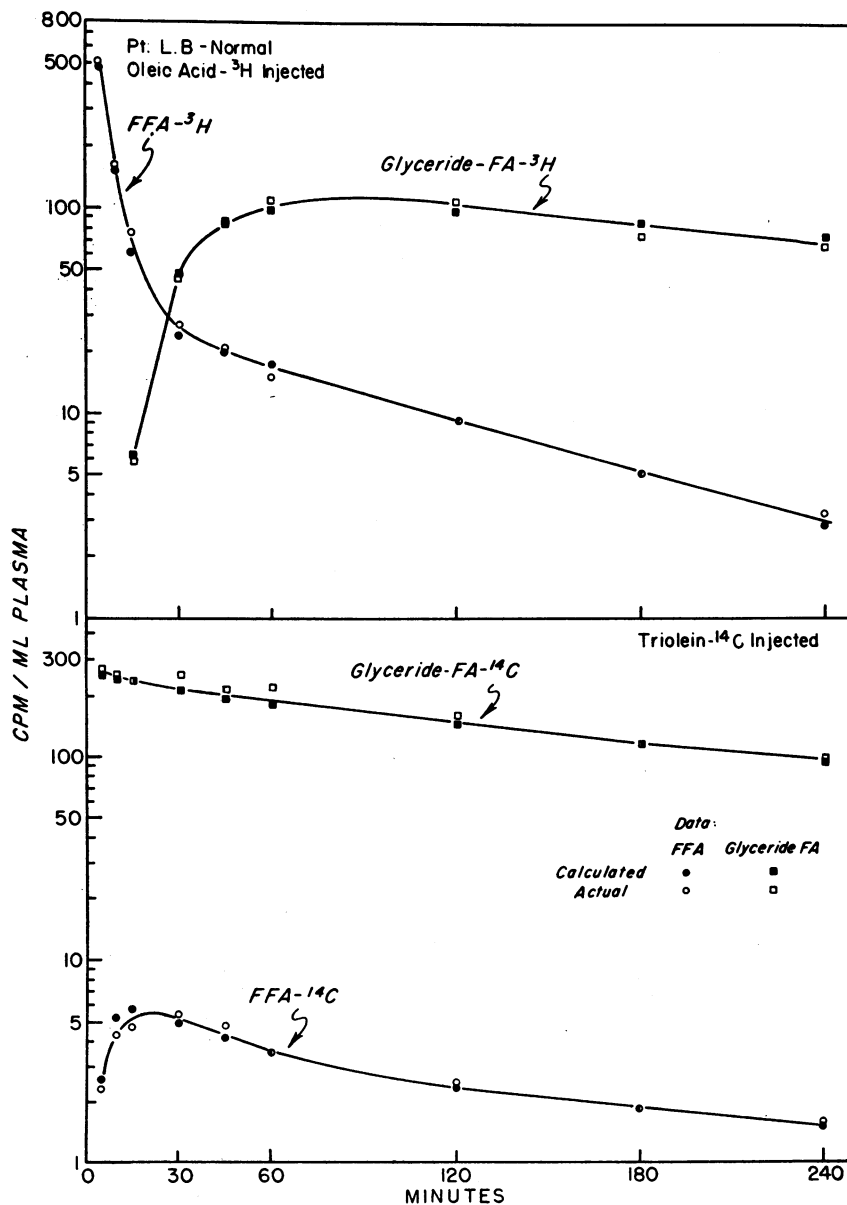


FIGURE 10 Plasma FFA and TGFA radioactivity as a function of time after simultaneous injection of radioactive FFA (oleic acid- $^3\text{H}$ ) and of lipoproteins labeled in vitro with TGFA (triolein- $^{14}\text{C}$ ) into a normal subject (L. B.). Upper panel, tritium data; lower panel,  $^{14}\text{C}$  data. Open circles, experimental values; closed circles, computer-generated data using model shown in Fig. 13.

model could be used and gave excellent fit for the data for all the subjects studied strengthens the presumption that it is an appropriate model.

Transport of TGFA was calculated in two ways. In the first, the value was calculated as the product of the rate constant for total TGFA transport ( $\lambda_{c,o}$ ) and the estimated pool size of TGFA (Table II, column 6). The TGFA pool, however, is not a single component. About 30% of the TGFA is present in HDL and LDL, both of which turn over at a much slower rate than the VLDL-TGFA. Furthermore, the HDL- and LDL-TGFA

are probably derived in part from the VLDL. The observed rate of TGFA decay,  $\lambda_{o,c}$ , probably reflects primarily the turnover of TGFA in the VLDL fraction but underestimates it to the extent that there is conversion to LDL and HDL, which were included in the TGFA analyzed. On the other hand, use of total TGFA pool (instead of the VLDL pool) in calculating transport tends to yield an overestimate since the rate constant, primarily reflecting VLDL transport, was applied to a pool larger by about 30%. We have evaluated the magnitude of error introduced by these approximations by

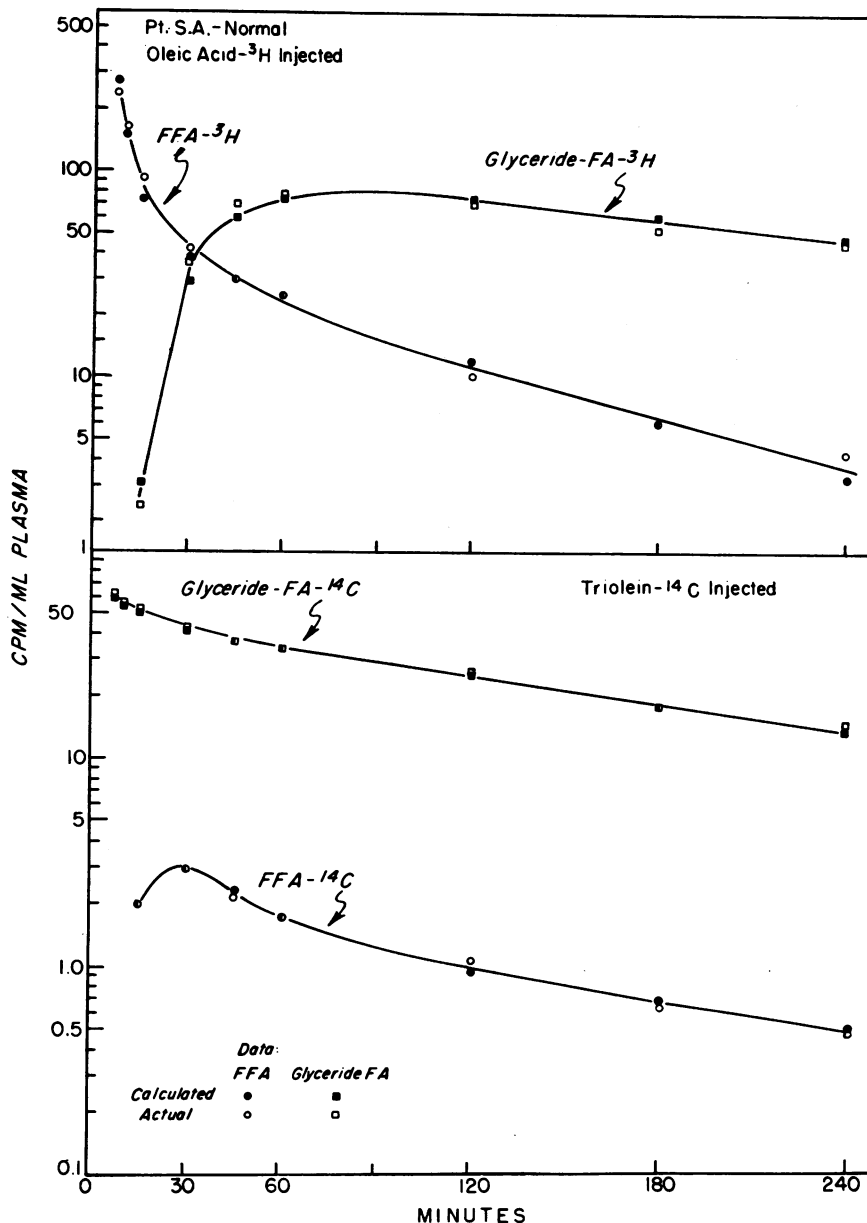


FIGURE 11 See legend to Fig. 10. Subject S. A.

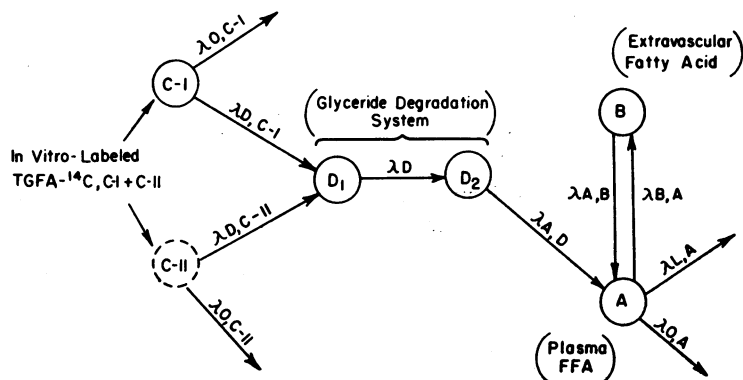


FIGURE 12 Multicompartmental model developed to be compatible with the observed kinetics for conversion of injected labeled TGFA ( $^{14}\text{C}$ -triolein-labeled lipoproteins) to FFA. As discussed in the text, inclusion of a second plasma TGFA compartment (C-II) was needed to give the optimal fit when TGFA labeled lipoproteins prepared in vitro were injected; it was not needed for fitting data when labeled FFA was injected. Compartment B represents all metabolic pools from which there is recycling of fatty acid back to the plasma FFA compartment *exclusive* of TGFA.

use of experimental data from recent studies in which HDL, LDL, and VLDL fractions were each isolated and analyzed as a function of time after injection of palmitic acid- $^{14}\text{C}$  as in the studies reported above (A. Frank, S. Quarfordt, D. Shames, M. Berman, and D. Steinberg, unpublished results). Values for total TGFA transport calculated as in the present studies and calculated from separate analysis of each lipoprotein fraction agreed within 5%.

TGFA transport was also determined from the calculated transport of FFA from plasma into the lipoprotein glyceride synthesis system ( $\lambda_{L,A} \times \text{FFA pool size}$ ) (Fig. 5). These values (Table II, column 8) represent minimum estimates for several reasons. First, it is possible that some slowly turning over FFA compartment, not detectable in a 4 hr experiment, could contribute to the transport. Second, although unlikely, there might be a TGFA component that turns over very rapidly so that

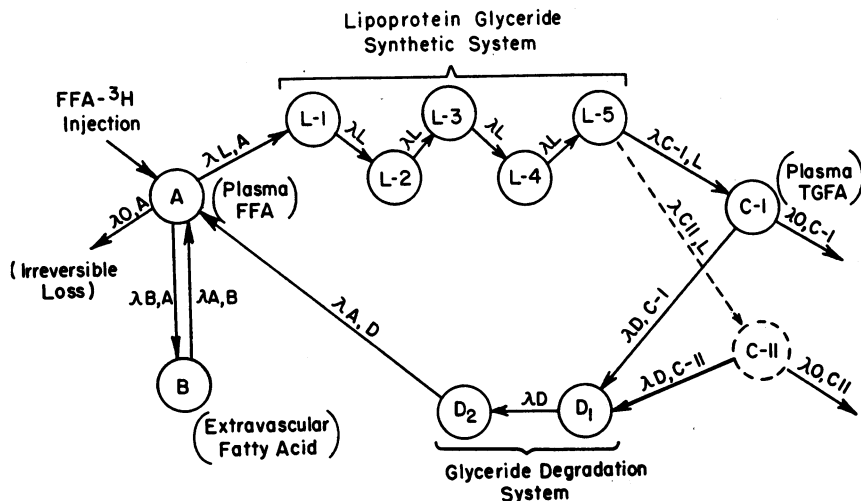


FIGURE 13 General multicompartmental model incorporating all features of the partial models previously discussed. Agreement between experimental data and data generated using this model is shown in Figs. 10 and 11. Values found for the various rate constants and pool sizes are given in Tables III and IV. The data obtained after injecting labeled FFA were fit equally well with or without compartment C-II in the model; hence the dotted line for  $\lambda_{C-II,L}$ .

TABLE III  
Kinetic Parameters for TGFA Transport after Simultaneous Injection of Lipoproteins  
Labeled *In Vitro* with TGFA-<sup>14</sup>C and of <sup>3</sup>H-Labeled FFA\*

Parameter	Subject L. B.	Subject S. A.
Plasma TGFA concentration, $\mu\text{Eq/ml}$	5.0	3.4
Plasma TGFA pool size <sup>†</sup> , $\mu\text{Eq}$	14,400	9850
Plasma TGFA half-life, <i>min</i>	177 $\pm$ 35	121 $\pm$ 36
Total plasma TGFA transport (C-1), $\mu\text{Eq/min}$	56 ( $\lambda_{C-I, C-I} = 0.0039 \pm 20\%$ )	56 ( $\lambda_{C-I, C-I} = 0.0057 \pm 30\%$ )
Recycling transport to plasma FFA (C $\rightarrow$ D $\rightarrow$ A) $\mu\text{Eq/min}$	35 $\pm$ 4 ( $\lambda_{D, C-I} = 0.0024 \pm 12\%$ )	38 $\pm$ 4 ( $\lambda_{D, C-I} = 0.0039 \pm 11\%$ )
Irreversible transport (C $\rightarrow$ 0), $\mu\text{Eq/min}$	21 $\pm$ 4 ( $\lambda_{C, C-I} = 0.0015 \pm 10\%$ )	18 $\pm$ 4 ( $\lambda_{C, C-I} = 0.0018 \pm 22\%$ )
Pool size of glyceride degradation system (D <sub>1</sub> + D <sub>2</sub> ), $\mu\text{Eq}$	96	290
Delay time in glyceride degradation system, <i>min</i>	3.5 ( $\lambda_D = 0.599 \pm 10\%$ )	19 ( $\lambda_D = 0.097 \pm 22\%$ )
Pool size of TGFA lipoprotein synthetic system <sup>§</sup> (L-1 through L-5), $\mu\text{Eq}$	1415	720
Delay time in TGFA lipoprotein synthetic system, <i>min</i>	25 ( $\lambda_L = 0.157 \pm 5\%$ )	26 ( $\lambda_L = 0.155 \pm 22\%$ )

\* Analysis according to model shown in Fig. 13. Experimental and computer-generated data shown in Figs. 10 and 11.

<sup>†</sup> Calculated from plasma volume and chemically determined TGFA, normalized for a 70 kg body weight.

<sup>§</sup> Calculated using the assumption of nonisotope input only from plasma FFA.

label reaching it is removed so quickly as to escape detection. Finally, unlabeled or slowly labeled precursors contributing to TGFA transport would not be included in such a calculation.

The rate constants calculated for total TGFA transport (Table II  $\lambda_{c,c}$ ) were defined with a mean SD of 14% (range 7–25%). The half-life values ranged from 89 to 321 min and were thus generally comparable to those reported by Friedberg, Klein, Trout, Bogdonoff, and Estes (121–530 min) using an integration method (28), and those reported by Nestel (200–300 min) using the first exponential extracted from the observed TGFA decay curve (29). Reaven, Hill, Gross, and Farquhar, using labeled glycerol and calculating from the first exponential extracted from the TGFA decay curve, found values of 72–348 min for the VLD fraction of 15 normal subjects, (30).

Estimated total TGFA transport in the present studies ranged from 25 to 81  $\mu\text{Eq/min}$ , including that portion recycling to FFA. Friedberg et al., using a quite different method of mathematical analysis, found values ranging from 20 to 65  $\mu\text{Eq/min}$  in their eight subjects (28). Carlson and Ekelund (31) concluded that over-all splanchnic net production of TGFA must be less than 100  $\mu\text{Eq/min}$  in normal man. As mentioned above, our values may err on the high side because of the weighting introduced by the relative preponderance of the very low density fraction. Our results agree in showing that

TGFA transport in man is quantitatively much less important than FFA transport and accounts for only a small fraction of over-all calorie consumption, at least in the fasting state.

In the hyperlipemic subject (L. W.), the rate constant for total TGFA transport,  $\lambda_{c,c}$ , was extremely small. The value could not be exactly defined but the available data indicated that the half-life was probably in excess of 500 min on the two occasions the patient was studied. A minimum estimate of TGFA transport could be made, however, by use of the second approach discussed above. Under basal conditions this minimum value was within the normal range. Thus, in the steady state, total lipoprotein production was not elevated, suggesting a primary defect in removal. During administration of norepinephrine, with FFA levels markedly elevated, there was an enormous increase in FFA going to TGFA, consonant with the increase in plasma TGFA from 20 to 27  $\mu\text{Eq/ml}$  during the infusion of norepinephrine. Dury and Treadwell (32) have previously reported increases in TGFA after infusions of norepinephrine in man and more recently Grasso, Michaels, and Kinsell have observed increases in plasma TGFA of hyperlipemic subjects owing to norepinephrine infusions (33).

In the patient with lipodystrophy (M. W.), the calculated half-life for TGFA was longer than that in any of the normal controls but not as strikingly so as in the case of the Type V hyperlipemic patient just discussed.

TABLE IV  
Kinetic Parameters for Plasma FFA Transport after Simultaneous Injection of Lipoproteins  
Labeled In Vitro with TGFA-<sup>14</sup>C and of <sup>3</sup>H-Labeled FFA\*

Parameter	Subject L. B.	Subject S. A.
Plasma FFA concentration, $\mu\text{Eq/ml}$	0.6	0.4
Plasma FFA pool size <sup>†</sup> , $\mu\text{Eq}$	1730	1160
Plasma FFA half-life, <i>min</i>	$2.9 \pm 10\%$	$2.7 \pm 10\%$
Extravascular FFA pool size, $\mu\text{Eq}$	$6620 \pm 8\%$	$4750 \pm 8\%$
Extravascular FFA half-life, <i>min</i>	43	29
Total plasma FFA transport, $\mu\text{Eq/min}$	418	294
	$(\lambda_{A,A} = 0.214)$	$(\lambda_{A,A} = 0.253)$
Exchange transport to extravascular FFA pool (A → B), $\mu\text{Eq/min}$	106	114
	$(\lambda_{B,A} = 0.061 \pm 10\%)$	$(\lambda_{B,A} = 0.098 \pm 8\%)$
Irreversible transport from plasma (A → 0), $\mu\text{Eq/min}$	272	164
	$(\lambda_{O,A} = 0.157 \pm 2\%)$	$(\lambda_{O,A} = 0.141 \pm 3\%)$
Transport to glyceride synthetic system (A → L), $\mu\text{Eq/min}$	40	16
	$(\lambda_{L,A} = 0.023 \pm 12\%)$	$(\lambda_{L,A} = 0.014 \pm 18\%)$
Input from extravascular fatty acid pool (B → A), $\mu\text{Eq/min}$	106	114
	$(\lambda_{A,B} = 0.016 \pm 8\%)$	$(\lambda_{A,B} = 0.024 \pm 8\%)$
Input from glyceride degradation system (D → A), $\mu\text{Eq/min}$	35	38
Input from fat depots and/or other tissue sites, $\mu\text{Eq/min}$	277	142

\* Analysis according to model shown in Fig. 13. Experimental and computer-generated data shown in Figs. 10 and 11.

† Calculated from plasma volume and chemically determined FFA, normalized for a body weight of 70 kg.

Total plasma TGFA was not abnormal and calculated transport was at the lower limits of the range in controls. It is clear that the calorie requirements in the fasting state in this patient were *not* being met by transport of TGFA. On the other hand, FFA transport was actually well above the normal range and could easily account for her calorie transport in the fasting state. The findings are similar to those reported recently by Havel, Basso, and Kane (34). As mentioned above, although the patient clinically was an example of "total lipodystrophy" some adipose tissue, albeit very little, could be demonstrated at postmortem. Barring the possibility that these patients can mobilize FFA from other tissues, it appears that what little adipose tissue they have must turn over very rapidly indeed in fasting. Only a small mass of adipose tissue would suffice. If the normal nonobese adult has about 10,000 g of stored fat and even during total fasting mobilizes 200 g of it daily, one can see that fat stores only 2% of normal might allow for brisk FFA transport at least during a short interval of fasting.

*Lipoprotein glyceride synthetic system.* Satisfactory fitting of data absolutely required the postulation of a "delay line" in the system for synthesis of plasma TGFA, i.e., a series of successive compartments (L-1 through L-5 in Fig. 5) causing a lag between injection of FFA and its appearance in plasma TGFA. A similar "delay line" was found by Baker and Schotz to be needed to fit

their data in the rat (12). In the present studies, the mean transit time or delay ranged from 31 to 40 min, while in the rat a 10 min delay gave satisfactory fitting of results (12). The available evidence strongly suggests that the liver is the primary site of synthesis of endogenous plasma TGFA (35-38), although there may be some contribution by the intestine (39, 40). However, the location and the nature of the compartments in the synthetic pathway cannot be directly inferred from kinetic studies such as these. All that can be said is that *at least* five compartments are needed to fit the data, but more complex systems would fit just as well. Actually we know that labeled FFA entering the liver must go through a series of chemically distinguishable "pools" even before it becomes TGFA (activation to the coenzyme A derivative, transacylation to form phosphatidic acid, etc.) The glyceride once formed can be shunted among pools before ultimate incorporation into a lipoprotein. Finally the lipoproteins may exist in separate pools within the liver. In short, the probability is that there are indeed more than the minimum five compartments found necessary here to fit the observed data. The calculated amount of fatty acid contained in the "hepatic" synthesis system (Table III) is clearly much less than the total liver TGFA. It has been shown that the hepatic TG pool in man and in animals is indeed heterogeneous (3-5, 7).

*Recycling of TGFA through FFA.* The present studies suggest that in man the fatty acids from endogenous plasma lipoprotein TGFA "recycle" to a significant extent through plasma FFA. The fraction of lipoprotein TGFA metabolized by this pathway was surprisingly large. In the glucose-fed rat, Laurell concluded that a significant fraction of endogenous TGFA recycled through FFA but did not assign explicit values to it (16). In the rabbit, Havel et al. calculated that endogenous plasma TGFA of density less than 1.006 was the source of approximately 10% of the circulating FFA (4). As they point out, the quantitative significance of this flow of TGFA to FFA as a pathway for removal of TGFA depends very much on the degree of equilibration between plasma and liver TGFA pools. In the case of exogenous chylomicron TGFA studied in the dog, degradation to plasma FFA was also demonstrated, and it can be calculated that as much as one-third of the TGFA may have followed this pathway (17).

In the analysis of data from the present studies involving injection of labeled FFA *alone* as precursor, the data for both TGFA and FFA were adequately fitted without a pathway from TGFA to FFA. The perturbation of the curve for FFA radioactivity due to such recycling would be too small to be defined with any accuracy in this type of study (injection of labeled FFA alone). Studies utilizing labeled glycerol as the TG precursor could not, of course, identify such a pathway, although it would be of interest to examine the kinetics of the labeled free glycerol in plasma after injecting glycerol-labeled lipoprotein TGFA.

The validity of the conclusion that the recycling of TGFA through plasma FFA demonstrated here properly reflects a physiologic pathway depends upon the extent to which the labeled lipoproteins prepared *in vitro* were handled exactly like endogenous, untreated lipoproteins. We have presented evidence suggesting that one portion of the *in vitro*-labeled material (component C-II) representing 15% of the total radioactivity of the preparation, is probably artifactual (e.g. representing denatured lipoprotein or some glyceride bound to plasma components other than the lipoproteins). This component decays more rapidly than any described lipoprotein species and constitutes a smaller portion (less than 6%) of the total plasma TGFA pool than any presently defined plasma lipoprotein species. For this reason the model shown in Fig. 11 is constructed so that this component is not used to evaluate the recycling of stable TGFA through plasma FFA. However, it is conceivable that component C-II is a real lipoprotein entity and that our experimental design was not adequate to establish its presence. Thus, the failure to identify such a component in the FFA-<sup>14</sup>C injection experiments might be attributable to the very

small amounts of FFA transported to TGFA (30  $\mu$ Eq/min) relative to the total FFA turnover (400  $\mu$ Eq/min) and to the small amount of endogenously synthesized TGFA potentially present in component C-II.

By a similar line of reasoning it is proper to question whether or not the remaining 85% of the injected *in vitro*-labeled TGFA (component C-I) behaves exactly like endogenous, untreated lipoprotein. Our evidence for its validity is indirect. This material decays with a plasma half-life (121 and 177 min) which is comparable to that determined for endogenously labeled TGFA (Table II) in our studies as well as in those of others (28-30). The volume of distribution of the material in both subjects was equal to the plasma volume and the model was thus constrained by this factor. The radioactivity was found to be distributed between the VLDL, LDL, and HDL fractions and this separation was found to be altered by the method of fractionation and/or the time taken to perform the fractionation. In a similar fashion the distribution of labeled fatty acid in these lipoprotein species labeled endogenously by prior injection of radioactive FFA is known to vary with time of sampling (i.e. the distribution being a function of relative rates of synthesis and degradation of the individual lipoprotein species). We conclude that this preparation may reasonably be used as a tracer in the study of TGFA metabolism, but that conclusions based solely upon its use should be regarded as tentative. Hopefully, further studies with individual lipoprotein fractions, similarly labeled *in vitro*, may help to establish the validity of this preparation for physiologic studies.

A further qualification applies to the absolute values calculated for recycling of TGFA to FFA. The computer analysis determines primarily the array of rate constants ( $\lambda$  values) that best fit the tracer data. As mentioned above, the transport values ( $\mu$ Eq/min) were calculated on the basis of the approximation that the plasma TGFA could be considered as a single compartment. This we know is not valid even though the observed data in these relatively short-term studies were satisfactorily described by such a one-compartment plasma TGFA model.

The mechanism by which the plasma TGFA may recycle through plasma FFA deserves comment. In the absence of heparin, it is unlikely that circulating levels of lipoprotein lipase activity can account for this on the basis of direct intravascular TGFA to FFA conversion. On the other hand, if the TGFA were first taken up into tissues and mixed with any large stores of fatty acids there before reentering the plasma, the FFA curves after injection of TGFA should have reflected this. The values for the calculated sizes of compartments  $D_1$  and  $D_2$  (Fig. 12) should have been large, but the calculated values were only about 25 mg and 75 mg, respectively, in

the two studies done in this way. Moreover, the transit time through these compartments was very short, 3.5 and 19 min respectively. That there is some delay suggests again that direct intravascular lipolysis of circulating TGFA is not an adequate explanation; that there is so little dilution and so short a transit time forces one to consider the possibility of lipolysis at the capillary surface or at the cell surface. This might entail some minor accumulation at the surfaces to create a kinetically defined compartment and thus a slight delay before appearance of FFA in the general circulation. One is reminded of the evidence that lipoprotein lipase is at or near the capillary endothelium (41).

*Extravascular FFA.* Analysis of the decay of plasma FFA-<sup>14</sup>C after the injection of labeled FFA alone requires the presence of a large exchanging compartment of fatty acid, designated as pool B (Fig. 2). Kinetically, this compartment is a distinct entity, described to a tolerance of  $\pm 20\%$ . Many different anatomical compartments may be represented by pool B including the extracellular space, the intracellular space in some tissues, and the lymphatic system. The movement of FFA into this large exchanging pool accounts for some 30% of the total plasma FFA transport. Thus, the actual maximum delivery of fat calories by way of plasma FFA is significantly lower than that estimated on the basis of the more common method of deriving a rate constant directly from the initial rate of decay. Thus instead of some 440  $\mu\text{Eq}/\text{min}$ , approximately 360  $\mu\text{Eq}/\text{min}$  at most are available for tissue oxidation (Table I). The plasma TGFA described in the general model (Fig. 13, Table III) contributes only an additional 20  $\mu\text{Eq}/\text{min}$  of fatty acid available for immediate tissue utilization, a small contribution beyond that available from FFA alone.

*General remarks.* One of the important advantages of the computer method used in the present study is that it permits assessment of the confidence limits within which each parameter is determined (9, 11). The ability to assign confidence limits proved especially useful in connection with the attempts to quantify recycling of TGFA to FFA. In most cases these confidence limits were fairly narrow, and it is clear that the proposed model is a suitable one. Of course, the actual system may be more complex but it cannot be simpler.

The data obtained in the two patients with disordered lipid metabolism were reasonably well fit using the general model, implying that their abnormalities lay in alterations of rates but without introduction of new abnormal pathways. Of course, it may be that in other disease states additional compartments or pathways will prove necessary to obtain appropriate fitting of data. Barring this possibility, it would appear that the model proposed could be useful in evaluating alterations in lipid transport in disease.

## ACKNOWLEDGMENT

We thank Dr. David M. Shames and Dr. Arthur Frank for helpful discussions on interpretations and analysis, Dr. Donald S. Fredrickson for making available for study the two patients with abnormal lipid metabolism, and Mr. William Briner for sterile preparation of substrates for injection.

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