Biochem. J. (1979) **184**, 73–81
Printed in Great Britain

Metabolism of Palmitate in Perfused Rat Liver

COMPUTER MODELS OF SUBCELLULAR TRIACYLGLYCEROL METABOLISM

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(*Received* 18 *May* 1979)

1. In the preceding paper [Kondrup (1979) *Biochem. J.* **184**, 63–71] the separation of two major fractions of hepatic triacylglycerol was described. One fraction contained triacylglycerol from the endoplasmic reticulum and from the Golgi apparatus. The other fraction contained triacylglycerol from the cytoplasmic lipid droplets. In the present paper possible precursor–product relationships between the two fractions were investigated by means of computer models. 2. The fatty acids present in di- and tri-acylglycerol in the fractions isolated in the time studies were analysed by gas chromatography. From this analysis the relative specific radioactivities, and contents, of palmitate in acylglycerols in the two fractions at the various time points were calculated. 3. A computer was used to predict relative specific radioactivities of pools in defined models of hepatic triacylglycerol metabolism. The acceptability of the models was evaluated by comparing predicted with measured relative specific radioactivities. 4. It is suggested that triacylglycerol in cytoplasmic lipid droplets does not originate (*a*) directly from triacylglycerol in the endoplasmic reticulum, (*b*) from a sub-pool of it or (*c*) directly from non-esterified fatty acids entering the cell. Rather, it is formed from diacylglycerol (and acyl-CoA) in the endoplasmic reticulum. Diacylglycerol, on the other hand, is furnished in part by hydrolysis of triacylglycerol in the endoplasmic reticulum. 5. This suggestion is discussed in relation to previous models of hepatic fatty acid metabolism.

Two subcellular pools of hepatic triacylglycerol have been separated and partially characterized with respect to purity and function (Kondrup, 1979). One pool, isolated in a high-speed pellet, contained triacylglycerol from the endoplasmic reticulum and the Golgi apparatus and was suggested to be the precursor for secreted triacylglycerol. The other pool was isolated as a floating-fat fraction dissolved in diethyl ether and contained lipid from the cytoplasmic lipid droplets. Morphological evidence suggests that triacylglycerol is formed in the endoplasmic reticulum and then deposited in the cytoplasmic lipid droplets (Stein & Stein, 1967). However, others have not been able to confirm these observations (Jones *et al.*, 1967).

Computer calculation on the basis of isotope experiments can be used to test possible precursor–product relationships in intermediary metabolism. This technique has been applied also to lipid metabolism in intact rats (Nikkilä *et al.*, 1966; Baker & Schotz, 1967; Haude *et al.*, 1972). Fitting of the isotope data in these studies necessitated the introduction of more than one hepatic pool of triacylglycerol. However, the existence of these pools was not verified experimentally. In the present study the metabolism of palmitate was investigated in the isolated perfused liver and two major pools of triacylglycerol were isolated. Further, in the previous studies one labelled species of fatty acids was injected and the calculations were based on all fatty acids present. It is known now that acylglycerols with different composition of fatty acids are metabolized differently (Sundler *et al.*, 1974). In the present experiments, the liver was perfused with a high concentration of palmitate (1 mM) and the calculations were based on the metabolism of palmitate alone.

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Materials and Methods

All data used in the present paper were based on the experiments described in the preceding paper (Kondrup, 1979). Di- and tri-acylglycerol in the high-speed pellet and triacylglycerol in the diethyl ether fraction were isolated by t.l.c., extracted from the silica gel and hydrolysed as described previously (Kondrup, 1979). The fatty acids were methylated in a closed glass ampoule by reaction with 25 μl of methanol/conc. H_2SO_4 (15:1, v/v) for 3h at 100°C and analysed by radio gas chromatography. A fully

automatized Perkin–Elmer 990 gas chromatograph, equipped with a 3 m column (4 mm internal diameter) packed with diatomite M-NAW (J. J. Chromatography, Kings Lynn, Norfolk, U.K.) coated with 12.5% diethylene glycol succinate, was used. The column effluent was split into two portions (1:9, v/v) and 90% was led through a column (700°C) of CuO to combust fatty acid to CO_2, mixed with 30% methane and counted for radioactivity in a 80 ml flow-through counter. The amount of palmitate was calculated from the estimated percentage of palmitate and from the total mass of fatty acids in that fraction. The total of fatty acids was calculated by multiplication of the content of glycerol in acylglycerols by 2 or 3 (Kondrup, 1979). The specific radioactivity of palmitate was calculated from the percentage of radioactivity in palmitate, the total amount of radioactivity in the fraction and from the amount of palmitate in the fraction (Kondrup, 1979).

For calculation of predicted specific radioactivities a computer program was constructed for calculation of flow between pools that change size constantly with time. For pool no. i, the change in size (Q) is given by:

$$\frac{dQ_i}{dt} = \sum V_{i_{in}} - \sum V_{i_{out}} \quad (1)$$

where $\sum V_{i_{in}}$ is the sum of all velocities into the pool and $\sum V_{i_{out}}$ is the sum of all velocities out of the pool.

If it is assumed that Q_i is a linear function of time, i.e.

$$Q_i = a_i t + b_i \quad (2)$$

(an assumption with which our results are compatible), then the velocities are constants and satisfy the linear constraints:

$$\sum V_{i_{in}} - \sum V_{i_{out}} = a_i \quad (3)$$

the number of which is equal to the number of pools. Consequently, some velocities, in all the total number of velocities minus the number of pools, may be chosen freely, whereafter the remaining velocities are determined from eqn. (3).

The radioactivity of pool no. i, q_i, is given by:

$$\frac{dq_i}{dt} = \sum V_{i_{in}} S_p - \sum V_{i_{out}} S_i \quad (4)$$

where S_p is the specific radioactivity of the precursor pool, p, and S_i is the specific radioactivity of pool no. i.

If q_i is replaced by $S_i Q_i$ in eqn. (4), the resulting factor dQ_i/dt is eliminated by means of eqn. (1) and the following equation emerges:

$$Q_i \frac{dS_i}{dt} = \sum V_{i_{in}} (S_p - S_i) \quad (5)$$

Eqn. (5) constitutes a set of linear differential equations, the solution of which is determined by the known initial values of the specific radioactivities at time $t = 0$. These equations are solved numerically by the method of Gear (1971).

It is noteworthy that the incorporation of radioactivity is not assumed to follow first-order kinetics, whereas it is assumed that molecules entering pool no. i have the same specific radioactivity as all molecules in the precursor pool(s).

The computer input consisted of: (1) the number of pools; (2) the number of velocities and their direction (structure of the model); (3) the number and magnitude of the freely chosen velocities; (4) the measured size of the pools at each time point; and (5) the relative specific radioactivity of exogenous [1-^{14}C]palmitate (= 100). The computer calculated the other dependent velocities from the given variations in pool size and also calculated the expected relative specific radioactivity of the pools at each time point according to all velocities. To avoid meaningless solutions some restrictions had to be laid on the choice of velocities and also on the acceptability of dependent velocities. Negative velocities were not accepted; other restrictions are discussed below. A Univac 1100 computer was used.

Results and Discussion

Gas-chromatographic analysis

Table 1 shows that at the earliest time point about 85% of the fatty acids in di- and tri-acylglycerol in the high-speed pellet was palmitate and that the percentage was fairly constant with time. The percentage in triacylglycerol in the diethyl ether fraction was considerably lower and increased with time. This difference serves as a further characterization of the two pools and supports the view that triacylglycerol in the high-speed pellet had a faster turn-over than triacylglycerol in the diethyl ether fraction (Kondrup, 1979). Apparently the liver can synthesize di- and tri-acylglycerol consisting almost exclusively of palmitate. The same has been reported with respect to diacylglycerol in hepatocytes (Sundler *et al.*, 1974). In the computer calculations that were based on the content of palmitate, it was therefore assumed that di- and tri-acylglycerol were formed from two or three molecules of palmitate respectively.

Linear regression

Fig. 1 shows the rates of accumulation of palmitate in the different fractions. When the computer formulated the differential equations specific for the observed accumulation of palmitate, it was presumed that the size of the pools changed constantly with time and the calculated r values justified this assumption.

Overall metabolism of palmitate

According to the results of Fig. 1 palmitate

Table 1. *Palmitate as a percentage of total fatty acids in di- and tri-acylglycerol in subcellular fractions isolated from perfused rat liver*

Livers from fed female rats were perfused with 1 mM-palmitate for 26 min and then with 1 mM-[1-^{14}C]palmitate for the periods indicated. Fatty acids in di- and tri-acylglycerol in the subcellular fractions were extracted and analysed by gas chromatography as described in the text. The results are presented as meaans ± S.D. ($n = 3$).

	Palmitate (mol/100 mol of fatty acid)		
	High-speed pellet		
	Diacylglycerol	Triacylglycerol	Diethyl ether-fraction triacylglycerol
10 min	85 ± 1	87 ± 1	58 ± 9
21 min	82 ± 3	85 ± 2	60 ± 7
60 min	86 ± 1	90 ± 3	74 ± 16
90 min	82 ± 3	85 ± 1	75 ± 8*

*$P < 0.05$ compared with values at 10 and 21 min.

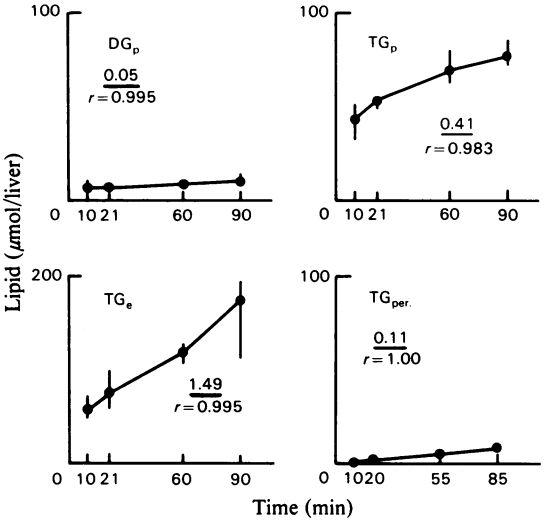

Fig. 1. *Accumulation of palmitate in di- and tri-acylglycerol in rat liver perfused with 1 mM-palmitate*

The content of palmitate was calculated by multiplying the content of acylglycerol in each fraction by 2 or 3 and by the individual percentage of fatty acids accounted for as palmitate. Fatty acids secreted as triacylglycerol from the liver were not analysed by gas chromatography. It was, however, assumed that the fatty acid composition of secreted triacylglycerol was similar to that in the high-speed pellet (as was the relative specific radioactivity). Therefore the accumulated secretion of palmitate at each time point was calculated by multiplying the mean secretion rate of glycerol in acylglycerol (Kondrup, 1979) by 3, by 0.85 (Table 1) and by time. The intrahepatic results are presented as the mean for three observations at each time point and the bars indicate the range of the observations. The values underlined are the slopes resulting from linear regression analysis of the means, expressed as μmol/min per liver. r values are the corresponding regression coefficients. Calculations were based on 12 individual experiments, three at each time point. Abbreviations used: DG$_p$, diacylglycerol in the high-speed pellet; TG$_p$, triacylglycerol in the high-speed pellet; TG$_e$, triacylglycerol recovered in the diethyl ether fraction; TG$_{per.}$, triacylglycerol recovered in the perfusate medium.

accumulated in total triacylglycerol at a rate of (0.41 + 1.49 + 0.11) = 2.01 μmol/min per liver. The incorporation of [1-^{14}C]palmitate into these fractions was (0.85 + 1.18 + 0.05) = 2.08 μmol/min per liver (Kondrup, 1979). Therefore secretion of palmitate was accepted as the only net exit of palmitate from hepatic triacylglycerol in the models. Fatty acids accumulated in these fractions at a total rate of (0.48 + 1.59 + 0.13) = 2.2 μmol/min per liver (Kondrup, 1979), which may suggest that non-palmitate fatty acids contributed to the formation of triacylglycerol at a rate of about 0.2 μmol/min per liver (see below). Di- and tri-acylglycerol in the low-speed pellet accounted for a negligible proportion of the total content and radioactivity of acylglycerols and were not included in the system. The incorporation of [1-^{14}C]palmitate into diacylglycerol in the high-speed pellet was 0.06 μmol/min per liver (Kondrup, 1979).

The content of phospholipid did not change significantly with time (Kondrup, 1979), and it was therefore taken to be constant at 516 μmol of fatty acids per liver, which is the mean of the estimations at the four time points. In the models, the flow of palmitate into phospholipid therefore had to be equalled by an exit of fatty acids from the phospholipid pool. The possible fate of these fatty acids is commented on below. Phospholipid recovered in the low-speed pellet accounted for a considerable proportion of the total content and radioactivity of phospholipid and was therefore included in the system. The incorporation of [1-^{14}C]palmitate into phospholipid in the two pellets was 0.36 μmol/min per liver (Kondrup, 1979).

The incorporation of [1-^{14}C]palmitate into lipids included in the system thus amounts to 2.5 μmol/min per liver, which accounts for 95% of the incorporation of [1-^{14}C]palmitate into all glycerolipids in all fractions (Kondrup, 1979); 2.5 μmol/min per liver was used as the inflow of exogenous palmitate into the system.

Relative specific radioactivity

The percentage of radioactivity in palmitate accounted for 98–100% of the total radioactivity, with traces present in the oleate fraction. Fig. 2 shows the measured relative specific radioactivities of palmitate at each time point.

The relative specific radioactivity of diacylglycerol rose rapidly, suggesting a fast turnover of precursors such as intracellular non-esterified fatty acids, acyl-CoA esters and phosphatidate. The same pattern, although much less pronounced, was seen in the relative specific radioactivity of triacylglycerol in the high-speed pellet. By contrast the relative specific radioactivity of triacylglycerol in the diethyl ether fraction and in the perfusate rose with some delay, suggesting that these pathways either had precursor pools of a significant size and/or that the flow of labelled palmitate was delayed by transport processes.

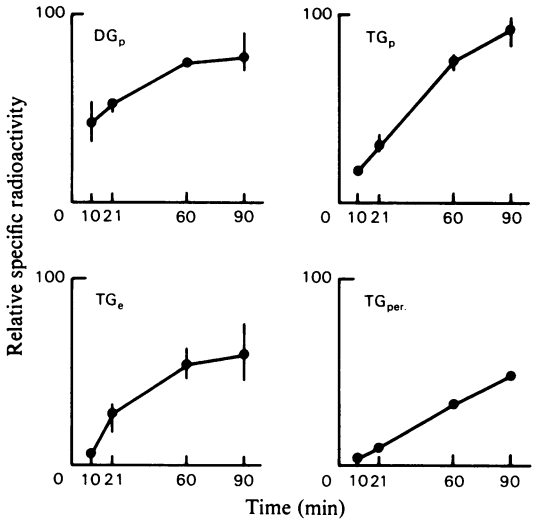

Fig. 2. *Relative specific radioactivities of palmitate in di- and tri-acylglycerol in rat liver perfused with* 1 mM-[1-^{14}*C*]-*palmitate* (= 100)

The relative specific radioactivity of palmitate in acylglycerols in each liver was calculated by dividing the amount of [1-^{14}C]palmitate present in each fraction (Kondrup, 1979) by the total amount of palmitate in that fraction (Fig. 1). The relative specific radioactivity of accumulated secreted triacylglycerol was calculated by interpolation of the results obtained at the time points indicated in Fig. 5 in the preceding paper (Kondrup, 1979). The calculation was corrected according to the assumption that palmitate accounted for 85% of the fatty acids in secreted triacylglycerol (cf. Fig. 1). The results concerning the intrahepatic pools are presented as means for three experiments for each time point. The bars indicate the range of the results for each time point. Abbreviations are defined in the legend to Fig. 1.

The relative specific radioactivity of diacylglycerol rose to a maximum of about 78 after 90 min of perfusion, whereas triacylglycerol in the high-speed pellet at the same time had a relative specific radioactivity of 96. Assuming that triacylglycerol formed in the high-speed pellet consisted of two palmitate molecules (from diacylglycerol) with a relative specific radioactivity of 78, and one exogenous palmitate molecule, with a relative specific radioactivity of 100, this would lead to a triacylglycerol molecule with a relative specific radioactivity of 85. This is considerably lower than the measured values and suggests that triacylglycerol was formed from a fraction only of diacylglycerol in the high-speed pellet. The heterogeneity of diacylglycerol was also suggested by the finding that at 60 min, 2.34 μmol of dipalmitoylglycerol in the high-speed pellet was unlabelled and at 90 min 2.39 μmol was unlabelled. When considering the flux through diacylglycerol (about 2 μmol/min per liver: see below) it is unlikely that about 20% of a single pool would remain unlabelled. Therefore, in the models to be presented, there are two pools of diacylglycerol to allow for these observations. The size of one 'unlabelled' pool was taken to be 2.37 μmol of palmitate. As a speculative suggestion for the role of this pool it was taken to be in equilibrium with the phospholipid pool, thus mimicking diacylglycerol associated with membrane phospholipid (Allan & Michell, 1977). It was therefore accepted that the 'unlabelled' pool of diacylglycerol was calculated to have a relative specific radioactivity equal to that of phospholipid, i.e. 7 at 90 min. The size of the main pool was taken to be the remainder of diacylglycerol.

The relative specific radioactivity of phospholipid in the low- and high-speed pellets was similar at each time point, which justifies phospholipid being included in the system as one pool. Fatty acids in phospholipid were not analysed by gas chromatography.

Test of models

Model A. The pools are designated for convenience as follows: DG_{p1}, diacylglycerol pool 1 in the high-speed pellet; DG_{p2}, diacylglycerol pool 2 in the high-speed pellet; TG_p, triacylglycerol in the high-speed pellet; TG_e, triacylglycerol in the diethyl ether fraction; and $TG_{per.}$, triacylglycerol secreted by the

liver and recovered in the perfusate medium. A pool, representing acyl-CoA esters of undetermined size and specific radioactivity, was inserted. The size was taken to be 0.3 μmol/liver (Kondrup & Grunnet, 1973). Because of this small size it had no significance in the models A, B and C. In relation to model D it is commented on below.

In a computer model study of hepatic fatty acid metabolism in intact rats (Haude *et al.*, 1972) a small pool of triacylglycerol with a rapid turnover was shown as the precursor to a larger pool with a much slower turnover. Stein & Shapiro (1959) demonstrated in a radioactive isotope study with intact rats that in the liver there is a small triacylglycerol pool, rapidly turning over and localized in the microsomal fraction, which equilibrates with a larger pool, recovered in the floating-fat fraction. In model A (Fig. 3) we examined whether triacylglycerol recovered in the high-speed pellet could be the precursor for that in the diethyl ether fraction.

The inflow of exogenous palmitate was given as

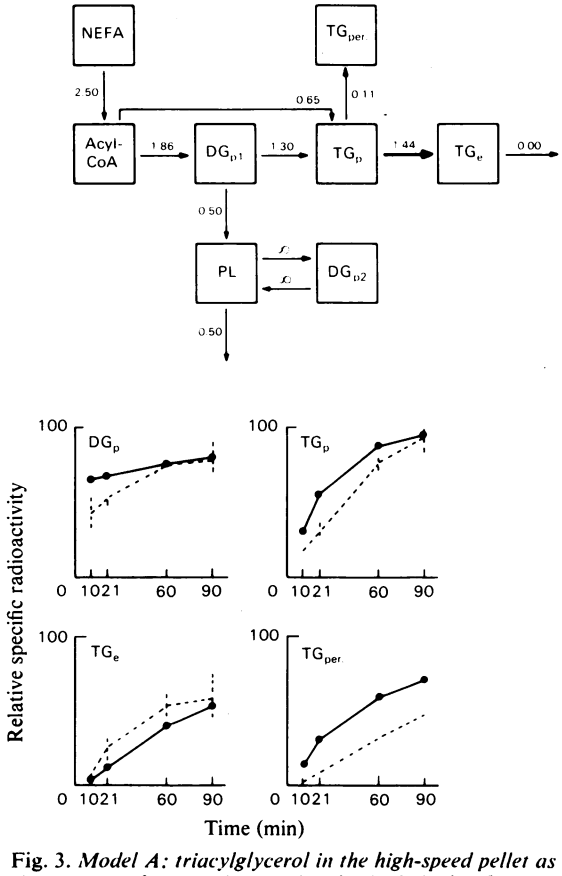

Fig. 3. *Model A: triacylglycerol in the high-speed pellet as the precursor for triacylglycerol in the diethyl ether fraction*

Abbreviations used: NEFA, external non-esterified fatty acids; PL, phospholipids. The evidence for the model and other abbreviations are explained in the text under 'Model A' and also in the legend to Fig. 1. The values represent the flux of palmitate, expressed as μmol/min per liver. The fluxes presented gave the best fit to the experimental data. From the relative specific radioactivity of exogenous palmitate (= 100) the computer calculated the relative specific radioactivities presented in the lower part of the figure as solid lines. For comparison the measured relative specific radioactivities (broken lines) are shown, with the range of observations at each time point.

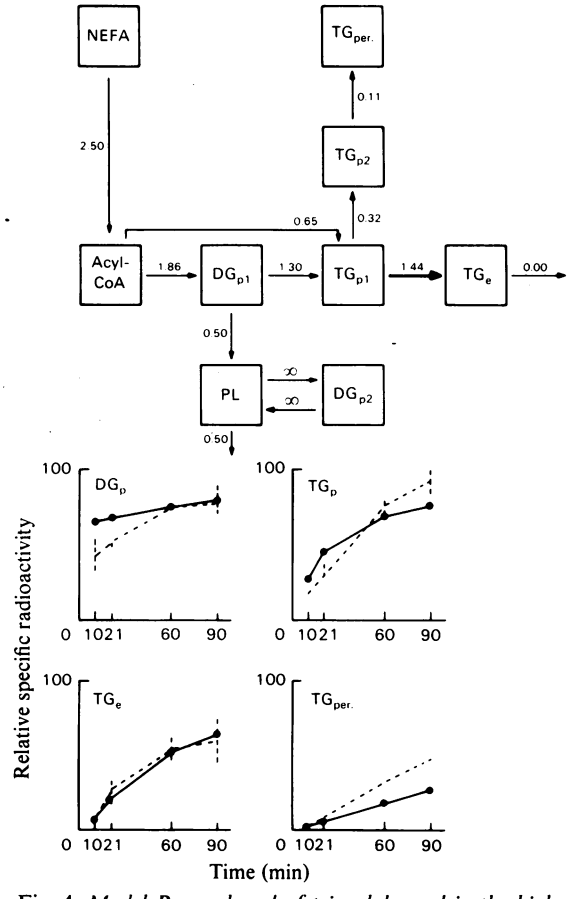

Fig. 4. *Model B: a subpool of triacylglycerol in the high-speed pellet as the precursor for triacylglycerol in the diethyl ether fraction*

Model A was changed as explained in the text. The computer calculated fluxes and relative specific radioactivities are from the same data as in model A. Calculated specific radioactivities are presented as solid lines and the measured values are presented as broken lines. Abbreviations used are defined in the text under 'Model A' and in the legends to Figs. 1 and 3.

discussed above. A ratio of 2:1 between the flow of palmitate from pool DG_{p1} and acyl-CoA to triacylglycerol was kept fixed. The export of triacylglycerol was 0.11 μmol/min per liver as determined chemically (Fig. 1). Inflow to phospholipid was adjusted to a value that gave the observed rate of incorporation of radioactivity. It emerges from these restrictions, combined with the measured variations in pool size, that the velocities in the model could vary within narrow limits only.

The infinite velocity between phospholipid and pool DG_{p2} reflects the equilibrium suggested above. (In the calculations a value of 2 μmol/min per liver was chosen.) The calculated specific radioactivities of pools DG_{p1} and DG_{p2} were re-calculated to give the specific radioactivity of one combined diacylglycerol pool, to make a direct comparison with the values measured.

This model evidently gave a rather poor fit to the measured specific radioactivities. Pools DG_p, TG_p and $TG_{per.}$ became too radioactive at the early time points. The specific radioactivity of pool TG_e rose too slowly indicating that pool TG_e was formed from a pool with a higher specific radioactivity than pool TG_p.

Model B. A more radioactive precursor pool could be a subpool of triacylglycerol in the high-speed pellet. Pool TG_p probably was a heterogeneous pool, since the high-speed pellet contained several types of organelles. Fig. 4 shows a model similar to model A, but containing two pools for TG_p (TG_{p1} and TG_{p2}). The size of pool TG_{p1} was varied until the calculated relative specific radioactivities of pool TG_e fitted the ones measured. In this way, a subpool of pool TG_p is postulated as a precursor pool for pool TG_e, and it is then examined how the other data fit into such a postulate. The specific radioactivities of pools TG_{p1} and TG_{p2} calculated by the computer were re-calculated to specific radioactivities for the combined pool TG_p to allow a direct comparison with the measured values. As can be seen in Fig. 4 only a poor fit was obtainable with respect to pools DG_p, TG_p and $TG_{per.}$.

Model C. It has been suggested that triacylglycerol in cytoplasmic lipid droplets in heart tissue may be synthesized directly from non-esterified fatty acids entering the cell (Christiansen, 1975). This possibility was investigated in the model depicted in Fig. 5. It is identical with model A, except that palmitate is directly incorporated into pool TG_e. In this model radioactivity rose too slowly in pool TG_p and too fast in pool TG_e.

Model D. In the previous models it was obvious that pool DG_{p1} became radioactive too fast compared with the measured data, even when pool DG_{p1} was not supplying fatty acids to pool TG_e. Also, when the size of pool DG_{p1} was taken to equal the total amount of diacylglycerol (pools $DG_{p1}+DG_{p2}$) its

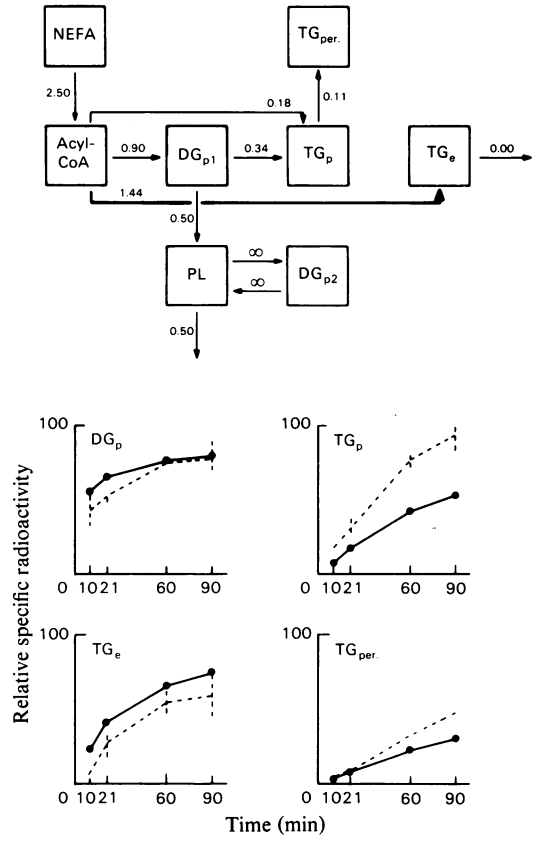

Fig. 5. *Model C: exogenous fatty acids as the precursor for triacylglycerol in the diethyl ether fraction*
Model A was changed as explained in the text. The computer calculated fluxes and relative specific radioactivities from the same data as in model A. Calculated specific radioactivities are presented as solid lines and the measured values are presented as broken lines. Abbreviations used are defined in the text under 'Model A' and in the legends to Figs. 1 and 3.

calculated specific radioactivity increased too fast. This suggests that the specific radioactivity of acyl-CoA (representing the precursors for pool DG_{p1}) was too high when equal to that of the infused palmitate. We therefore tried to replace acyl-CoA by a larger pool representing intracellular non-esterified fatty acids. The size of this pool was taken to be 7 μmol/liver, assuming that the intracellular fatty acid concentration was equal to the concentration in the perfusion medium (1 mM). This had no effect on the calculated specific radioactivity of diacylglycerol, probably because the flow through 'intracellular non-esterified fatty acids' (2.5 μmol/min per liver) was high compared even with that pool size. Similarly

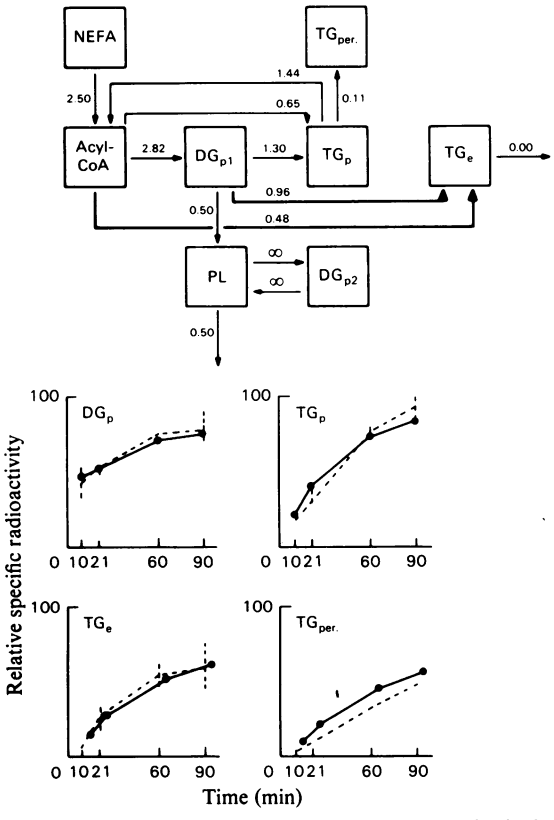

Fig. 6. *Model D: hydrolysed triacylglycerol in the high-speed pellet as the precursor for triacylglycerol in the diethyl ether fraction*

Model A was changed as explained in the text. The computer calculated fluxes and relative specific radioactivities are from the same data as in model A. Calculated specific radioactivities are presented as solid lines and the measured values are presented as broken lines. Abbreviations used are defined in the text under 'Model A' and in the legends to Figs. 1 and 3.

insertion of a pool representing phosphatidate into the model would have no effect, when assuming a content of about 1 μmol/liver (Åkesson *et al.*, 1970). Therefore there appeared to be some other unlabelled source of palmitate furnishing acyl-CoA and pool DG_{p1}. This other source could not remain unlabelled during the whole experimental period since the relative specific radioactivity of pool TG_p could not increase to about 100 if the other source continuously contributed significant amounts of unlabelled palmitate. Therefore a pool, unlabelled at the beginning and highly labelled at the end of the experiment, was looked for. The unlabelled pool had to have a considerable size to obtain sufficiently low specific radioactivities of pool DG_{p1} at 10 and 21 min. Pool TG_p was tested, as depicted in Fig. 6.

When this modification was tried with a direct pathway from pool TG_p to pool TG_e (as in model A), the calculated specific radioactivities of pool TG_p remained too high, whereas those of pool TG_e were too low. Therefore the model was further elaborated as shown in Fig. 6. Pool TG_e is formed at the same rate as in model A, but the precursors are acyl-CoA and pool DG_{p1} (in the ratio 1:2). In this model pool TG_p serves as a precursor pool for pool TG_e, albeit indirectly via acyl-CoA, which is formed from pool TG_p at a rate similar to the formation of pool TG_e.

The observed 5 min delays in the appearance of label in pools TG_e and $TG_{per.}$ (Kondrup, 1979) have also been included in model D. The relative specific radioactivities of pools TG_e and $TG_{per.}$ calculated by the computer were put into the model with delays of 5 min. In this way the delays reflect transport processes in addition to dilution in precursor pools.

With this model the computer-calculated relative specific radioactivities fitted within the ranges of the measured values except in two cases; pool DG_p at 60 min and pool TG_p at 21 min. Pool $TG_{per.}$ cannot be directly compared because the relative specific radioactivity of triacylglycerol in the perfusate medium was estimated at the different time points only in the three livers perfused for 90 min. At this last time point the range of the measured relative specific radioactivities was 80–110% of the mean value and the computer calculated a value that was 108% of the measured mean. The calculated relative specific radioactivities of phospholipid were 0.6, 1.3, 4.4 and 6.9 at the four time points compared with the measured values of 0.6, 1.7, 4.5 and 6.7 respectively.

Model E. Model E (Fig. 7) is similar to model D, only pool TG_p is partly directed to pool DG_{p1} as well as to acyl-CoA (also in a ratio of 2:1). This model fits the experimental data as well as model D.

Discussion of models D and E

Radioactive label in phosphatidate was included in phospholipid in the present investigation, which is an inaccuracy. However, it was considered to be of little importance. As discussed above, it would be of no significance to insert phosphatidate between acyl-CoA and pool DG_{p1}, because of its small pool size. Likewise phosphatidate probably accounted for little of the total radioactivity in phospholipid at the later time points (about 5% at 90 min, assuming 100% labelling of both fatty acids in phosphatidate and a constant content of 1 μmol/liver).

The flux of palmitate into phospholipid in models D and E is shown to be 0.5 μmol/min per liver. Without efflux this would lead to an accumulation of $0.5 \times (26+90) = 58$ μmol of palmitate in phospholipid, which would add about 10% to phospholipid initially present. This would not necessarily be detected because of the large variation in the esti-

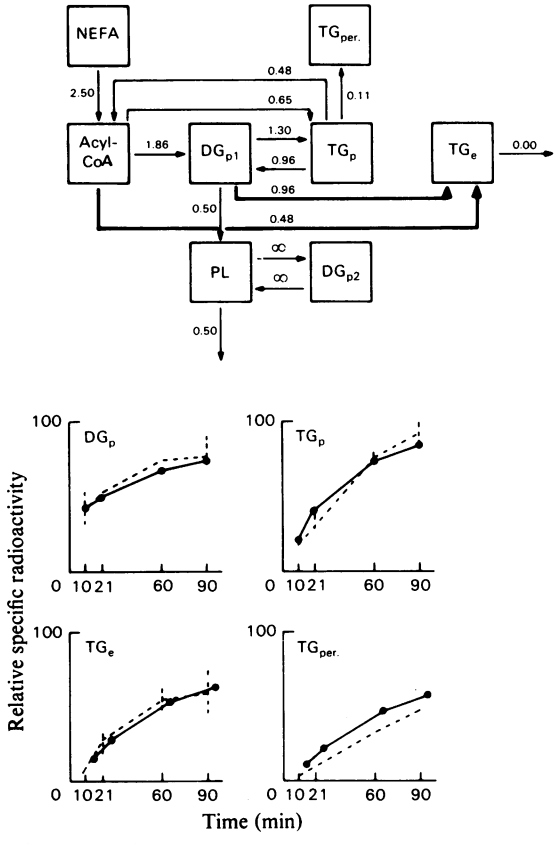

Fig. 7. *Model E: partially hydrolysed triacylglycerol in the high-speed pellet as the precursor for triacylglycerol in the diethyl ether fraction*

Model A was changed as explained in the text. The computer calculated fluxes and relative specific radioactivities from the same data as in model A. Calculated specific radioactivities are presented as solid lines and the measured values are presented as broken lines. Abbreviations used are defined in the text under 'Model A' and in the legends to Figs. 1 and 3.

mations of phospholipid (Kondrup, 1979). However, as discussed previously, the size of the phospholipid pool was taken to be constant and therefore an exit from phospholipid equal to the inflow had to be accepted. Fatty acids leaving phospholipid may be in the form of palmitate as well as other fatty acids.

It was suggested above that fatty acids other than palmitate contributed to the formation of triacylglycerol, at a rate of 0.20 μmol/min per liver. Phospholipid may have been the source of these other fatty acids. Also, phospholipid accumulated in the diethyl ether fraction at a rate equal to 0.04 μmol of fatty acids/min per liver (Fig. 4 in Kondrup, 1979). In this way 0.24 μmol of the 0.50 μmol/min per liver could be accounted for. Phospholipid secreted in conjunction with triacylglycerol was not determined, neither was phospholipid secreted into bile. These two pathways may account for a part of the remainder. The flux of fatty acids not accounted for would be (0.50−0.24)/3.21 = about 8 % of the fatty acid uptake.

In summary, this computer study is not compatible with the concept that triacylglycerol in the cytoplasmic lipid droplets is formed directly from triacylglycerol in the endoplasmic reticulum, nor from a subpool of it. In addition, diacylglycerol appears to be present in at least two subfractions, one of which does not take part in the formation of triacylglycerol or phospholipid. Diacylglycerol also appears to be furnished with palmitate from a relatively large endogenous source that becomes labelled during the experimental period. Models D and E represent a coherent interpretation of these observations and an acceptable fit between calculated and measured relative specific radioactivities.

The models suggest that excess triacylglycerol in the endoplasmic reticulum is (partially) hydrolysed and re-esterified when triacylglycerol is deposited in cytoplasmic lipid droplets. Bar-on *et al.* (1971) concluded from a study with a double-label technique that triacylglycerol in cytoplasmic lipid droplets is a precursor for secreted triacylglycerol and that triacylglycerol in the lipid droplets was hydrolysed and subsequently re-esterified in the endoplasmic reticulum (microsomal fraction) before secretion. Models D and E suggest that a similar mechanism is involved when triacylglycerol is transferred from the endoplasmic reticulum to deposition in the cytoplasmic lipid droplets.

In the endoplasmic reticulum triacylglycerol appears to be synthesized as intracisternal lipoprotein particles and subsequently transferred either to cytoplasmic lipid droplets or to the Golgi apparatus (Glaumann *et al.*, 1975). Partial hydrolysis of triacylglycerol, as suggested by model E, may be a prerequisite for transport of fatty acids in triacylglycerol through the membrane to the cytoplasmic compartment. Here triacylglycerol could be reassembled on the outside of the endoplasmic reticulum and then deposited in the cytoplasmic lipid droplets.

The models proposed by previous investigators were based on the metabolism of all fatty acids in intact rats, without separation of hepatic triacylglycerol pools. Further, they did not measure the relative specific radioactivity of diacylglycerol; the specific radioactivity of fatty acids taken up by the liver was calculated and the fatty acid uptake by the liver was not measured. Our solution is similar to the models suggested by Schotz *et al.* (1964) and by Haude *et al.* (1972) in suggesting two hepatic triacylglycerol pools, one with a fast and one with a slow turnover.

Also, both of these studies included delay pools to allow for the delayed appearance of radioactive label in secreted triacylglycerol. However, in their models the arbitrary slowly turning-over pool was 6–10-fold larger than the one with a fast turnover. In our estimations the two pools were approximately the same size at the beginning of the experiment. Further, their flow diagrams showed an outflow from the slowly turning-over pool that was 80–100% of the inflow. No metabolic fate for these fatty acids was suggested. In our models the exit from pool TG_e was calculated to be zero, i.e. the increase in pool size accounted for the total inflow. An exit from pool TG_e would imply an equally lower flux to phospholipid since the overall metabolism of the system must be kept unaltered. A lower flux to phospholipid (e.g. 0.4 μmol/min per liver) would lead to a calculated relative specific radioactivity of phospholipid at 90min that was 5.3 instead of 6.9 (measured mean value, 6.7; range, 5.5–7.3). A minor exit from pool TG_e cannot be excluded by these data, but it is unlikely to be larger than 0.1 μmol/min per liver.

Bustos (1970) labelled hepatic triacylglycerol in intact rats with [1-^{14}C]palmitate and separated triacylglycerol in microsomal fraction from that in floating fat. He also investigated the relationship between these pools by means of computer models, but could not obtain an acceptable fit between experimental and calculated data. The calculated relative specific radioactivity of triacylglycerol in floating fat was too low when microsomal triacylglycerol was taken to be the precursor pool. This is similar to the situation in our model A. As one possible explanation for the poor fit Bustos (1970) suggested that there existed an intermediate compartment, e.g. diacylglycerol (or phospholipid), between microsomal and floating-fat triacylglcyerol. Our models D and E are in agreement with this suggestion.

We thank Dr. J. Knudsen, University of Odense, for his kind co-operation in performing the gas-chromatographic analysis. The work was supported by the Danish Medical Research Council.

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