Monitoring of changes in hepatic fatty acid and glycerolipid metabolism during the starved-to-fed transition *in vivo*

Studies on awake, unrestrained rats

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1. The technique of selective labelling of hepatic fatty acids *in vivo* [Moir and Zammit (1992) Biochem. J. **283**, 145–149] has been used to monitor non-invasively the metabolism of fatty acids in the livers of awake unrestrained rats during the starved-to-refed transition. Values for the incorporation of labelled fatty acid into liver and plasma glycerolipids and into exhaled carbon dioxide after injection of labelled lipoprotein and Triton WR 1339 into rats with chronically cannulated jugular veins were obtained for successive 1 h periods from the start of re-feeding of 24 h-starved rats. 2. Starvation for 24 h resulted in marked and reciprocal changes in the incorporation of label into glycerolipids and exhaled $^{14}CO_2$, such that a 4-fold higher value was obtained for the oxidation/esterification ratio in livers of starved rats compared with fed animals. 3. Refeeding of starved rats did not return this ratio to the value observed for fed animals for at least 7 h; during the first 3 h of refeeding the ratio was at least as high as that for starved rats. Between 4 h and 6 h of refeeding the ratio was still approx. 70 % of that in starved animals, and 2.5-fold higher than in fed rats. 4. These data support the hypothesis that the capacity of the liver to oxidize fatty acids is maintained at a high level during the initial stages of refeeding [Grantham and Zammit (1986) Biochem. J. **239**, 485–488] and that control of the flux of hepatic fatty acids into the oxidative pathway is largely lost from the reaction catalysed by mitochondrial overt carnitine palmitoyltransferase (CPT I) during this phase of recovery from the starved state. 5. Refeeding also resulted in a rapid (< 1 h) increase in hepatic malonyl-CoA concentrations to values intermediate between those in livers of fed and starved animals. The sensitivity of CPT I to malonyl-CoA inhibition in isolated liver mitochondria was only partially reversed even after 5 h of refeeding. 6. Re-feeding resulted in an acute 35 % inhibition of the fraction of synthesized triacylglycerol that was secreted into the plasma; the maximal effect occurred 2–3 h after the start of refeeding. The inhibition of the fractional secretion rate was fully reversed after 5 h of refeeding. 7. The amount of ^{14}C label that was incorporated into phospholipids as a fraction of total glycerolipid synthesis was doubled within 2 h of the start of refeeding. This partitioning in favour of phospholipid synthesis was maintained for the initial 5 h of refeeding, and was followed by a rapid reversal of the effect.

INTRODUCTION

The partitioning of hepatic long-chain fatty acids between esterification to the glycerol moiety (leading to glycerolipid synthesis) and formation of acylcarnitines is an important determinant of the rate of fatty acid oxidation (and hence ketogenesis) in the liver (see [1]). The pioneering work of Mayes and Felts [2] and McGarry et al. [3] demonstrated that livers isolated from rats in different physiological states showed intrinsic differences in relative fluxes of fatty acids that are channelled into the esterification or oxidative pathways. The discoveries (i) that malonyl-CoA inhibits the overt carnitine palmitoyltransferase (CPT I) of mitochondria [4], and (ii) that the sensitivity of this enzyme to malonyl-CoA changes with physiological state [5,6], focused attention on the potentially important regulatory role that the activity of CPT I *in vivo* can play in determining the fate of intrahepatic fatty acids and especially on the amplification that the system displays [7]. In previous work [8,9] we have shown that the degree of control exerted by CPT I on the flux of fatty acids channelled to oxidation is likely to vary depending on which phase of a given ketotic episode is studied. In particular, we suggested that the strength of control exerted at the CPT I step is considerable during the induction of the ketogenic state that accompanies the onset of starvation, but that it is markedly diminished during the initial phases of refeeding [8]. These

inferences were made from the observations (i) that the sensitivity of CPT I to malonyl-CoA in mitochondria isolated from starved–refed rats takes a much longer period of time to return to the level observed in mitochondria from normal, fed animals than does the decline in blood concentrations of ketone bodies [3,10], and (ii) that the maximal activity of the enzyme (expressed per mg of mitochondrial protein) remains elevated for an even longer period [8]. Hence the determination of the flux of fatty acids to oxidation in general, and to ketogenesis in particular, was postulated to pass to other sites, both proximal (i.e. fatty acid supply to the liver through lipolysis in adipose tissue) and distal (e.g. inhibition of hydroxymethylglutaryl-CoA synthase [11,12]) to CPT I. The central implication of these conclusions was that, for a period of several hours after refeeding of starved rats, the liver retains a relatively high capacity for acylcarnitine formation, even though the flux through the pathway is necessarily much diminished due to curtailment of fatty acid supply to the liver [3,10] following the anti-lipolytic action of the feeding-induced rise in circulating insulin concentration [13]. The physiological consequences of this delay in the inhibition of fatty acid diversion towards oxidation would include the maintenance of gluconeogenesis through continued activation of pyruvate carboxylase by mitochondrial acetyl-CoA (see [8]).

Experimental proof of this hypothesis is difficult to obtain. The technique that has hitherto been available, namely the

isolation of intact livers from the animals after different periods of refeeding and their perfusion *in vitro*, necessitates the introduction of artificial conditions. Thus the perfused liver is exposed to arbitrarily chosen concentrations of fatty acids [14] and other substrates (especially lactate [3]), perfusate flow-rates and levels of oxygenation [15], all of which affect the partitioning of fatty acid metabolism. Consequently, it would be greatly advantageous to use a method that is able quantitatively to monitor the partitioning of fatty acids between oxidation and esterification in the unrestrained, awake rat *in vivo*, with the retention of all the conditions that the liver is exposed to during the progressive metabolic changes that accompany refeeding of starved rats. For such a method to be useful, the acyl-CoA pool of the liver has to be labelled selectively so that the products formed, including labelled glycerolipids retained in the liver or secreted into the plasma, as well as exhaled CO_2, are known to originate almost exclusively from hepatic metabolism or the consequences of it.

In this paper we have extended our previous work on the selective labelling of hepatic fatty acids *in vivo* [16] in order to obtain such information in a non-invasive manner. We demonstrated previously [16] that the technique yields valid data about the relative rates of synthesis and secretion of glycerolipids (in anaesthetized rats) that are entirely consistent with previous observations. We have now extended the method to include the monitoring of oxidation of the labelled hepatic fatty acids in awake, non-restrained rats, and have obtained time courses for the partitioning of the fatty acids between glycerolipid formation and oxidation. The data support the hypothesis that the capacity of the oxidation pathway to compete successfully for long-chain acyl-CoA is largely undiminished in the liver for several hours after the commencement of refeeding of starved rats.

We also show that refeeding results in the inhibition of the fractional rate of triacyglycerol secretion by the liver and in a 2-fold increase in the proportion of the total glycerolipid synthesized that appears in the phospholipid fraction.

MATERIALS AND METHODS

Animals

Animals used as recipients of cholesteryl [1-^{14}C]oleate- and [^{3}H]cholesteryl oleoyl ether-labelled lipoproteins were female Wistar rats (160–180 g) which were fed *ad libitum* on a standard laboratory rat diet (56% carbohydrate, 19% protein, 3% fat; Special Diet Services, Edinburgh, U.K.). They were each surgically fitted with a jugular cannula under pentobarbitone anaesthesia and allowed to recover for 7–9 days before being used for an experiment. Cannulae were flushed twice daily with citrated saline and the animals regained their normal food intake during this period. Donor animals were male rats (400–600 g) that were given a 10% fructose solution to drink for the 48 h period before being used for the preparation of remnant lipoproteins.

Measurement of relative rates of fatty acid esterification and oxidation

Apolipoprolein C-poor remnant lipoproteins ($d < 1.015$) were obtained from fructose-fed rats as described previously and labelled with cholesteryl [1-^{14}C]oleate and [^{3}H]cholesterol oleoyl ether [13]. An aliquot (0.25 ml) of lipoprotein solution was injected into the animals through the jugular cannula at the start of the period to be monitored. The animals were then placed in

a chamber (1.5 l) through which air was drawn at the rate of 5 l/min. Where appropriate, the rats had access to food while in the chamber and water was available throughout. Collection of $^{14}CO_2$ was by passing the air through 50 ml of a mixture containing ethanolamine and ethylene glycol monomethyl ether (1:2, v/v). After 15 min, the animals were injected (through the cannula) with 1 ml per 200 g body weight of a 10% solution of Triton WR 1339 as described previously [16]. Two series of experiments were performed. In the first series, time courses were obtained for the appearance of label in exhaled $^{14}CO_2$ and [^{14}C]glycerolipids in the liver and plasma of animals. These experiments showed that no $^{14}CO_2$ was exhaled by any of the animals during the first 15 min after injection of label. At the end of the 3 h period the animals were removed from the chamber, anaesthetized, and a sample of blood obtained from the aorta. The left lateral lobe of the liver was immediately freeze-clamped. Samples of other tissues (including spleen, mixed hind leg muscle, heart, parametrial adipose tissue) were also taken and frozen in liquid nitrogen. Parallel time courses for the incorporation of label into glycerolipids were obtained from other animals that were sampled at the times indicated in Figure 1. In subsequent
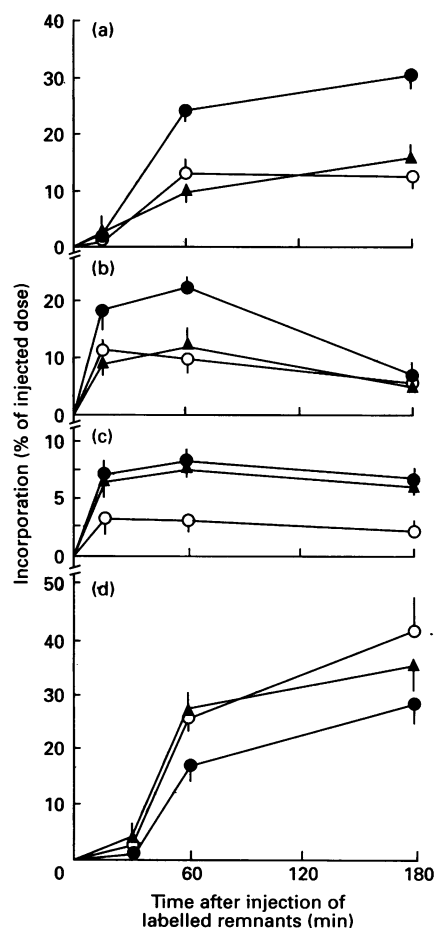

Figure 1 Time courses for the appearance of ^{14}C label in (a) plasma triacylglycerol, (b) liver triacylglycerol, (c) liver phospholipid and (d) exhaled CO_2 in rats injected intravenously through a jugular cannula with cholesteryl [1-^{14}C]oleate-labelled remnants at zero time

Animals were either fed *ad libitum* (●), starved for 24 h (○) or 24 h-starved and refed for 1 h (▲) prior to injection of label. The parameters were measured over the subsequent 3 h as described in the methods section. Values are means (± S.E.M.) for 3 to 5 separate animals for each physiological state.

Table 1   Incorporation of ^{14}C label into hepatic and plasma triacylglycerol and phospholipids 60 min after injection of remnants labelled with cholesteryl [1-^{14}C]oleate into fed, 24 h-starved and starved–refed rats

Values are means ± S.E.M. for the number of different animals shown in parentheses. The parameters are expressed as a percentage of the total net hepatic hydrolysis of cholesteryl [^{14}C]oleate during this period.

| | Incorporation (%) | | | |
| | Liver | | Plasma | |
	Triacylglycerol	Phospholipid	Triacylglycerol	Phospholipid
Ad libitum fed (5)	25.8 ± 1.6	9.8 ± 1.3	32.1 ± 3.8	0.5 ± 0.2
24 h-starved (4)	13.1 ± 1.4	5.8 ± 1.1	19.6 ± 1.9	0.3 ± 0.2
Refed 0–1 h (5)	11.6 ± 1.0	8.3 ± 0.8	12.0 ± 1.8	0.2 ± 0.2
Refed 1–2 h (5)	16.6 ± 3.4	12.6 ± 1.1	10.3 ± 2.2	0.2 ± 0.1
Refed 2–3 h (4)	14.6 ± 1.8	12.4 ± 2.9	12.2 ± 1.9	0.3 ± 0.2
Refed 3–4 h (4)	16.5 ± 1.4	13.9 ± 1.7	15.6 ± 3.7	0.2 ± 0.2
Refed 5–6 h (4)	18.4 ± 0.9	16.6 ± 4.9	18.5 ± 4.5	0.3 ± 0.1
Refed 7–8 h (3)	21.4 ± 2.9	11.6 ± 0.9	27.3 ± 4.1	0.3 ± 0.2

experiments the incorporation of label into $^{14}CO_2$ and glycerolipids was measured over initial 1 h periods after injection of label during sucessive periods of refeeding.

Extraction of total lipids and separation of the different lipid classes by t.l.c. were performed as described previously [16,17].

Measurement of malonyl-CoA concentrations

Rats were anaesthetized with pentobarbitone (60 mg/kg) and allowed to stabilize for 20 min before the abdominal cavity was opened and the left lateral lobe of the liver was freeze-clamped. Malonyl-CoA concentrations were measured in $HClO_4$ extracts of the frozen tissue by the method of Singh et al. [18] using [1-^{14}C]butyryl-CoA as substrate.

Sensitivity of CPT I to malonyl-CoA inhibition

The isolation of liver mitochondria and measurement of CPT I activity (at 37 °C) in the presence of different concentrations of malonyl-CoA were performed as described previously [8,9].

Materials

The sources of biochemicals and radiochemicals were as described previously [16,17]. In addition, ethanolamine and ethylene glycol monomethyl ether were obtained from Sigma (Poole, Dorset, U.K.). [1-^{14}C]Butyryl-CoA was prepared as described previously [18] and fatty acid synthetase was purified from rat liver [19].

RESULTS AND DISCUSSION

The rationale behind the present experiments is that the hydrolysis of cholesteryl [1-^{14}C]oleate, delivered selectively to the liver [16], gives rise to the labelling of a pool of fatty acids that is used both for glycerolipid synthesis as well as for oxidation. We previously showed [16] that the patterns of glycerolipids

synthesized, as well as that of their secretion by the liver, suggest that the pool of fatty acids labelled through the hydrolysis of internalized cholesteryl [1-^{14}C]oleate corresponds to that which results from metabolism of preformed, exogenously delivered fatty acids, as previously assessed from studies on cultured hepatocytes [20–22]. That the labelled intrahepatic fatty acids are also available for oxidation was already apparent in [16], as the proportion of total hepatic fatty acid label that was incorporated into glycerolipids was higher in normal fed animals than in 24 h-starved or diabetic rats. The large proportion of total injected dose that could be accounted for by exhaled $^{14}CO_2$ in the present studies (Figure 1) provides direct experimental evidence for this contention. Exhaled $^{14}CO_2$ is presumed to have two origins, namely direct formation of $^{14}CO_2$ by the liver and the oxidation of [^{14}C]ketone bodies in peripheral tissues. The lag in the appearance of label in exhaled CO_2 (Figure 1) results from the time taken for equilibrium of $^{14}CO_2$ with the whole-body bicarbonate pool [23,24], as well as that for the delivery of ketones to, and their oxidation in, peripheral tissues. However, the turnover of ketone bodies appeared to be high, as insignificant amounts of label were recovered in the acid-soluble fraction of whole blood (results not shown). (Although, theoretically, [^{14}C]-ketone bodies could also be incorporated into [^{14}C]triacylglycerol in lipogenic tissues, the extent of this must have been very minor, as less than 2 % of the injected dose was recovered in the total lipid fraction of white adipose tissue 60 min after injection of label; results not shown.)

Competition between fatty acid oxidation and esterification

The effective pulse of hepatic fatty acid labelling achieved through the rapid hydrolysis of the internalized cholesteryl [1-^{14}C]oleate (see [16,25]) gave us the opportunity to monitor the partitioning of hepatic fatty acids between oxidation and esterification over successive periods of time (or 'windows'). The time courses obtained (Figure 1) showed that glycerolipid synthesis from labelled fatty acid was very rapid, and that between 30 and 60 min after injection of label there was substantial secretion of the labelled triacylglycerol by the liver. Similarly, there was rapid production of exhaled $^{14}CO_2$ by the animals between 30 and 60 min which was followed by a slower phase of production of exhaled $^{14}CO_2$ over the following 2 h. In view of these time courses, it was considered that the monitoring of the above parameters over the initial 1 h period after injection of label would provide the most suitable 'window' for the time-dependent changes in the partitioning of hepatic fatty acid metabolism during a physiological perturbation such as refeeding, especially as incorporation of label into glycerolipids was maximal at 60 min. It was also evident that underestimation of the extent of incorporation of label into oxidation products was inevitable, as $^{14}CO_2$ continued to be exhaled after the initial 1 h period (although at a much slower rate). However, it was considered that measurement of the initial rate of production of $^{14}CO_2$ (up to 60 min) would reflect the total amount of oxidation products formed from the pulse of label provided during the 0–60 min interval. As described in [16], the total amount of cholesteryl oleate delivered to the liver was quantified by injection of remnants that had been simultaneously labelled with cholesteryl [1-^{14}C]oleate and [^{3}H]cholesteryl oleoyl ether. Consequently, the net amount of label metabolized in the liver was derived from the amount of ^{3}H recovered in the liver (due to uptake of unhydrolysable cholesteryl ether) minus the amount of ^{14}C still associated with the cholesteryl ester fraction 60 min after injection of label. Routinely, the former was 90–95 % and the latter was 1–3 % of the injected dose for all conditions studied.
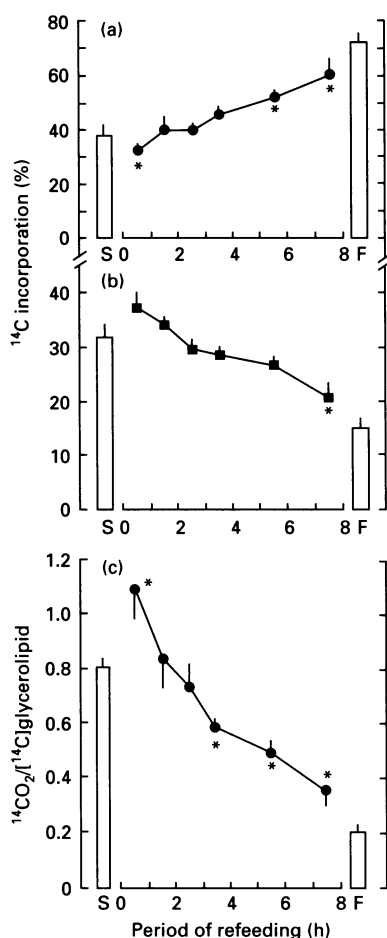
Figure 2   Changes in the incorporation of ^{14}C label into (a) total glycerolipids and (b) exhaled CO_2 in 24 h-starved (bar S), *ad libitum*-fed (bar F) and 24 h-starved/refed rats; (c) shows the progressive change in the ratio between the two parameters

Refed rats were injected with labelled remnants either simultaneously with presentation of food (0–1 h) or at the indicated periods after the start of refeeding. Values are expressed as percentages of net cholesteryl [1-^{14}C]oleate hydrolysis that occurred in the individual rats. They are means ± S.E.M. for 3–5 different rats for each time period. Where indicated by *, values are statistically significantly different ($P < 0.001$) from those obtained for 24 h-starved rats.

The data confirm that the present technique (see also [16]) reproduces for awake, unrestrained rats the well-established observation that a lower proportion (approx. 50 % less) of labelled hepatic fatty acid is metabolized to acylglycerols in starved than in fed animals (Table 1 and Figure 2a). This decrease was accompanied in the 24 h-starved rat by a doubling of the amount of $^{14}CO_2$ exhaled (Figure 2b). Consequently, the ratio ($^{14}CO_2$ exhaled)/(total [^{14}C]glycerolipid) increased from 0.21 ± 0.02 in fed animals to 0.79 ± 0.03 after 24 h starvation (Figure 2c), reflecting the expected shift in the relative abilities of the esterification and oxidation pathways to compete for hepatic long-chain acyl-CoA. In Figure 2(c), values for this $^{14}CO_2$/[^{14}C]glycerolipid ratio are plotted for successive 1 h periods during the refeeding of 24 h-starved rats. It is apparent that the ability of the fatty acid oxidation pathway to compete with esterification remained high during the first 1 h period of refeeding (indeed, it tended to increase) and, during the subsequent 2 h, was maintained at a value not significantly different from that found for the starved rat. Only during the fourth hour

of refeeding was there a decline in the ratio to below the 'starved' value. However, even then and during the 6th hour of refeeding the ratio was still 2.5-fold higher than in fed animals. Consequently Figure 2(c) illustrates that normalization of the relative abilities of the oxidation and esterification pathways to compete for their common substrate takes more than 7 h after the commencement of refeeding to be achieved.

These data demonstrate experimentally that, under *in vivo* conditions, the prediction (see Introduction) made from the changes in the kinetic properties of CPT I in isolated mitochondria is fulfilled. It is noteworthy that this conclusion is based solely on the values of the ratio between the two parameters and is valid irrespective of the absolute flux of fatty acid through the two pathways. (For example, the supply of non-esterified fatty acids to the liver falls substantially upon refeeding of starved animals [3,10].) Therefore the data demonstrate that for the particular (and continuously changing) intrahepatic acyl-CoA concentrations that exist *in vivo* during the initial phase of refeeding, the ability of CPT I to compete with glycerol 3-phosphate acyltransferase for their common substrate (long-chain acyl-CoA) was maintained at a high level. Consequently, the rapid decrease in the rate of ketone body production that occurs *in vivo* during the initial 30–60 min of refeeding [3,10] cannot be determined to any significant extent at the step catalysed by CPT I. It is likely that the curtailment of fatty acid delivery to the liver due to the anti-lipolytic action of insulin on adipose tissue (see above) accounts for a large proportion of the decreased ketogenic flux, but factors distal to CPT I (see, e.g., [11,12]) may also be involved. However, with respect to the latter, it is noteworthy that in studies with isolated livers perfused with fixed, high concentrations of oleate [3], the rate of formation of ketone bodies was still 3-fold higher than basal (fed) levels in livers from rats that had been refed for 2 h prior to the 1 h perfusion period [3]. Similarly, the ketone body/long-chain acylcarnitine concentration ratio in the liver remains elevated during the first 2 h of refeeding [23], suggesting that the flux from intramitochondrial acetyl-CoA to ketones is not substantially impaired compared with the curtailment of fatty acid delivery to the liver.
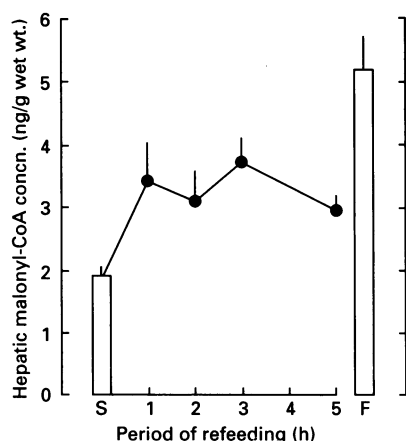
Between the 3rd and 8th hours of refeeding, the fatty acid oxidation pathway in the liver gradually lost most of its starvation-induced ability to compete for acyl-CoA (Figure 2c). It is noteworthy that this drop in the ratio coincided with the partial re-establishment, at 5 h, of the sensitivity of CPT I to malonyl-CoA in 24 h-starved rats (Figure 3) (and in 48 h-starved rats [8]). During this period there was no statistically significant decrease in the maximal activity of the enzyme, although the value at 5 h of refeeding tended to be slightly decreased (legend to Figure 3 and [8]). Interestingly, the hepatic malonyl-CoA concentration increased to values intermediate between those obtained for livers of starved and fed animals (Figure 4). This increase was very rapid (1 h) but not progressive. Consequently, the gradual decrease in the ability of the oxidative pathway to compete for acyl-CoA was best correlated with changes in the sensitivity of CPT I to malonyl-CoA rather than with the changes in malonyl-CoA concentrations themselves. This confirms, for the *in vivo* situation, the importance of changes in CPT I sensitivity to malonyl-CoA in the amplification of the signal represented by changes in hepatic malonyl-CoA concentration, as originally suggested [7].

The rapid change in malonyl-CoA concentration to 'fed' values after refeeding agrees with the equally rapid dephosphorylation of acetyl-CoA carboxylase we described previously [26]. The intermediate values of malonyl-CoA are exactly as expected from the observation that the effective activity of acetyl-CoA
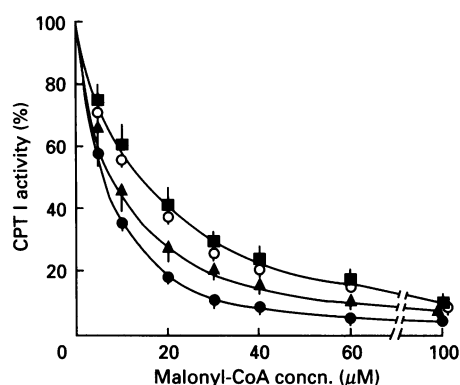
Figure 3   Sensitivity of CPT I to malonyl-CoA inhibition in mitochondria isolated from fed (●), 24 h-starved (○) and starved animals refed for 2 h (■) and 5 h (▲)

Values are means ± S.E.M. for three or four separate mitochondrial preparations from rats in each physiological condition. The maximal activity of CPT, measured in the presence of 300 μM palmitoyl-CoA and in the absence of malonyl-CoA, were (nmol/min per mg of protein at 37 °C): fed, 19.2 ± 1.4 ($n = 4$); starved, 35.7 ± 3.7 (4); refed 2 h, 33.6 ± 3.0 (3); refed 5 h, 27.4 ± 3.9 (3).

Figure 5   Induction of preferential synthesis of phospholipid as a fraction of total acylglycerol synthesis upon refeeding of 24 h-starved rats

Values were obtained for fed (bar F), 24 h-starved (bar S) and 24 h-starved/refed (●) rats. The incorporation of label into hepatic and plasma phospholipids is expressed as a fraction of the total ^{14}C incorporation into plasma and liver glycerolipids. Values are means ± S.E.M. for three to five separate rats for each conditions.
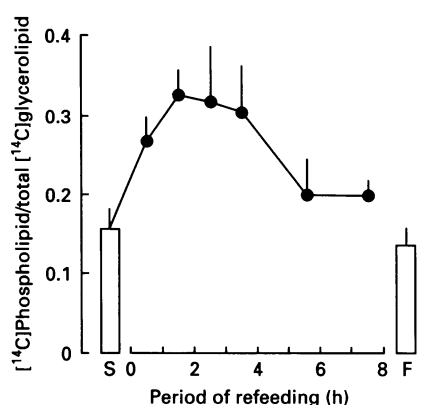
Figure 4   Time course of the increase in hepatic malonyl-CoA concentration during the early stages of refeeding of 24 h-starved rats

Bars denoted by S and F represent values obtained for 24 h-starved and fed rats respectively. Values are means ± S.E.M. for four to six determinations on separate rat livers.

label into phospholipids and triacylglycerol (Figure 5). The amount of [^{14}C]fatty acid label that was incorporated into phospholipids as a proportion of total glycerolipid synthesis increased 2-fold within the first 2 h of refeeding. Between 5 and 7 h of refeeding there was a sharp reversal of this effect, such that the [^{14}C]phospholipid/total [^{14}C]glycerolipid ratio declined towards normal 'fed' values. It is noteworthy that this ratio did not differ significantly for livers of fed and 24 h-starved animals, and that its acute biphasic response to refeeding was limited to the first few hours of this physiological perturbation. We suggest that this increased diversion of fatty acids towards phospholipid formation during refeeding may represent a protective mechanism of the liver to maintain phospholipid synthesis under conditions associated with a rapid decline in availability of acyl-CoA substrate, prior to full re-establishment of endogenous fatty acid synthesis. It is also possible that the response may reflect the rapid remodelling of phospholipid acyl chain composition in liver membranes. Several membrane-located functions have been suggested to be sensitive to the phospholipid composition of membrane lipids [29]. Remodelling of the fatty acid composition of phospholipids upon refeeding may serve to alter the molecular ordering of the lipid in specific membranes and thus affect the function of these membrane-associated processes in response to the rapidly changing physiological conditions.

carboxylase would be limited, not so much by its rate of dephosphorylation, but by the loss of enzyme protein [26]. It is possible that the relationship between the rate of lipogenesis and intrahepatic malonyl-CoA concentration is altered during refeeding, as observed for other transitions [27]. This may result from an altered acetyl-CoA carboxylase/fatty acid synthase activity ratio, which determines intrahepatic malonyl-CoA concentrations, irrespective of the absolute lipogenic flux [28].

Changes in the proportions of incorporation of label into phospholipids and acylglycerols

Notwithstanding the slowness with which partitioning of fatty acids into total glycerolipids recovered during refeeding, marked changes did occur more acutely in the relative incorporation of
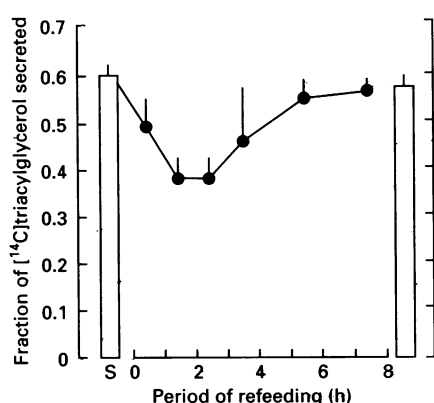
Changes in the proportion of triacylglycerol secreted by the liver

There was no significant difference between fed and starved rats in the proportion of labelled triacylglycerol that was secreted by the liver (Figure 6). However, upon refeeding there was a 40 % decline in this parameter. After 7 h of refeeding the fraction of secreted triacylglycerol had recovered to the values observed in fed or starved animals. To our knowledge this is the first demonstration of a meal-induced inhibition of the proportion of triacylglycerol secreted by the liver in vivo. It is possible that it is caused by the increase in plasma insulin that occurs upon refeeding [30]. That such inhibition of very-low-density lipoprotein (VLDL) secretion would occur in vivo had been inferred previously [31,32] from the inhibitory effects of insulin on triacylglycerol and apolipoprotein B secretion by cultured hepatocytes [33–36]. In addition, Vogelberg et al. [37] had shown

Figure 6   Decrease in the proportion of labelled triacylglycerol synthesized in the liver that was secreted into the plasma during successive 1 h periods after the start of refeeding of 24 h-starved rats

Values were obtained by expressing the amount of ^{14}C incorporated into plasma triacylglycerol as a fraction of the total incorporation of ^{14}C into plasma plus liver triacylglycerol. Results are means ± S.E.M. for three to five determinations on separate rats for each condition. Bars S and F refer to values for 24 h-starved and fed rats respectively.

that the VLDL-triacylglycerol production rates in hypertri-glyceridaemic human subjects was negatively correlated with the hepatic clearance rate of insulin, and that portal infusion of insulin resulted in an inhibition of VLDL secretion. Moreover, if the stimulation of triacylglycerol secretion by non-esterified fatty acids observed for cultured hepatocytes [38] occurs in vivo, the effects of elevated insulin levels on VLDL secretion would be reinforced by those due to the rapid decrease in plasma non-esterified fatty acids that occurs upon refeeding.

It is interesting that, in the present work, the inhibition of hepatic triacylglycerol secretion was reversed within a few hours. This suggests either that rather high (but physiological) concentrations of insulin (attained during a post-prandial spike of insulin release [30]) are required for the effect to occur, or that the liver becomes refractory to the effect of insulin after the first few hours of exposure to the elevated plasma insulin concentrations that occur in the fed state compared with the starved state. In this respect, the work described in [20] suggested that longer periods of exposure of cultured hepatocytes to insulin resulted in an attenuation of the inhibitory effect of the hormone on VLDL secretion. However, the possibility of synergy between insulin and a gastric hormone cannot be excluded. Irrespective of the mechanism, it is of interest that the time courses for the increased diversion of fatty acids into phospholipids and the inhibition of triacylglycerol secretion were identical and mirrored each other, suggesting that a common signal is responsible for the two effects.

The fraction of labelled phospholipid secreted was always very low and did not show any statistically significant changes under any of the conditions studied.

Differences between anaesthetized and awake rats

In our previous study [16] on selective labelling of hepatic fatty acids we had found that starvation caused significant changes in the proportion of fatty acid label that was incorporated into acylglycerols and phospholipids, and in the fraction of triacylglycerol secreted by the liver. Those studies were conducted on anaesthetized rats. It is evident from the present data that these effects of starvation were much less pronounced, if detectable at

all, when awake unrestrained rats were used. This suggests that the administration of pentobarbitone to the animals may have different effects on liver metabolism depending on prior physiological state, and reinforces the desirability of using non-invasive methods in monitoring hepatic fatty acid metabolism in vivo.

Conclusions

The selective labelling of hepatic fatty acids achieved through the liver-specific direction of cholesteryl [1-^{14}C]oleate in awake rats appears to provide a valid method for assessing several parameters of hepatic fatty acid metabolism in vivo. The most important of these, from the perspective of the regulation of CPT I, is the partitioning of label between esterification and oxidation products. Novel information has also emerged on the acute in vivo effects of refeeding on glycerolipid synthesis and triacylglycerol secretion by the liver, namely that refeeding is associated with (i) an acute inhibition of the proportion of synthesized triacylglycerol that is secreted by the liver, and (ii) a transient doubling of the proportion of phospholipids synthesized. The dynamics of the uptake of injected lipoproteins by the liver combined with the short half-life of the labelled cholesteryl ester result in a temporal window for which the parameters of interest can be quantified. The use of successive periods of observation of this kind yields valuable information about the progressive metabolic changes that occur in the liver in vivo during specific metabolic perturbations.

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