REVIEW ARTICLE

Role of insulin in hepatic fatty acid partitioning: emerging concepts

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INTRODUCTION

The effects of insulin action on hepatic lipid metabolism are increasingly emerging as central factors in the integration of the metabolic status of the whole animal. In humans, insulin resistance results in derangements of liver metabolism which are associated with such clinically important conditions as obesity, diabetes and atherosclerosis. The perceived role of the liver in the aetiology of these conditions has attracted extensive research in the area, and several reviews have appeared in recent years that deal with aspects of the role of the hormone in the control of liver lipid metabolism (see, e.g., [1–3]). The aim of the present one is not to duplicate these, but rather to suggest possible ways in which newly acquired information can be combined with other well-established phenomena in the formulation of an integrated theory of control of the hepatic partitioning of fatty acid metabolism by insulin. The approach adopted is purposely speculative, in the anticipation that this is most likely to stimulate novel ideas and work.

The liver plays a central role in orchestrating the delivery of substrates to peripheral tissues. In the process, it partly determines the circulating concentrations of metabolites and lipoproteins. The traffic is bidirectional and, under any particular set of physiological conditions, a balance is established as a result of the rates of secretion by the liver, uptake by peripheral tissues and re-uptake by the liver. This is perhaps best exemplified by lipid substrates, because the liver plays such a central role in the metabolism of fatty acids and cholesterol. Both can be synthesized by the liver, and are also delivered to it in the circulation. Fatty acids are esterified to produce triacylglycerols (TAGs) and phospholipids which, together with unesterified cholesterol and cholesteryl esters, make up the lipid components of very-low-density lipoproteins (VLDL) secreted by the liver. Their secretion, combined with the ability of the peripheral tissues to metabolize them and of the liver itself to clear the remnants or products of the metabolism of VLDL (and chylomicrons secreted from the gut), determines the levels of such important parameters as the plasma concentrations of triacylglycerol, esterified and unesterified cholesterol and of the remnants themselves. Hepatic fatty acid and cholesterol metabolism is therefore intimately linked, and insulin plays an important role in determining the outcome of their metabolism within the liver.

Insulin affects hepatic lipid metabolism directly through its actions to modulate the expression of enzymes or secretory proteins [e.g. apolipoprotein B (apoB); see below], and indirectly by determining the rate of delivery of esterified or non-esterified fatty acids to the liver, through its antilipolytic effect on adipose tissue and its influence on the expression of lipoprotein lipase activity. Much of the interest in the effects of insulin has centred on the role of the liver in the aetiology of non-insulin-dependent diabetes mellitus and its associated insulin resistance. In this respect, it has become increasingly appreciated that a deficient response of lipid metabolism to diurnal changes in insulin secretion accompanying food intake may directly affect postprandial triglyceridaemia and influence the development of other aspects of insulin resistance, such as the impaired response of glycogen synthesis to insulin in muscle [2,3].

Under normal conditions, the liver is regularly exposed, via the portal circulation, to major increases in insulin concentration in response to food intake. Consequently, the most physiologically relevant studies of the response of hepatic lipid metabolism to the effects of insulin are those that involve meal-induced changes in the secretion of the hormone. However, the effects of insulin during such physiological changes are difficult to study because of the concomitant changes in counter-regulatory hormone concentrations. Consequently, frequently the effects of insulin have had to be studied by using the isolated perfused liver or cultured hepatocyte preparations, with the limitations that these impose. As a result, considerable controversy has arisen in the literature over the significance of certain effects of insulin *in vitro* when different preparations are used in different laboratories. Attempts to overcome these difficulties have been made through the development of methods to study hepatic fatty acid metabolism *in vivo* (through the specific labelling of hepatic fatty acids [4]), and particularly the effects thereon of changes in insulin status of the animals, either when meal-induced or after pharmacological manipulation.

ENZYMES INVOLVED IN THE PARTITIONING OF FATTY ACIDS BETWEEN OXIDATION AND GLYCEROLIPID FORMATION

Insulin favours the esterification of fatty acids to form glycerolipids. In all liver preparations used *in vitro*, insulin has always been found to stimulate TAG synthesis [5–9]. The enzymes that catalyse the first reactions that commit long-chain acyl-CoA either to glycerolipid formation (the glycerol-3-phosphate acyltransferases, GPATs) or to intramitochondrial β-oxidation (overt carnitine palmitoyltransferase, CPT I) necessarily compete for their common substrate, and the outcome of this competition is directly influenced by insulin [10].

The hepatic mitochondrial and microsomal forms of GPAT respond differently to insulin-deficient states in the rat, suggesting that their expression may be differentially affected by insulin *in vivo*. Thus it is specifically the activity of mitochondrial GPAT that is decreased in the diabetic- or starved-rat liver [11,12]. Moreover, insulin acutely increases mitochondrial GPAT activity in the perfused rat liver [13]. This effect is analogous to the effect of insulin on cardiomyocyte and adipocyte GPAT [14,15]. These may be considered to be the direct actions of insulin to favour esterification. However, insulin also exerts indirect effects on β-oxidation of fatty acids (see Figure 1).

The esterification of the long-chain acyl moiety to carnitine is catalysed by a family of carnitine acyltransferases [known commonly as carnitine palmitoyl- or octanoyl-transferases (CPTs

Abbreviations used: ACC, acetyl-CoA carboxylase; apoB, apolipoprotein B; CPT, carnitine palmitoyltransferase; CT, CTP:phosphocholine cytidylyltransferase; DAG, diacylglycerol; DGAT, DAG acyltransferase; ER, endoplasmic reticulum (rER, rough ER; sER, smooth ER); GPAT, glycerol-3-phosphate acytransferase; mHMG-CoA, intramitochondrial 3-hydroxy-3-methylglutaryl-CoA; NEFA, non-esterified fatty acids; PDH, pyruvate dehydrogenase; TAG, triacylglycerol; VLDL, very-low-density lipoproteins.

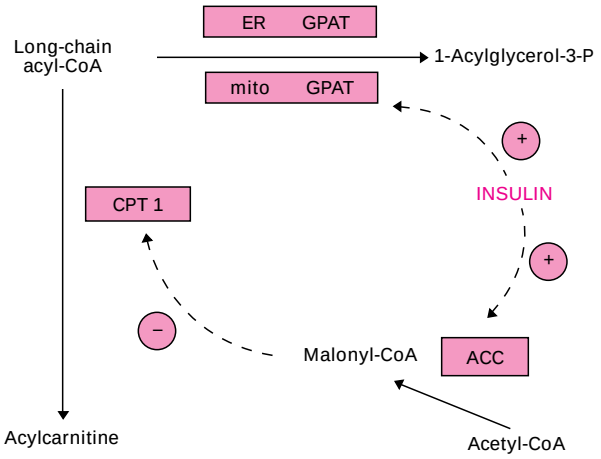

Figure 1 Direct and indirect effects of insulin on the partitioning of cytosolic acyl-CoA between oxidation and esterification

Insulin activates mitochondrial GPAT and also ACC (see the text). The latter effect results in the elevation of intrahepatic malonyl-CoA concentration, which indirectly inhibits β-oxidation of fatty acids through inhibition of CPT I. Abbreviation: mito, mitochondrial.

or COTs respectively)], which are found in mitochondria, the microsomal fraction and peroxisomes. They are all immunologically distinct [16–19], and sequencing of the cDNA of several of them has so far indicated that they are separate gene products [20–23]. Interestingly, the pattern that appears to be emerging from ongoing studies is that each of these membrane systems possess two isoforms of CPT, the activity of only one of which is sensitive to malonyl-CoA inhibition [23–29]. The functions of the microsomal and peroxisomal enzymes can only be speculated on, at present. In peroxisomes, the carnitine acyltransferases may be involved in the transfer, out of organelles, of the chain-shortened products of the oxidation of very-long-chain fatty acids [30,31]. The presence of two CPTs in the microsomal fraction, a membrane-bound cytosol-facing one which is malonyl-CoA-sensitive, and a second one that appears to be lumenal [24,29], suggests that carnitine-dependent transfer of acyl moieties across the microsomal membrane may occur. Alternatively, the lumenal enzyme may be involved in the buffering of intralumenal CoA concentrations. If net carnitine-dependent transfer does occur *in vivo* across the endoplasmic-reticular membrane, it would be important to determine in which direction net transfer is mediated. Intralumenal acyl-CoA esters may be required for processes such as protein acylation [32]. However, it is likely that significant fluxes would only be involved if the transported acyl chains were to be used as intermediates in the synthesis of a major secretory product, e.g. TAG. The possible involvement of such a mechanism in the synthesis of secreted TAG is discussed below. Moreover, it should be emphasized that alternative functions (phospholipase, thiol protease) have been proposed for at least one of these proteins that display CPT activity (see discussion in [24, 33]).

Mitochondrial β-oxidation is quantitatively by far the most important route for the oxidation of the most abundant fatty acids and, consequently, plays a dominant role in determining the partitioning of hepatic fatty acid metabolism. The CPT system of mitochondria (overt CPT I and latent CPT II) is much better understood than the other membrane systems of the cell. The activity of CPT I to convert acyl-CoA into acylcarnitine, and the reversal of this reaction by CPT II in the inner-membrane-matrix compartment (connected by the transport of the acyl-carnitine by a specific carrier located in the inner membrane), results in the effective transfer of long-chain fatty-acyl molecules across the inner membrane while leaving the acyl-CoA pools in the cytosolic and matrix compartments separate. CPT I resides in the outer membrane of the mitochondria [34] and is an integral membrane protein [35,36], whereas CPT II is a peripheral protein of the inner membrane, with its active site facing the matrix space. CPT I is the one that is sensitive to malonyl-CoA. This property not only makes the reaction it catalyses a potentially important control site, but also provides a mechanism through which insulin can effect acute control over acylcarnitine synthesis, and thus β-oxidation of fatty acids.

Malonyl-CoA is the product of the reaction catalysed by acetyl-CoA carboxylase (ACC), which commits cytosolic acetyl-CoA to the synthesis of fatty acids. Because of the high fatty acid synthase/ACC activity ratio, and the relatively low affinity of fatty acid synthase for malonyl-CoA, the concentration of the inhibitor of CPT I is determined largely by the activity of ACC [37]. Insulin can potentially activate ACC through a variety of mechanisms (see [38]), including dephosphorylation of serine residues in the protein [39], the phosphorylation of which results in enzyme inhibition [40], direct (activatory) phosphorylation at an 'insulin site' [41], or possibly through the action of a low-molecular-mass effector [42]. However, it is not certain that all these mechanisms are necessarily applicable to the liver enzyme, as most of the studies on post-translational modification of the enzyme have been conducted on adipose-tissue ACC. The net result, however, is that conditions characterized by high insulin/glucagon concentration ratios in the portal circulation are accompanied by high hepatic activity of ACC and elevated concentrations of malonyl-CoA, whereas absolute or relative insulin deficiency results in low malonyl-CoA concentrations [43–47]. Consequently, the rate of fatty acid oxidation would be expected to be inhibited at the CPT I reaction simply through the effects of insulin on ACC activity. However, acylcarnitine synthesis has the potential to be much more sensitive to the effects of insulin, because hypoinsulinaemic or insulin-resistant states result in a desensitization of CPT I to malonyl-CoA [48–53]. Consequently, the effects of changes in the absolute concentration of malonyl-CoA in the liver are amplified by the adaptation of CPT I kinetic properties during conditions characterized by absolute or relative insulin deficiency [38,54,54a].

The ability of CPT I to alter its properties under different physiological conditions is thought to result directly from its strong interaction between the hydrophobic regions present in its primary structure [22] (including two potential membrane-spanning domains [55]) and the lipid components of the mitochondrial outer membrane. The changes in its kinetic characteristics observed in mitochondria isolated from rats in different conditions probably occur as a result of changes in the composition of the annular lipids that interact directly with these and other hydrophobic stretches in the primary sequence of the protein. Thus the densitization to malonyl-CoA observed *in vivo* can be mimicked *in vitro* by decreasing the lipid order (i.e. increasing the fluidity of the lipid bilayer) in purified outer-membrane preparations, whether this is induced chemically [56] or thermally [56–58]. In addition, studies on the overall lipid composition of outer membrane isolated from normal fed, starved and streptozotocin-diabetic rats indicate that there is a correlation between the mobility of a hydrophobic molecular probe (diphenylhexatriene) within the membrane (a measure of 'fluidity'), the cholesterol/phospholipid ratio in the membrane and the degree of desensitization of CPT I to malonyl-CoA (C. G. Corstorphine and V. A. Zammit, unpublished work). Ad-

dition of phospholipids to mitochondria [59], or treatment with phospholipases [60], has also shown that the sensitivity of CPT I to malonyl-CoA can be altered *in vitro*. Insulin may be involved in modulating membrane lipid composition, e.g. through its effects on cholesterol metabolism [61,62] and/or the remodelling of membrane phospholipids. It is noteworthy that the malonyl-CoA-sensitive CPTs in the other membrane systems of hepatocytes (microsomes, peroxisomes) also undergo changes in sensitivity similar to those of the CPT I of mitochondria [24,25].

The levels of expression of mitochondrial CPT I and II proteins have been shown to increase several-fold in ketogenic conditions, the effect being much more marked for CPT I [17]. Experiments using cultured hepatocytes have shown that fatty acids and cyclic AMP-elevating agents increase the rate of transcription of the CPT I gene, but not of the CPT II gene. Insulin antagonizes the effect of cyclic AMP and inhibits the expression of CPT I [63]. Incubation of cultured foetal rabbit hepatocytes with long-chain fatty acids also induces the expression of CPT I [64]. Similarly, studies on neonatal-rat liver have shown that the increase in insulin/glucagon concentration ratio that accompanies weaning of the pups on to a high-carbohydrate diet is accompanied by a rapid decline in the CPT I mRNA content, but not in that of CPT II [65]. Interestingly, the expression of CPT I protein showed a much more gradual decline than that of its mRNA, indicating that the protein has a long half-life. The same inference was drawn from measurement of CPT I activity in mitochondria isolated from livers of starved–refed [66] or insulin-treated streptozotocin-diabetic rats [67].

The slowness of the changes in the expression of CPT I activity during physiological transitions is matched by that shown by changes in the sensitivity to malonyl-CoA. Thus, it takes 12–14 h of starvation of rats before any decrease in sensitivity to malonyl-CoA occurs (L. Drynan and V. A. Zammit, unpublished work). This is considerably later than the large decrease in plasma insulin concentrations that occurs after 6 h of starvation [68]. Similarly, reversal of the effects of starvation or streptozotocin-induced diabetes in rats only starts 6 h after refeeding of the animals and is not complete even after 12 h after the start of refeeding [66] or insulin treatment [67] respectively. The requirement for several hours to achieve the observed modification of CPT I kinetics has been confirmed in experiments using cultured hepatocytes exposed to insulin or glucagon [63,69] and in rats subjected to a hyperinsulinaemic/euglycaemic clamp [70]. The time scale over which these changes occur is commensurate with the involvement of changes in lipid composition of the mitochondrial outer membrane (see above) in determining the properties of CPT I. It also has important implications for the distribution of metabolic control over the fatty acid oxidative pathway, especially during the reversal of oxidative (ketogenic) conditions. When starved rats are refed [66] or when diabetic animals are treated with insulin [67], there is a rapid decline in circulating ketone-body concentrations, reflecting a decreased rate of ketone-body production in the liver. The questions arise, therefore, as to the involvement of insulin in this response, as well as to the site at which the effect is exerted.

If, as the enzymological data appear to indicate, the expression of CPT I remains high in spite of the rapid decline in CPT I mRNA (because of the long half-life of the protein) and its desensitized state with respect to malonyl-CoA inhibition persists during the reversal of ketogenic states [66,67], then it would be anticipated that this reaction no longer exerts major control over the rate of fatty acid oxidation during these metabolic transitions. It has been suggested that intramitochondrial 3-hydroxy-3-methylglutaryl-CoA (mHMG-CoA) synthase becomes an important locus of control under these conditions [71,72]. Insulin treatment of starved or diabetic rats results in the rapid decline in the hepatic concentration of mHMG-CoA synthase mRNA and protein [72,73]. In addition, the enzyme reverts to the succinylated (inhibited) state during refeeding of starved rats [74]. Although these changes suggest that a lower proportion of intramitochondrial acetyl-CoA is diverted towards ketone-body formation under these conditions, it is unlikely that the overall partitioning of fatty acids between oxidation and glycerolipid formation is greatly influenced by this. Indeed, when metabolic-control-analysis experiments are performed by using the CPT-specific inhibitor tetradecylglycidic acid (TDGA) to titrate CPT I activity in hepatocytes isolated from fed, starved and starved–refed or insulin-treated starved rats, the control strength of CPT I over the ketogenic rate from exogenously provided palmitate is uniformly high (L. Drynan and V. A. Zammit, unpublished work). These data therefore suggest that if control is lost from the reaction catalysed by CPT I, it is transferred to a step that is proximal to the reaction catalysed by CPT I (e.g. fatty acid supply to the liver) rather than one distal to it (such as mHMG-CoA synthase).

The inference to be drawn from the above is that the potential of the oxidative pathway to compete for cytosolic acyl-CoA remains high during the initial phases of reversal of episodes characterized by high rates of fatty acid oxidation. Such an inference is difficult to test *in vivo*, although evidence for it has existed for some time. Thus insulin treatment of diabetic rats does not result in an immediate reversal of the enhanced ability of isolated hepatocytes or perfused liver to oxidize fatty acids; reversal of the high oxidative capacity requires about 6 h of insulin treatment [75]. Similarly, the ability of hepatocytes isolated from diabetic rats to oxidize fatty acids remains high even after several hours of insulin treatment of the rats from which the hepatocytes are prepared [76]. It is difficult to demonstrate that this lag in the response of the liver actually occurs *in vivo*, because such sustained partitioning of fatty acids in favour of oxidation has to be discerned over and above the rapidly declining rates of absolute rates of fatty acid oxidation, due to the curtailment of non-esterified fatty acid (NEFA) delivery to the liver through the antilipolytic action of insulin on adipose tissue.

The way this difficulty can be overcome is to monitor the partitioning of long-chain fatty acid metabolism, rather than the actual flux. This can be achieved *in vivo* through the specific labelling of the hepatic fatty acid pool, by using cholesteryl [1-^{14}C]oleate-labelled remnants of previously TAG-rich lipoproteins (VLDL and/or chylomicrons) to deliver the ester to the hepatocyte population [4,77]. The labelled ester is rapidly and selectively taken up by these cells and hydrolysed with a half-life of about 15 min [78] to yield an effective pulse of intrahepatic radioactively labelled oleate [4,77]. The rate of the label can then be monitored by measuring the accumulation of label in the oxidation and esterification products in the liver, plasma and exhaled gases of the animals (see [79,80] for further details). This approach has been used to try to elucidate the role of insulin in the partitioning of hepatic fatty acid metabolism *in vivo*, not only between oxidation and esterification but also at the main branch-points of glycerolipid metabolism (see below).

Two experimental models in which acute and well-defined increases in insulin concentration occur are the starved–refed rat and the meal-fed rat. The latter animals are trained to consume their daily food intake within 3 h every day for 15 days before the experiment. Consequently, they are effectively starved for 21 h diurnally before being allowed to consume their diet. The insulin-secretory response to feeding is more pronounced, although

other physiological adaptations (e.g. increased absorption from the gut) are also apparent [81]. In both models, the ingestion of a meal results in a rapid decline in circulating NEFA concentrations, thus curtailing the availability of substrate (acyl-CoA) for both oxidation and esterification. But when the partitioning of hepatic acyl-CoA between oxidation and esterification is monitored *in vivo*, it is apparent that the response of the liver is very different between the two conditions. Whereas in starved–refed rats acylcarnitine synthesis remains able to compete successfully for cytosolic acyl-CoA for several hours after the start of refeeding, in meal-fed rats the ingestion of food brings about a much more rapid switch of utilization of acyl-CoA from oxidation to esterification [79,80]. The difference is due to a combination of the adaptation in the properties of CPT I (it is desensitized and over-expressed in starved–refed, but not in meal-fed, animals) and to the response of hepatic malonyl-CoA concentrations to food intake. The latter rises only moderately upon refeeding of starved rats, but overshoots the 'fed' value within 2 h of the start of feeding in meal-fed animals [80]. Evidently, the greater expression of ACC and its more extensive kinetic changes, as well as the accelerated dephosphorylation of pyruvate dehydrogenase (PDH), in meal-fed rat livers result in a much higher flux through the lipogenic pathway [82]; hence the rapid rise in malonyl-CoA concentrations. The effect is amplified by the relatively greater sensitivity of CPT I to the inhibitor in meal-fed animals [80]. Consequently, whereas in starved–refed animals, as expected, the inhibition of acylcarnitine synthesis (i.e. CPT I activity) plays little or no role in mediating the very rapid decline in the absolute rate of fatty acid oxidation (i.e. CPT I no longer exhibits high control strength), in meal-fed animals control is shared, much earlier during the prandial/early-absorptive period, between extra- and intra-hepatic mechanisms, namely antilipolysis in adipose tissue and inhibition of CPT I. The possible physiological rationale behind this difference in terms of the different requirements, in the two models, for continued utilization of pyruvate for the synthesis of glucosyl units during the prandial period has been discussed elsewhere [77]. Of interest in the present context is the role of insulin. The rapid activation of PDH in meal-fed rats is unlikely to be due to the direct action of the hormone, as insulin is known to activate PDH only to a small extent, if at all, in the liver [83,84,84a]. So the PDH effect is likely to be substrate-driven, e.g. through a rise in intramitochondrial pyruvate concentration, which will inhibit PDH kinase [85]. The rapid decline in oxidation of fatty acids will also result in a decrease in the intramitochondrial acetyl-CoA/CoA ratio, which would favour dephosphorylation of PDH [85]. However, the extrahepatic control exerted on this parameter (i.e. NEFA supply) is not likely to be very different between the two models, such that the role of insulin in mediating extrahepatic control is likely to be very similar.

It was of interest to determine whether, if the insulin-secretory response to a meal is highly attenuated, hepatic metabolism can still respond by altering the partitioning of fatty acids between oxidation and esterification. When starvation–refeeding experiments were performed on severely diabetic rats and the fate of [1-^{14}C]oleate was monitored, it was found that the liver was able to switch off acylcarnitine synthesis very rapidly, and before any significant rise in hepatic malonyl-CoA concentration occurred [86]. One possibility to explain this finding is that substrate-driven effects are sufficient to bring about the rapid diversion of fatty acyl-CoA flux from oxidation to esterification entirely through intrahepatic mechanisms [86]. The net result *in vivo* was that, whereas NEFA concentrations rose in these adipose-tissue-depleted starved diabetic rats upon refeeding, the circulating ketone-body concentrations did not increase, just as expected from the partitioning data [86]. In these experiments, a late, modest and transient rise in malonyl-CoA concentration was observed, which suggested that the desensitization of CPT I to malonyl-CoA inhibition, characteristic of the diabetic state [54a], was somehow overcome. A possible mechanism may be a putative acute decrease in the cytosolic pH during the prandial/absorptive period, such as might be associated with the observed rapid increase in hepatic lactate/pyruvate ratio. This would result in the effective sensitization of CPT I to malonyl-CoA, as the enzyme is much more sensitive to malonyl-CoA inhibition at the lower end of the physiological range of pH [53]. Another mechanism may involve the inhibition of CPT I activity by increased hepatocyte cell volume [87], which may accompany any increase in the portal concentrations of osmotically active metabolites and/or ions (see below).

ACUTE CHANGES IN GLYCEROLIPID PARTITIONING DURING THE PRANDIAL/EARLY-ABSORPTIVE PHASE

The secretion of hepatic VLDL-TAG is an important source of fatty acids for peripheral tissues. Even though the expression of lipoprotein lipase in peripheral tissues may be diminished in insulin-deficient and/or -resistant states, they still utilize VLDL-TAG for an important part of their energy requirements [88,89]. Consequently, under these conditions hepatic VLDL secretion is substantial in absolute terms, even if the fraction of fatty acids metabolized by the liver that is esterified is lower than in insulin-replete states. The increased rates of delivery of fatty acids to the liver makes this possible. (Although it has been found repeatedly that rates of VLDL-TAG secretion by isolated cultured hepatocytes and the perfused livers obtained from starved and diabetic rats have a much lower rate of secretion of TAG [90–92], these experiments may have underestimated the rates attainable, due to the inadequacy of the mixture of substrates provided to these preparations *in vitro*, as demonstrated in [93].)

The same considerations may apply to the fasted/post-absorptive state, i.e. a substantial rate of VLDL-TAG secretion would be expected to be maintained by the liver in the post-absorptive state [94]. Upon resumption of food intake, the absorption of fat from the gut results in the secretion of TAG-rich chylomicrons that compete with VLDL for lipolysis by lipoprotein lipase [95,96]. Consequently, if the liver were to continue to secrete VLDL at pre-prandial rates, the hypertriglyceridaemia experienced during the absorptive phase would be exacerbated [96]. It would be expected that, when the plasma NEFA concentrations are acutely decreased upon refeeding, due to the anti-lipolytic action of insulin on adipose tissue, the magnitude of the flux of acyl chains entering the TAG-synthetic route would be automatically curtailed and that this would be sufficient to inhibit hepatic TAG synthesis and secretion. However, the liver does not appear to depend solely on diminished substrate supply to achieve a decline in TAG secretion during the prandial period.

Evidence for this comes from several lines of study. Thus studies in humans have shown that intraportal infusion of insulin in humans results in a decrease in the secretion of VLDL-TAG [97]. Similarly, when humans are treated with insulin, the secretion of apoB_{100}-associated TAG is decreased [98]. Although a large proportion of this effect can be attributed to the decrease in circulating NEFA concentrations due to the anti-lipolytic action of insulin, even when the concentration of NEFA was kept high, insulin still gave a decrease in hepatic VLDL secretion rate of about 30% [98]. In humans, the concentrations of both apoB_{48} (from the gut) and of apoB_{100} (from the liver) increase with very similar time courses in the post-prandial state [99]. Although this had previously been considered to demonstrate

that hepatic VLDL-TAG secretion increases during the absorptive phase because [^{3}H]leucine incorporation into circulating apoB_{100} was increased [100], it is now appreciated that the reason for the increase in apoB_{100} during the post-prandial period is that chylomicron TAG competes with VLDL-TAG for hydrolysis by lipoprotein lipase in peripheral tissues [101]. The fall in apoB_{100} content of the intermediate-density lipoprotein fraction during peak post-prandial triglyceridaemia probably reflects both this competition between chylomicrons and VLDL, and an inhibition of VLDL-TAG secretion rate by the liver [96], but direct evidence for the latter is difficult to obtain in humans. In experiments in which the fatty acid pool of the liver in awake rats is specifically labelled *in vivo* [4,77], changes are observed in the partitioning of label between TAG and phospholipid synthesis, as well as in the fractional rate of secretion of newly labelled TAG, that suggest that acute inhibition in the proportion of synthesized TAG that is secreted occurs during the ingestion of a meal [79,80]. Finally, studies *in vitro* also suggest that a prandial increase in insulin secretion should inhibit TAG secretion by the liver, e.g. culture of rat hepatocytes or HepG2 cells in the presence of insulin stimulates TAG synthesis, but inhibits its secretion.

Insulin may act at several different loci to exert such acute effects. In order to be able to discuss these possibilities, a brief summary of the metabolic models that have recently emerged to explain the route taken by fatty acyl moieties in reaching the secretory TAG pool is given.

ASSEMBLY OF VLDL PARTICLES IN THE LIVER

VLDL particles are made of a core of hydrophobic lipids (TAGs, cholesteryl esters) associated with a molecular of apoB, a large protein with many, relatively short, hydrophobic stretches of amino acid sequence that interact with this core lipid. (In humans apoB_{100} is the hepatic form secreted; in species like the rat both apoB_{48} and B_{100} are synthesised and secreted by the liver.) In addition, a layer of polar, surface, lipids, including cholesterol and phospholipids, covers the surface area left unoccupied by apoB and provides the interaction required with the aqueous phase in the plasma [102]. The requirements for cholesterol [103,104] and for phosphatidylcholine synthesis [105] for the efficient production and secretion of VLDL by isolated perfused liver preparation or cultured hepatocytes have been described. Some general principles about apoB assembly with the hydrophobic core have been well established, but others remain to be determined.

ApoB is co-translationally inserted into the rough endoplasmic reticular (rER) membrane [106], but only a fraction of the nascent polypeptide is translocated into the lumen of the rER. This translocation is accompanied by the acquisition of a TAG core, and thus the formation of a nascent lipoprotein particle. A model that has been suggested to account for this postulates that, during insertion of apoB across the membrane and into the lumen of the rER, the TAG passes through the disrupted membrane and is accepted into a hydrophobic pocket formed by the apoB molecule itself [102,107,108] (see inset in Figure 2). Assembly of TAG-rich apoB lipoproteins has an obligatory requirement for a lumenal microsomal triacylglycerol transfer protein [109]. It catalyses the transfer of TAG and other non-polar lipids between membranes and liposomes *in vitro* [110] and its function *in vivo* may be to facilitate the transfer of TAG across the ER membrane and into the nascent apoB polypeptide. It is noteworthy that this lumenal protein occurs as a heterodimer in association with protein disulphide-isomerase (which is essential for the correct folding of secretory proteins involving disulphide bridges) in the lumen of the ER [110]. Its apparently obligatory involvement in VLDL assembly and secretion may reflect a putative dual function in the correct folding of apoB and the concomitant transfer of TAG to the nascent VLDL particle.

The subsequent events involved in the acquisition of the full complement of core lipids by apoB have proved controversial to determine. Several accounts of the detailed arguments in favour of the different models have appeared (see, e.g., [108,111–113]). It will suffice to summarize the three models that have emerged as being the most plausible, each apparently based on good experimental evidence. In one model, nascent VLDL obtains its full complement of core lipids in the rER. This is supported by lipid-composition studies on apoB-containing particles isolated from the ER lumen [112]. A two-step model (see Figure 2) has also been suggested in which part of the TAG content of the mature particles is incorporated into the apoB-containing particles in the rER during the co-translational insertion of the apoprotein into the ER lumen, whereas the rest is derived through enlargement with intralumenal TAG that occurs in globules which reside within the smooth ER (sER) [102]. This is supported by electron-micrographic evidence showing that TAG globules, which are not associated with apoB, occur within the lumen of the sER [114]. A third model is an elaboration of the two-step hypothesis and envisages that the lipoprotein particles originating in the rER are relatively lipid-poor, and that core lipids are added as the particles traverse the secretory system from ER through to the Golgi [115]. Interestingly, the work of Boren et al. [116], in which McA-RH7777 cells were used, indicated that apoB_{48}-VLDL is almost exclusively assembled through a two-step process, whereas a substantial proportion of apoB_{100}-containing particles formed during the co-translational insertion of apoB_{100} into the sER lumen occur in the VLDL density range. More recent experimental data obtained from the work on rat liver [116a] showed that, even in the less dense nascent particles (*d* 1.006), the amount of TAG associated with each particle was only approximately half that present in the mature VLDL present in the Golgi, and presumably secreted as VLDL. A denser type of particle within the rER (*d* 1.006–1.020), which contained almost exclusively apoB_{48}, was almost devoid of TAG. These data suggest that, if rER particles are precursors to Golgi VLDL, additional core lipids would need to be added to them to bring their composition up to that present in secreted VLDL. This would imply, in agreement with [102], that co-translational insertion of apoB into the ER lumen is accompanied by substantial acquisition of an amount of core lipid dictated by the size of the nascent polypeptide, with additional core lipid being added in a second step. The work in [116a] suggests that the two-step core-lipidation process could be extended to encompass not only the rER and sER, but also the Golgi, as previously suggested [117].

In all three models, it is suggested that the amount of apoB that is translocated into the lumen is only a fraction of the total synthesized, and that the protein destined for early degradation remains in the outer leaflet of the ER membrane [115]. In common with [102], the work in [115] suggests that a sizeable proportion of nascent particles that progress through the secretory pathway are not secreted, but are targeted for degradation. Both this suggestion, and the proposal that a separate pool of TAG exists within the sER within non-apoB-associated globules, raise the prospect that TAG metabolism (synthesis and/or lipolysis) within the lumen of the microsomal fraction can occur independently of that of apoB. For example, if the TAG within the nascent particles targeted for degradation is hydrolysed, the existence of an intralumenal lipase activity would need to be envisaged, and the fate of the constituent acyl chains (e.g. whether they are transported out of the lumen back into the

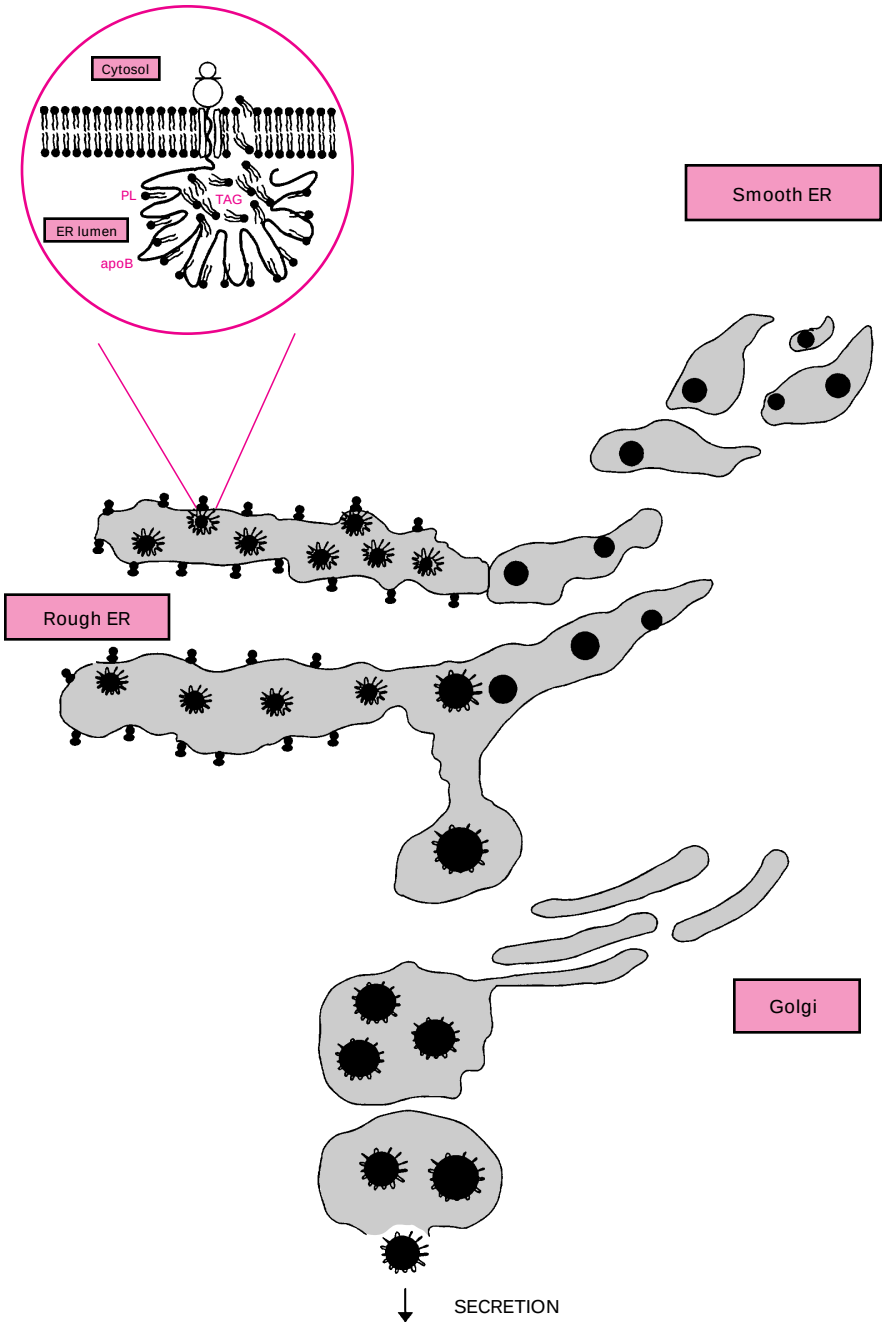

Figure 2 Diagrammatic representation of the two-step model for the assembly of VLDL in the liver

The inset shows the way in which co-translational insertion of $apoB_{100}$ into the lumen of the rER may be accompanied by the stabilization of the protein through the simultaneous acquisition of a hydrophobic core of TAG. The addition of TAG to nascent particles after fusion of sER and rER could be particularly important for the formation of $apoB_{48}$-VLDL. The diagram summarizes the suggestions made in [102,108,114,116,117].

cytosol; see discussion on the possible roles of microsomal carnitine acyltransferases, above) would need to be considered.

POOLS OF FATTY ACIDS, DIACYLGLYCEROL (DAG) AND TAG IN THE LIVER

There is heterogeneity with respect to the use of different intrahepatic pools of fatty acids for phospholipid or TAG synthesis. Fatty acids synthesized *de novo* by the liver are preferentially utilized for phospholipid synthesis, and these phospholipids are also preferentially secreted within VLDL [118]. Heterogeneity also exists in the route through which fatty acyl moieties reach the secretory TAG pool(s). The existence of multiple pools of TAG in the cytosol, ER membrane and ER lumen, each with distinct rates of turnover, has been established for some time [119–122]. The relationship between the cytoplasmic 'storage' pool of TAG and secreted TAG has been difficult to elucidate. It is evident from experiments conducted on

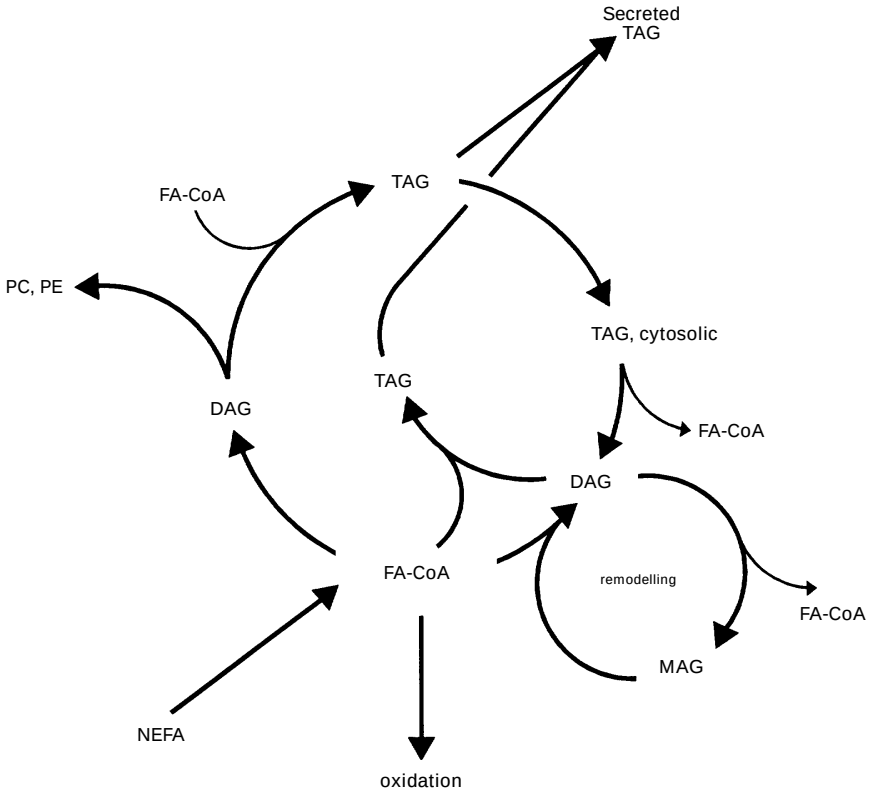

Figure 3 Schematic representation of the possible inter-relationships between different pools of intracellular TAG and DAG with respect to the secretion of VLDL-TAG by the liver

The scheme is based on proposals by several authors (see [120–125]). Further abbreviations: MAG, monoacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

isolated hepatocytes [123] and *in vivo* [124] that only a minor proportion of the flux leading from cytosolic TAG to secreted TAG is due to transfer *en bloc* of intact molecules of TAG. Rather, the cytosolic TAG has to undergo a degree of hydrolysis and re-esterification before being utilized for secretion. The work of Yang et al. [125], in which the acyl-chain composition of secreted TAG was compared with that of intrahepatic glycerolipids, suggested that the hydrolysis of cytosolic TAG only proceeds down to the DAG level, rather than completely to the component fatty-acyl moieties. It further suggests that, after remodelling of the DAG acyl composition, it is re-esterified to TAG, which is then secreted. Accordingly, there should exist at least two pools of DAG within microsomal membranes (in agreement with [121]), as well as two distinctly localized activities of diacylglycerol acyltransferase (DGAT). Evidence for the latter has emerged from fractionation studies performed on HepG2 cells [115]. It is of interest that the proportion of secreted TAG that has been calculated to be derived from cytosolic TAG in [125] is very similar to those calculated from experiments performed on isolated rat liver perfused with exogenous fatty acids [126, 127]. In this preparation, labelled fatty acids abstracted from the perfusate do not equilibrate with the cytosolic pool before they appear in the secreted TAG [126,127]. These same experiments all suggest, however, that a cycle of synthesis (with consequent dilution of label) and mobilization of cytosolic TAG accounts for about half of the overall TAG secretion rate, even when the liver is perfused with relatively high concentrations of fatty acid [126]. Moreover, this proportion does not appear to depend on the initial level of cytosolic TAG in the livers [126]. A model that would accommodate the recent and the previous observations is given in Figure 3, in which it is envisaged that TAG is synthesized *de novo* from component fatty acyl chains and glycerol phosphate, via phosphatidate, on the cytoplasmic face of the ER. This pool of TAG is only partly used directly for assembly into nascent lipoprotein particles by association with apoB during the protein's co-translational insertion through the membrane into the lumen of the ER. The TAG that is not immediately utilized for this purpose is directed into the cytosolic pool, which is constantly used to generate a second, distinct, pool of DAG. This pool of DAG, after remodelling of its acyl chain composition, is used to form TAG, presumably through the involvement of a second DGAT activity. It is not known whether this second pool of DAG is more likely to give rise to the non-apoB-associated TAG in the sER, where, in density gradients of homogenates of HepG2 cells, a distinct high-specific-activity peak of DGAT occurs [115]. In this context, it is noteworthy that the inability of HepG2 cells to secrete large, TAG-rich, VLDL [128] coincides with their inability to form apoB-free TAG particles (i.e. the absence of a 'second' step) [102], and that this is associated with the existence of a much lower rate of turnover (hydrolysis) of their cytosolic pool of TAG [129].

The scheme depicted in Figure 3 suggests that the phosphatidate-derived DAG pool that gives rise to the initial synthesis of TAG would also be available for phospholipid (phosphatidylcholine, phosphatidylethanolamine) synthesis, and therefore would represent a branch-point at which the control of partitioning between TAG and phospholipid synthesis can be exerted. In addition, as indicated in Figure 4, it would

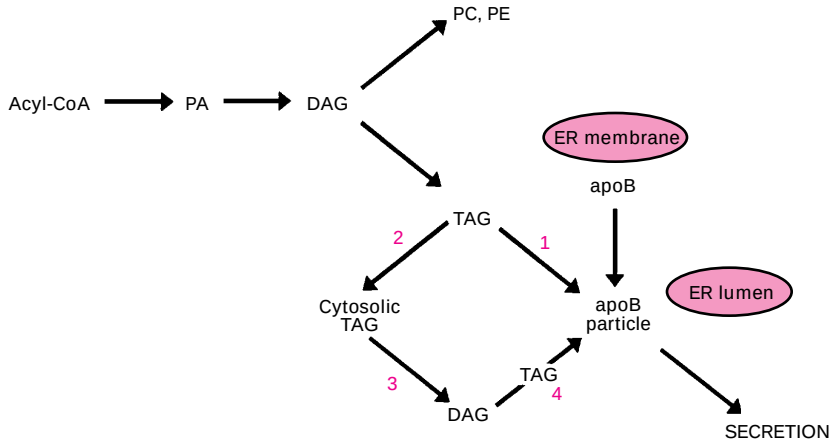

Figure 4 Dual routes may be involved in the incorporation of TAG into VLDL in the liver to determine the number and size of the particles secreted

TAG synthesized on the ER is considered to be partitioned between association with apoB to form nascent particles (step 1) and the formation of cytosolic TAG (step 2). The latter is hydrolysed (step 3) to form partial glycerides and fatty acyl-CoA. TAG is synthesized from the remodelled DAG (step 4) to give a second source of secretory TAG. This scheme is based on proposals presented in [123,161]. Further abbreviations: PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

imply that the partitioning of TAG between retention in the liver and secretion into the plasma can be achieved through two mechanisms: (i) by altering the fraction of apoB available for assembly of TAG into nascent particles in the rough ER (i.e. between steps 1 and 2 in Figure 4), and (ii) by altering the rate of cytosolic TAG hydrolysis (step 3). Such dual loci of regulation would be expected to result in the control of both the size of the cytosolic TAG pool and the size of the secreted VLDL particles. There is no evidence at present as to whether distinct TAG pools are preferentially utilized for the 'first' and 'second' step phases of incorporation of TAG into the hydrophobic core of VLDL. If they were to be so used, then the existence of separate routes for TAG secretion would enable control to be exerted on the number and size of the secreted particles, and thus allow these parameters to be differentially affected by the supply of fatty acid and carbohydrate substrates to the liver [130,131]. Insulin is known to decrease the availability of apoB for secretion by increasing the rate of early degradation of the newly synthesized protein (see [132]). In addition, insulin appears to be able to inhibit utilization of the cytosolic pool of TAG for secretion [9,133], although, paradoxically, the hormone does not affect the rate of turnover of cytosolic TAG [123] (but see below).

As mentioned above, the existence of a separate pool of DAG that is utilized to form non-apoB-associated TAG within the lumen of the ER would imply the existence of a second population of DGAT enzyme molecules. Evidence for two peaks of DGAT activity, one associated with the rER and one (the larger) with the sER, already exists (see above). As pointed out in [125], although the formation of DAG through hydrolysis of cytosolic TAG would have the advantage of producing a membrane-permeant intermediate, the current consensus is that DGAT activity is exclusively located on the cytosolic face of the ER membrane. In fact, this is not entirely supported by the data in [134], which show that a substantial proportion of DGAT activity may be latent in intact rat liver microsomes. This is in contrast with the case for the activities of all the other enzymes involved in glycerolipid synthesis, with the possible exception of DAG cholinephosphotransferase [134]. It is possible, therefore, that DAG generated from cytosolic TAG permeates the ER membrane and is used for TAG resynthesis on the lumenal side of the membrane, i.e. acyl moieties would have to be transported across the ER membrane for esterification of DAG to occur. The possibility that a substantial flux of acyl chains may occur across the microsomal membrane has emerged from the above-mentioned observation that microsomal membranes have separate cytosol-facing and lumenal carnitine acyltransferase activities [24,29]. If a carnitine–acylcarnitine carrier analogous to that present in the mitochondrial inner membrane exists in the microsomal membrane, then it would be possible for carnitine-dependent transfer of acyl chains to occur across the membrane and regenerate acyl-CoA within the lumen of the ER. However, there is no suggestion that these activities are enriched in either rER or sER [29], and the chain-length specificity of the cytosol-facing enzyme suggests that it is not particularly active with long-chain acyl-CoA esters as substrates [29]. However, teleologically, carnitine-dependent transfer of acyl chains across the ER membrane would provide an additional locus of control by insulin, as the cytosol-facing microsomal carnitine acyltransferase is sensitive to malonyl-CoA inhibition [24,29]. Consequently, insulin, by raising cytosolic malonyl-CoA concentrations, would simultaneously increase the overall rate of fatty acid esterification (by inhibiting fatty acid oxidation at the reaction catalysed by mitochondrial CPT I; see above) and inhibit the transfer of fatty acids into the lumen for re-synthesis of TAG from DAG derived from the hydrolysis of cytosolic TAG. Such a role would resolve the paradox as to how insulin is able to inhibit the utilization of cytosolic TAG for secretion [9,133] without diminishing the rate of turnover of this pool [123]. If insulin were to be able not only to enhance apoB degradation, but also to inhibit secretory TAG resynthesis from DAG (step 4 in Figure 4), it would be able simultaneously to decrease the number and size of the VLDL particles secreted. Evidence that insulin achieves the inhibition of TAG secretion in cultured hepatocytes through both mechanisms has been presented [135]. However, these suggestions are necessarily highly speculative at present.

EXPERIMENTAL OBSERVATIONS FOR A ROLE OF INSULIN IN TAG SECRETION

The effect of insulin on hepatic TAG secretion has been controversial to determine. Thus, whereas experiments conducted with isolated perfused rat liver showed that insulin stimulates

both synthesis and secretion of TAG [136,137], many subsequent studies conducted on cultured hepatocytes have found that insulin, although promoting synthesis of TAG, inhibits its secretion [6,8,133,138,139]. The reasons for this discrepancy between the two sets of data may reside in the use of different experimental systems (perfused liver, cultured cells) and in the choice of the precise perfusion or culture conditions, by different laboratories. Thus Topping et al. [137] have pointed out that the perfusion rate, haematocrit and level of oxygenation of the medium used to perfuse the liver *in vitro* all affect the action of insulin. Laker and Mayes [136] suggest that cultured cells are not very secretion-competent compared with the perfused liver and may not be the best system in which to study the effects of insulin. Conversely, Sparks and Sparks [113] suggest that the stimulatory effect of insulin on TAG secretion observed in the perfused liver may have been related to glucocorticoid effects. In addition, it is apparent that TAG synthesis and secretion respond to different concentrations of insulin in cell-culture experiments, higher ones being required to inhibit secretion [8]. There also seems to be disagreement as to whether insulin only affects apoB degradation [139], or whether it also affects apoB synthesis [140]. Moreover, prolonged exposure (5 days) of cultured rat hepatocytes to insulin enhanced $apoB_{48}$ secretion by promoting apoB mRNA editing [141].

Experiments using cultured rat hepatocytes and HepG2 cells have indicated that insulin could have a biphasic effect on TAG secretion. Culture of hepatocytes with insulin for 6–48 h results in the inhibition of secretion, whereas more prolonged culture (up to 72 h) results in stimulation of secretion (over a diminishing control value) [142–144]. Although this phenomenon has not always been observed [6,145], it has been interpreted as indicating that insulin acts acutely on the liver to inhibit TAG secretion, whereas, when the liver is chronically exposed to high levels of insulin, it becomes resistant to the hormone and the effect is lost. Consequently, it has been suggested that acute exposure of the liver to surges in portal insulin concentration (such as would occur during the prandial period) would result in the inhibition of TAG secretion, whereas more prolonged exposure of the liver to hyperinsulinaemic conditions (e.g. in insulin-resistant states) would make it refractory or resistant to this effect (see, e.g., [8,132,146]). If these observations can be extrapolated to situations *in vivo* characterized by chronic hyperinsulinaemia and insulin resistance (e.g. non-insulin-dependent diabetes mellitus, obesity), they would offer an explanation for the apparently paradoxical observations that hyperinsulinaemic conditions are normally associated with hypertriglyceridaemia, partly due to increased hepatic TAG secretion [147,148]. They would imply that, as the rate of delivery of NEFA to the liver is increased, because of the diminished anti-lipolytic action of insulin on adipose tissue, the increased rate of TAG synthesis would not be counteracted by insulin-mediated inhibition of secretion. Consequently, insulin resistance would be accompanied by an increased rate of hepatic TAG secretion, which would contribute towards the dyslipidaemia associated with these conditions because of the failure (through insulin resistance) of both extra- and intrahepatic mechanisms [2,3].

In order to start addressing these questions *in vivo*, experiments in my laboratory have been performed on acute increases in insulin concentrations and prolonged insulin deficiency. Acute exposure of the liver to insulin occurs during the prandial period. Consequently, experiments using the specific labelling of hepatic fatty acids *in vivo* [4,77] have been aimed at addressing the question as to whether the partitioning of hepatic glycerolipid flux is altered during the prandial period upon refeeding of starved rats or in animals subjected to a meal-feeding regime. Detailed time courses were obtained for the partitioning of acyl-CoA (see above), DAG (between the labelling of TAG and of the major phospholipids) and of TAG between retention within the liver and secretion into the circulation. These experiments led to the description of concomitant acute decreases in the proportion of DAG that is used for the synthesis of TAG, and of the latter that is secreted [4,77,79,80]. The effects were apparent within 1 h of the refeeding of starved rats, reached a peak at 2–3 h and were relatively rapidly reversed thereafter, although they persisted longer in meal-fed animals. The time courses for the two effects are very similar, suggesting that they are mediated by the same mechanism or hormonal action. The synchronization of the two effects is noteworthy in that the two metabolic branch points concerned occur sequentially. Consequently, the overall effect of the two acute changes in metabolic partitioning is multiplicative; the proportion of the acyl chains esterified to the glyceryl moiety that is secreted by the liver as VLDL-TAG would be expected to be acutely and markedly diminished. In absolute terms, the rate of secretion of TAG would additionally be affected by the acute decrease in the rate of delivery of NEFA to the liver during the prandial period [149]. The role for intrahepatic mechanisms that specifically curtail TAG synthesis and fractional rate of secretion may arise from the inability to decrease NEFA supply to the liver rapidly enough and to a sufficiently low level (see [4]) to achieve the required degree of inhibition.

Two questions arise about these acute changes in DAG and TAG partitioning within the liver. Firstly, which enzymes/processes are likely to be involved? Secondly, are the changes in partitioning mediated (solely) by insulin?

PARTITIONING OF DAG BETWEEN PHOSPHOLIPID AND TAG SYNTHESIS

Two mechanisms could be involved in mediating the change in partitioning of DAG observed *in vivo*. It is possible that the affinity of DGAT for DAG in the microsomal membrane is lower than that of CDP-choline and CDP-ethanolamine acyltransferases, as suggested in [150,151], such that a decreased rate of DAG synthesis (owing to a decreased rate of delivery of NEFA to the liver) would automatically favour its partitioning into phospholipids [152–154]. As fatty acids are known to activate DGAT [154], the sharp decline in NEFA supply to the liver upon refeeding of starved rats could result in the attenuation of DGAT activity (see below). Evidence from experiments on permeabilized hepatocytes indicates that phosphatidylcholine and TAG syntheses utilize the same pool of DAG [155], although, as discussed above, at least one additional pool of microsomal membrane DAG may exist [121,122,125]. Alternatively, altered DAG partitioning could involve acute changes in the activities of the enzymes that exert major control over the respective pathways through post-translational mechanisms. Little is known about the possible mechanisms involved in the acute control of phosphatidylethanolamine biosynthesis. However, in the case of phosphatidylcholine biosynthesis, the properties of the enzyme that catalyses the main rate-controlling step (CTP:phosphocholine cytidylyltransferase, CT) are well characterized (see [156] for recent review). The activity of CT is affected by the availability of fatty acids, which enhance the association of this ambiquitous enzyme with a membranous fraction of the cell, where its substrate resides, thus effectively recruiting inactive, soluble, enzyme to become active at the site of phosphatidylcholine synthesis [157]. This translocation on to the membrane could involve an increase in the DAG content of the membrane [158,159], although, as intimated above, in normal rats during the prandial/early-absorptive period, this is unlikely to be due to

an increase in the supply of substrate for DAG synthesis. However, other mechanisms for increasing membrane DAG may exist (see below). Interactions of CT with membrane lipids (through a hydrophobic stretch in its primary amino acid sequence) result in its activation [160]. The particulate fraction with which the enzyme becomes associated has been conventionally assumed to be the microsomal membrane, but relatively recent immunocytochemical evidence suggests that translocation occurs to the nuclear membrane [161,162]. The significance of such a localization is not clear at present. The translocation process has also been suggested to be affected by the reversible phosphorylation of CT by cyclic AMP-dependent protein kinase [163]. Although addition of cyclic AMP analogues to intact hepatocytes or the elevation of endogenous cyclic AMP concentrations does not appear to affect either the phosphorylation or the membrane association of CT with the membrane [164], it has been suggested that the effects of increased cellular cyclic AMP could be mediated through a decrease in membrane content of DAG [158]. Therefore, it may be relevant that in starved-rat liver the activity of cyclic AMP-dependent protein kinase and the concentration of cyclic AMP are increased, and that during refeeding they are both rapidly decreased [68,165]. Evidently experiments need to be conducted, on liver samples obtained *in situ*, to find out directly whether changes in membrane DAG content and translocation of CT are involved in the acute increase in the partitioning of TAG towards phospholipids.

The activity of DGAT is thought to be modulated by reversible phosphorylation; incubation of rat liver microsomal fractions with ATP and Mg^{2+} inhibits the activity of DGAT [166,167]. Conversely, the activity of the enzyme is increased by fatty acids [153,154]. Consequently, it is possible that the decreased availability of NEFA to the liver during the prandial period could be involved in lowering the activity of DGAT, and hence the relative flux of DAG directed towards TAG formation. No information is available as to whether DGAT activity is acutely affected by exposure of the liver to insulin.

PARTITIONING OF TAG BETWEEN SECRETION AND RETENTION IN THE LIVER

The possibility that TAG may follow two separate routes to join the secretory pathway raises the prospect that partitioning of TAG between secretion and retention in the liver (i.e. the fractional rate of secretion) is determined through control at two loci. As discussed above, an amount of TAG is required for the formation of nascent apoB-containing particles during the co-translational insertion of the apoprotein through the ER membrane. Consequently, the proportion of apoB available for secretion would be expected to be involved in determining the fractional rate of secretion of TAG at this site, with the 'excess' TAG being diverted towards the cytosolic pool. This concept is supported by early work on the isolated perfused liver which showed that TAG secretion is proportional to the rate of fatty acid uptake (i.e. perfusate fatty acid concentration) until a maximal rate is reached, beyond which TAG starts to accumulate in the liver [168]. Insulin may exert a major effect at this step, as it enhances the rate of degradation of apoB, thus making less of the protein available to the secretory pathway. ApoB is synthesized at a rate in excess of that required to meet the requirements of its rate of secretion in VLDL. Its rate of secretion does not appear to be determined at the transcriptional level (see [169] for review), although there is one report that insulin inhibits secretion of apoB partly through the inhibition of apoB synthesis and partly through the stimulation of its degradation [140]. In general, however, the main determinant of the rate of apoB secretion appears to be the rate of intracellular degradation of the protein. Newly synthesized polypeptide that remains associated with the ER membrane, rather than being translocated into the lumen, is degraded. In addition, a proportion of apoB-containing nascent particles within the lumen are also targeted for degradation [108]. This possibility is supported by the results of experiments on HepG2 cells, in which a 70 kDa product of apoB degradation was found associated partly with the lumenal contents of the ER [170]. Insulin-enhanced degradation only affects a proportion of total apoB and appears to have different characteristics from constitutive degradation [113,140]. However, it is not known whether it is the availability of apoB that determines the fraction of TAG that is channelled towards secretion, or vice versa; evidently increases in TAG secretion can occur in the absence of any increase in apoB secretion [130]. From experiments conducted on cultured hepatocytes obtained from fed or starved rats, Davis et al. [171] concluded that the availability of $apoB_{48}$, which is the major B apoprotein in rat liver, determines the rate of TAG secretion. The question has also been addressed by Boren et al. [108], working on HepG2 cells. They concluded that it is the availability of TAG that determines the proportion of apoB that is committed to secretion and spared degradation within the lumen of the ER; increased availability of TAG did not determine the fraction of apoB translocated into the ER lumen, but increased the number of mature VLDL particles formed [108]. More recent work from this group [116] has further supported this conclusion, and the two-step hypothesis in general.

When the meal-induced effects on the partitioning of DAG and on the fractional rate of secretion of TAG were first described for rat liver *in vivo* [77,79,80], it was assumed that they were necessarily mediated by first-phase insulin release [113,132]. This conclusion appeared to be supported by the fact that the effects were slightly more pronounced and longer-lasting in meal-fed rats, in which the prandial release of insulin is more pronounced. In an attempt to determine more directly the role of insulin in the diversion of acyl-CoA away from TAG synthesis and secretion during the prandial period *in vivo*, refeeding experiments were performed on starved–refed streptozotocin-diabetic rats [172]. In spite of a much attenuated insulin-secretory response to food intake [86], the liver of these animals showed the same pattern of acute changes in the partitioning of DAG and in the fractional rate of secretion of TAG (see Table 1 and Figure 5). Indeed, the effects occurred more rapidly and were longer-lasting than in

Table 1 Effects of starvation and refeeding or insulin treatment of normal or diabetic rats on the partitioning of [^{14}C]oleate label between phospholipid and TAG labelling and on the fractional rate of [^{14}C]TAG secretion *in vivo*

Rats were starved for 24 h before being refed (for 2 h) or given an intraperitoneal injection of insulin (15 units/kg) 2 h before being used. [^{14}C]Oleate label was delivered specifically to the liver as described in [77]. Abbreviations: PL, phospholipids; GL, glycerolipids. Data are from [77,79,80,86,172,173].

	^{14}C in PL as % of ^{14}C in total GL	[^{14}C]TAG secreted as % of total labelled
Fed (6)	14.2 ± 2.0	57.1 ± 3.3
Starved (6)	16.7 ± 1.8	60.5 ± 2.4
Starved, refed (4)	33.3 ± 2.1	39.2 ± 3.4
Diabetic (4)	15.9 ± 1.1	68.9 ± 4.6
Diabetic, starved (4)	24.0 ± 3.0	75.2 ± 3.0
Diabetic, starved–refed (3)	78.3 ± 6.3	27.4 ± 12.9
Diabetic, insulin treated (3)	23.8 ± 2.5	57.9 ± 1.7

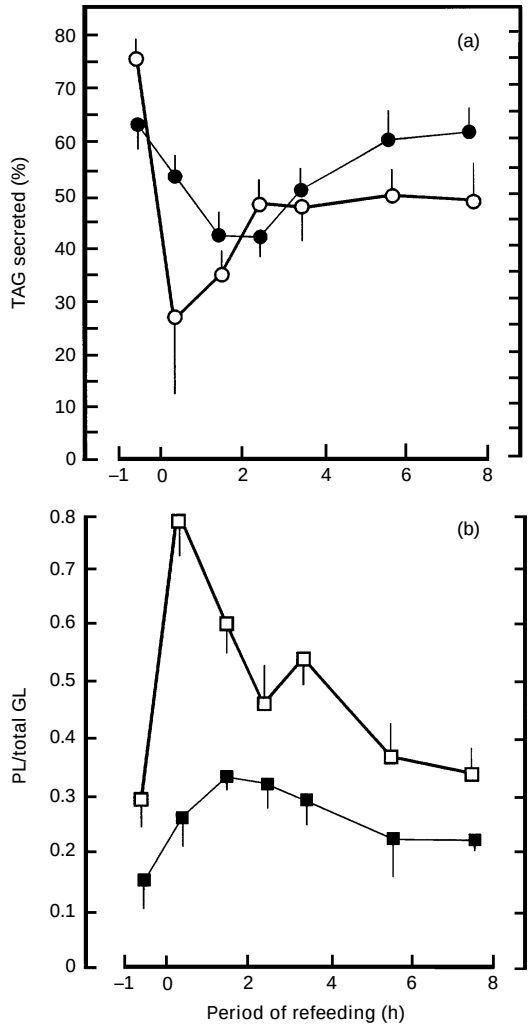

Figure 5 Comparison of the time-courses for changes in (a) the fractional rate of secretion of newly synthesized [^{14}C]TAG, and (b) the partitioning of [^{14}C]oleate between phospholipids (PL) and total glycerolipids (GL) after specific labelling of hepatic oleate *in vivo* in 24 h-starved diabetic (○, □) and normal (●, ■) rats refed for the periods indicated

Data are from [77] and [172].

normal rats. By contrast, when diabetic rats were treated with insulin, there was no inhibition of the fractional rate of secretion of TAG, and only a minor decrease in the proportion of total glycerolipid labelling that was accounted for by TAG [173]. Consequently, it is unlikely that insulin has an obligatory role in mediating the effects of food intake on hepatic glycerolipid partitioning. Other mechanism(s) must exist to act in synergy with the hormone in normal animals, and/or to compensate for the attenuation of its putative effect in diabetic animals.

As discussed above for the case of acyl-CoA partitioning between oxidation and esterification, an additional mechanism through which liver metabolism could be affected during the prandial phase involves acute changes in hepatocyte hydration state (i.e. cell volume). Increased concentrations of osmolyte amino acids or metal ions (e.g. K^+) would increase hepatocyte water content (see [174]). Increased cell volume is known to affect hepatocyte metabolism in many respects, including the stimulation of glycogen and fatty acid synthesis, inhibition of fatty acid oxidation and the induction of gene transcription (see [175] for recent review). Experiments have therefore been conducted to determine whether the presumed hepatocyte swelling that would be induced *in vivo* during the absorptive phase could provide the signal whereby DAG is diverted towards phospholipid synthesis and the fractional rate of TAG secretion is lowered. Isolated rat hepatocytes were incubated *in vitro* under conditions that increase their water content and the intracellular volume (i.e. either by incubation with the glutamine or in hypo-osmotic media) to check whether cell swelling can mimic the effects on glycerolipid metabolism observed *in vivo* during the prandial/early-absorptive phase. Several key observations have emerged from these studies [176]. Hepatocyte swelling results in (i) an increase in the proportion of exogenously supplied [^{14}C]palmitate that is incorporated into phospholipids, at the expense of TAG labelling (this effect is counteracted by concentrations of fatty acids above 0.5 mM), and (ii) an inhibition of the overall rate of [^{14}C]TAG secretion, as well as of the fraction of newly synthesized TAG that is secreted [176]. Thus, both effects observed *in vivo* on the partitioning of DAG and TAG can be mimicked *in vitro* by exposure of isolated hepatocytes to conditions that result in a 10–20 % increase in cell volume. These observations may explain why the changes in glycerolipid metabolism that accompany food intake are unimpaired in severely diabetic rats [172], especially since the rate of Na^+ co-transport of amino acids such as glutamine and alanine is induced several-fold in diabetic-rat hepatocytes [177]. They do not exclude the possibility, however, that in the normal animal the effects of increased portal substrate/ion concentrations on cell volume could act synergistically with the effects of insulin. Indeed, insulin itself could exert part of its action on TAG secretion through the effects it exerts on cell volume in its own right, as it increases cell volume in the isolated perfused rat liver [178] and cultured rat hepatocytes [179]. The hormone may therefore act to decrease apoB and TAG secretion through an effect on hepatocyte volume. It may be relevant that both insulin treatment and hypotonic swelling of cultured rat hepatocytes result in the activation of several, and not entirely overlapping, peaks of mitogen-activated protein (MAP) kinase activity in hepatocyte extracts [180]. Thus insulin could act synergistically with portally delivered substrates to induce hepatocyte volume increases to feed into overlapping signalling mechanisms. In insulin-deficient or -resistant states, the substrate-mediated insulin-independent effects would still be operative, provided that the peak concentrations of volume-active metabolites and/or ions in the portal vein are sufficiently high. As mentioned above, if activation of osmolyte amino acid transport across the plasma membrane of hepatocytes is associated with relative or absolute insulin deficiency (e.g. starvation, diabetes [177,181,182]), the effects of osmolyte amino acids in the portal circulation could be amplified to compensate for the attenuation or absence of insulin action. It would therefore be expected that the composition of the diet is likely to be important in determining whether the liver is able to respond by decreasing its rate of TAG secretion during the prandial period, especially in insulin-deficient or -resistant states. The physiological requirement for the curtailment of hepatic TAG secretion during the prandial/early-absorptive phase is possibly more acute in individuals suffering from these conditions, owing to the exacerbation of hypertriglyceridaemia due to a diminished rate of clearance of chylomicron-TAG by peripheral tissues [183,184]. It would therefore be of interest to study the dependence of the extent of $apoB_{100}$-associated post-prandial hypertriglyceridaemia, in response to a given fat load in humans, on the ability of the other components of the meal to generate osmotically active substrates for hepatocyte plasma-membrane

transporters. Such considerations are important, in view of the association between the degree of post-prandial lipaemia and the development of coronary heart disease [101,185]. The effects of increased hepatocyte volume on TAG secretion suggest that detrimental effects of absolute or relative insulin deficiency on the severity of post-prandial lipaemia can be counteracted by appropriate dietary intake.

CONCLUDING REMARKS

As the complexities of the regulation of hepatic fatty acid metabolism are unravelled, it becomes increasingly evident that the control of the pathways involved is distributed over several loci. This is true both for the partitioning of acyl-CoA between oxidation and esterification, and for control within these two major pathways themselves. An example of this has emerged recently from studies on the distribution of control exerted *in vivo* by dietary fatty acids on the different partitioning steps discussed in this review [186]. Insulin can affect hepatic fatty acid metabolism at several different points in the two pathways. Moreover, it can act on the liver indirectly through its effects on NEFA supply to the tissue. The observation that NEFA concentration can interact with the effects of insulin on glycerolipid metabolism in the perfused liver [7], and with the effects of cell swelling in isolated rat hepatocytes [176], as well as on apoB degradation and secretion [7,113] in cultured cells, emphasizes the degree of integration that is possible between the intra- and extra-hepatic effects of the hormone. The emergence of the modulation of cell volume as a regulatory mechanism through which portal substrates, as well as insulin itself (and other hormones), may partly exert their metabolic effects has raised the possibility that substrate-driven effects may act synergistically with the hormone to achieve acute changes in hepatic metabolism in response to food intake. Both fatty acid oxidation and TAG synthesis and secretion appear to be candidates for this mode of synergy between the effects of insulin and of the hydration state of the hepatocyte.

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