Effects of hydration state on the synthesis and secretion of triacylglycerol by isolated rat hepatocytes

Implications for the actions of insulin and glucagon on hepatic secretion

Victor A. ZAMMIT

Hannah Research Institute, Ayr, Scotland KA6 5HL, U.K.

The effects of hepatocyte volume on the secretion of triacylglycerol were studied in order to test the suggestion that increases in the portal concentrations of osmolyte amino acids and metal ions during the prandial/early-absorptive phase may be involved in mediating the acute changes in glycerolipid metabolism observed *in vivo* [Zammit (1995) *Biochem Soc. Trans.* 23, 506–511]. Incubation of isolated rat hepatocytes with hypo-osmotic medium or in the presence of glutamine (in the presence or absence of leucine), conditions which gave an increase in cell water content of between 8 and 27%, resulted in a decrease in the rate of [14 C]triacylglycerol (TAG) secretion when [14 C]palmitate was used as substrate. The inhibition was proportional to the increase in cell water content. At low exogenous palmitate concentration (0.05 mM), the inhibition of [14 C]TAG secretion was accompanied by a marked shift in the incorporation of label from TAG to phospholipid. In the presence of 0.5 mM palmitate this effect was attenuated, and in the presence of 1 mM palmitate it was abolished. Increased cell volume associated with incubation

of hepatocytes with glutamine (in the presence or absence of leucine) also resulted in a decrease in the fraction of newly labelled TAG that was secreted into the medium. Decreased cell volume, achieved by incubation of hepatocytes with hyper-osmotic medium (sufficient to decrease cell water content by approx. 9%) decreased overall [14 C]TAG secretion, but did not affect the amount of label that was incorporated into phospholipid as a fraction of that incorporated into total glycerolipids. Cell shrinkage, however, diminished the fraction of newly labelled [14 C]TAG that was secreted. When intracellular TAG was prelabelled with [3 H]glycerol, it was found that cell shrinkage markedly inhibited (preformed) [3 H]TAG secretion, whereas cell swelling did not affect this route of TAG secretion. The data are discussed in terms of the possible action of changes in cell hydration at the different loci at which hepatocyte TAG secretion is controlled, with reference to previous observations that both insulin and glucagon are able to inhibit TAG secretion in cultured rat hepatocytes and HepG2 cells.

INTRODUCTION

During the prandial/early-absorptive period, the liver undergoes acute changes in the partitioning of glycerolipid metabolism that are thought to result in the inhibition of triacylglycerol (TAG) secretion [1,2]. The evidence for this emerged from experiments *in vivo* in which the hepatic fatty acid pool was specifically labelled and the glycerolipids that accumulated in the liver or were secreted into the plasma were monitored over 1 h periods during refeeding of 24 h-starved [3], meal-fed [4] or starved-diabetic rats [5]. Specifically, the proportion of the [14 C]oleoyl moiety that is recovered in phospholipids (PL) as a fraction of total glycerolipid labelling is increased 3–4-fold over the first 2 h of feeding, and gradually returns to normal (about 15% of total) over the following few hours. Concomitantly, there is an acute inhibition of the fraction of newly labelled TAG that is secreted into the circulation, with correspondingly more being retained within the liver [2,6]. This phasic inhibition of the fractional rate of secretion of TAG displays a very similar time course to that observed for partitioning between PL and TAG labelling. Therefore, the suggestion has been made that the two effects are mediated by a common mechanism [4].

Insulin inhibits TAG and apolipoprotein B (apoB) secretion in primary cultures of rat hepatocytes and HepG2 cells [7–10]. Moreover, several studies conducted on humans have shown that intravenous infusion of insulin acutely decreases the rate of very-low-density-lipoprotein (VLDL) secretion by the liver [11–13]. Consequently, the prandial effects on diacylglycerol (DAG) and TAG partitioning could be interpreted as reflecting the

action of insulin on the liver in awake, unrestrained, rats during the prandial period [4,14–16]. However, the observation that these changes in glycerolipid metabolism are observed even in severely diabetic rats, in which refeeding is not accompanied by significant insulin secretion [5], suggests that, even if insulin may be involved in the response observed in normal animals, the hormone may act synergistically with other meal-induced stimuli to elicit the hepatic responses. These alternative stimuli would require to compensate for the absence of a normal insulin-secretory response in diabetic animals (see [6]).

One insulin-independent stimulus that the liver receives during the prandial period is an increase in the portal concentrations of metal ions (e.g. K^+) and of amino acids that are co-transported with Na^+ across the plasma membrane, and are therefore powerful osmolytes. Such an increase in portal amino acid concentrations is thought to be sufficient to increase hepatocyte volume to an extent (10–20%) at which several metabolic processes are affected (see [17] for recent review). For example, increases in hepatocyte volume have been shown to activate glycogen and fatty acid synthesis and to inhibit fatty acid oxidation [18]. In general, the effects mediated by amino acid osmolytes (e.g. glutamine) can be mimicked by increased cell hydration induced by incubation of hepatocytes, or perfusion of the isolated liver, with hypo-osmotic medium [18–20], although specific effects due to intracellular accumulation of glutamate and chloride on specific protein phosphatases have been described [20].

Therefore, in the present study the possibility that the acute changes in glycerolipid metabolism observed *in vivo* could be

Table 1 Effects of different incubation conditions on the hepatocyte wet-weight/dry-weight ratio after 60 min of incubation in the presence of fatty acid substrate

Values are expressed as a percentage of that in iso-osmotic medium for the same preparation. Asterisks indicate values that were statistically significantly different ($P < 0.01$) from controls. The 100% values for iso-osmotic medium were 3.98 ± 0.20 , 3.80 ± 0.06 and 4.66 ± 0.42 for incubations containing 0.05 mM, 0.5 mM and 1.0 mM palmitate respectively.

Medium	Palmitate concn. ...	Wet-wt./dry-wt. ratio		
		0.05 mM ($n = 3$)	0.5 mM ($n = 4$)	1.0 mM ($n = 3$)
Iso-osmotic		100	100	100
Hypo-osmotic		$109.5 \pm 1.8^*$	$108.6 \pm 1.3^*$	105.4 ± 1.6
Hyperosmotic		$91.8 \pm 0.8^*$	—	$91.3 \pm 0.8^*$
Iso-osmotic + glutamine (10 mM)		—	$113.1 \pm 2.3^*$	—
Iso-osmotic + glutamine (10 mM) + leucine (2.5 mM)		$128.8 \pm 3.8^*$	$117.6 \pm 1.6^*$	$120.4 \pm 4.4^*$

mediated by increases in cell volume induced by increases in the portal concentrations of osmolytes absorbed from the gut has been investigated through the use of isolated rat hepatocytes incubated under conditions that result in a range of hydration states. The data indicate that the diversion of DAG towards PL synthesis and the inhibition of the fractional rate of secretion of TAG can be mimicked in isolated hepatocytes by cell swelling induced by incubation with amino acids or under hypo-osmotic conditions. The former effect is antagonized by increasing fatty acid concentrations. Cell shrinkage induced by a hyperosmolar medium also affects TAG secretion, but through a distinct mechanism. The implications of these findings for the synergistic effects of increased portal amino acid and/or ion osmolyte concentrations, insulin secretion and decreased plasma non-esterified fatty acids (NEFA), as well as for the action of glucagon upon VLDL-TAG secretion, are discussed.

MATERIALS AND METHODS

Animals

These were female Wistar rats (200–250 g) fed *ad libitum* on a standard laboratory-rat diet (see [3]). They were kept under a 12 h-light/12 h-dark cycle and were used for hepatocyte preparation 3 h into the light phase.

Preparation and incubation of hepatocytes

Hepatocytes were isolated by the method of Seglen [21], as previously described [22]. Unless otherwise indicated, they were incubated at 37 °C, with shaking, in silicone-treated 25 ml conical flasks containing 4 ml of medium, at a density of approx. 4×10^6 cells/ml. Before any of the experimental protocols were started, hepatocytes were equilibrated for 20 min in the respective media used. Iso-osmotic Krebs medium [23] contained 120 mM NaCl, whereas hypo-osmotic medium contained only 75 mM NaCl. Hyperosmotic medium had the same ionic composition as iso-osmotic medium, but in addition contained 80 mM sucrose. Where indicated, amino acids were present at concentrations of 10 mM (glutamine) and 2.5 mM (leucine). All media contained a final concentration of 2% BSA (defatted and dialysed). When the effects on newly synthesized TAG were studied, [14 C]palmitate (Na^+ salt) was added at zero time (0.4 μCi per flask) complexed to BSA to obtain the concentrations indicated. A parallel series of flasks from which [14 C]palmitate was omitted, but which were otherwise identically treated, was run concurrently and used to obtain packed cell pellets, the wet and dry weights of which were determined 30 and 60 min after

the addition of substrate. Radioactive incubations were stopped at the times indicated by centrifugation of the cells and removal of the supernatant. Total lipid extracts of cell pellets and supernatants were prepared by using chloroform/methanol (2:1, v/v). Separation of the different lipid classes was performed by TLC as described previously [3]. When the effects of incubation conditions on the secretion of preformed cellular TAG were studied, the cellular TAG was pre-labelled by incubation in bulk with 10 mM [^3H]glycerol (1 $\mu\text{Ci}/\mu\text{mol}$) for 45 min at 37 °C in iso-osmotic medium (in the presence or absence of 1 mM palmitate and 2% BSA, as indicated). They were then washed twice with the same medium, and samples (1 ml) were added to flasks containing media (3 ml) whose composition was adjusted so that, upon dilution with these samples, the same compositions as those given above for iso-osmotic, hypo-osmotic and hyperosmotic media were attained. Incubations were then allowed to proceed at 37 °C for up to 90 min, and at the times indicated they were stopped as described above.

Materials

[14 C]Palmitate and [^3H]glycerol were obtained from Amersham International (Amersham, Bucks., U.K.).

RESULTS AND DISCUSSION

Experiments were performed at three different concentrations of added fatty acid (0.05, 0.5 and 1.0 mM palmitate) in the presence of a constant amount of BSA (2%), in order to investigate the interaction between the effects of hepatocyte hydration state and changes in ambient fatty acid concentration. The amount of labelled material was held constant. In all instances, most of the fatty acids (> 90%) in the medium were utilized within the first 30 min of incubation. The different incubation conditions did not affect this parameter. The hydration state of the isolated hepatocytes was altered by incubation in hypo-osmotic (by lowering the NaCl concentration) or hyperosmotic (by adding 80 mM sucrose) media. In addition, experiments in which glutamine (10 mM) was added, either alone or in the presence of 2.5 mM leucine, to iso-osmotic medium were also performed (leucine does not, by itself, increase cell volume, but it enhances the effect of glutamine [18]). Cell hydration state was assessed by measurement of wet and dry cell weight (Table 1). As expected, incubation with glutamine and leucine resulted in a larger increase in cell wet-weight/dry-weight ratio, with intermediate values being obtained after incubation in hypo-osmotic medium or in the presence of glutamine alone. Hyperosmotic medium resulted in the expected decrease in cell weight.

Figure 1 Effect of hypo-osmotic and amino-acid-induced swelling, and of hyperosmotic-medium-induced shrinkage, of rat hepatocytes on the rate of [^{14}C]TAG secretion during incubation with (a) 0.05 mM, (b) 0.5 mM and (c) 1.0 mM [$1\text{-}^{14}\text{C}$]palmitate

Cells were preincubated for 20 min at 37 °C with shaking in iso-osmotic medium only (●), or in the additional presence of 10 mM glutamine (◆) or 10 mM glutamine and 2.5 mM leucine (○). Other cells were incubated with hypo-osmotic (△) or hyperosmotic (■) medium before addition of palmitate complexed to BSA (see the Materials and methods section). Values of [^{14}C]TAG secretion are given as a percentage of the maximal amount observed after 90 min in iso-osmotic medium for the same preparation of cells. The numbers of separate cell preparations were as shown in Table 1.

Table 2 Interaction between cell swelling and fatty acid concentration on the relative degree of [^{14}C]palmitate incorporation into PL as a proportion of total glycerolipid (GL) labelling after 30 min incubation

Values are means ($\pm$ S.E.M.) for separate hepatocyte preparations. Those significantly different ($P < 0.02$) from iso-osmotic controls are indicated (*).

Medium	Palmitate concn.	[^{14}C]PL/[^{14}C]GL		
		0.05 mM ($n = 3$)	0.5 mM ($n = 4$)	1.0 mM ($n = 3$)
Iso-osmotic		0.41 ± 0.05	0.31 ± 0.01	0.24 ± 0.02
Hypo-osmotic		$0.55 \pm 0.05^*$	0.35 ± 0.02	0.27 ± 0.04
Hyperosmotic		0.37 ± 0.05	—	0.24 ± 0.02
Iso-osmotic + glutamine (10 mM)		—	$0.38 \pm 0.3^*$	—
Iso-osmotic + glutamine (10 mM) + leucine (2.5 mM)		$0.69 \pm 0.06^*$	$0.41 \pm 0.02^*$	0.27 ± 0.02

Secretion of TAG

In Figure 1 the appearance of [^{14}C]TAG in the incubation media, as a function of time, is shown for experiments conducted at different palmitate concentrations with cells incubated under conditions that resulted in cell swelling or shrinkage. An increase in cell weight of less than 10% (Table 1), induced by incubation in hypo-osmotic medium, resulted in a small (15–20%) but significant decrease in the rate of [^{14}C]TAG secretion at all three palmitate concentrations used. A more pronounced increase in cell swelling, such as induced by glutamine alone (about 15%) or in combination with leucine (approx. 25%), gave much more marked inhibition of TAG secretion. (Glutamine alone was only tested at 0.5 mM palmitate.) The degree of inhibition of the rate of [^{14}C]TAG secretion during incubation with 0.5 mM [^{14}C]palmitate was generally proportional to the degree of swelling induced. Incubations in hyperosmotic medium, which resulted in a decrease in cell wet/dry-weight ratio of approx. 10% (see Table 1), gave the same degree of inhibition of [^{14}C]TAG secretion as that observed in hypo-osmotic medium (10% increase in wet/dry-weight ratio). Because numerous previous studies [17–20] have shown that hepatocyte swelling and shrinkage have opposite effects on numerous metabolic parameters, the fact that hypo- and hyper-osmolarity affected TAG secretion in the same direction, although not to the same

extent, suggested that different steps in the pathway of secretion of [^{14}C]TAG by hepatocytes were being affected by the two sets of conditions. Analysis of the distribution of the ^{14}C label between the different lipid classes in the cells and medium supported this explanation.

PL versus TAG labelling

The time courses for the appearance of label into total TAG and PL (cells plus medium) for incubations conducted under iso-osmotic and hypo-osmotic conditions were obtained for incubations in the presence of 0.05 mM and 1.0 mM palmitate. Two effects were discerned. Firstly, in the presence of a low concentration of palmitate (0.05 mM), incubations of cells under hypo-osmotic conditions, sufficient to yield a $\leq 10\%$ increase in wet/dry-weight ratio of the cells, resulted in a large increase in the proportion of glycerolipid that could be accounted for as phospholipid (Table 2). This suggested that cell swelling favoured the partitioning of DAG towards PL labelling at the expense of TAG labelling. This effect was more pronounced when the extent of cell swelling was increased further by incubation of cells in iso-osmotic medium in the presence of glutamine and leucine. Cell shrinkage induced by incubation of hepatocytes in hyperosmotic medium had no effect on this

parameter (Table 2). Secondly, increasing the concentration of palmitate antagonized the effect of increased cell hydration to favour PL labelling. The effect of moderate swelling (hypo-osmotic medium) was completely reversed by 0.5 mM palmitate, although the effects of more pronounced swelling induced by glutamine plus leucine were only partially reversed (Table 2). Incubation with 1 mM palmitate totally prevented the effect of amino acid-induced cell swelling on this parameter. Increasing the palmitate concentration in the medium tended to blunt the increase in cell volume (e.g. glutamine plus leucine resulted in cell wet/dry-weight ratios that were $127.5 \pm 4.5\%$, $120.7 \pm 2.8\%$ and $123.3 \pm 1.0\%$ of the respective iso-osmotic controls in the presence of 0.05 mM, 0.5 mM and 1.0 mM palmitate respectively), but this effect was not pursued further. It is suggested that the effects of increasing palmitate concentration represent a direct effect of fatty acids on the partitioning of DAG, with increasing fatty acid concentrations favouring TAG synthesis over PL synthesis (see [24]). Consequently it is evident that, *in vivo*, simultaneous lowering of NEFA concentrations in the plasma would be expected to amplify the inhibition of TAG labelling and the activation of PL synthesis induced by cell swelling. Cell shrinking brought about by incubation of hepatocytes in hyper-osmotic medium had no effect on the partitioning of label between PL and TAG at either low or high palmitate concentrations (Table 2).

Fractional secretion of [14 C]TAG

The percentage of total [14 C]TAG that was secreted into the medium was relatively variable between different cell preparations, and also depended on the concentration of palmitate used, being optimal at 0.5 mM palmitate. Thus the percentage of [14 C]TAG secreted after 90 min incubation by cells incubated under iso-osmotic conditions was $10.1 \pm 2.0\%$, $21.0 \pm 3.0\%$ and $8.5 \pm 3.2\%$ after incubation when initial concentrations of palmitate were 0.05 mM, 0.5 mM and 1.0 mM respectively. This bimodal pattern may be explained by saturation of TAG secretion at intermediate (0.5 mM) palmitate concentrations, such that at higher concentrations (1 mM) a lower fraction of total newly synthesized TAG is secreted. In Figure 2, the effects of cell swelling on the fractional rate of secretion of [14 C]TAG are shown for hepatocytes incubated in the presence of 0.5 mM palmitate. The fraction of [14 C]TAG secreted was not affected after moderate swelling induced by incubation in hypo-osmotic medium. Consequently, the entire effect of this level of cell swelling on the overall rate of [14 C]TAG secretion (Figure 1) can be accounted for by its stimulation of 14 C labelling of PL at the expense of that of TAG (Table 2), rather than on any effects on the fraction of labelled TAG that was secreted. More pronounced swelling induced by glutamine, especially in the presence of leucine, resulted in very significant decreases in the fractional rate of secretion of [14 C]TAG (Figure 2). In the case of glutamine as the sole amino acid, this effect would totally account for the inhibition of [14 C]TAG secretion, as at this concentration of palmitate (0.5 mM) the effect of the amino acids on DAG partitioning is fully attenuated (Table 2). In the presence of glutamine plus leucine, the fractional rate of [14 C]TAG secretion was very markedly depressed. The effect was progressive, such that between 60 and 90 min no further [14 C]TAG secretion was observed (Figure 2). Since at 0.5 mM palmitate the effect of glutamine plus leucine on the partitioning between PL and TAG labelling is still substantial (Table 2), effects on the partitioning of both DAG (Table 2) and TAG (Figure 2) are likely to contribute towards the very marked overall inhibition of

Figure 2 Effects of amino-acid-induced and hypo-osmotically induced cell swelling on the fractional rate of secretion of [14 C]TAG synthesized from [14 C]palmitate (0.5 mM) in isolated hepatocytes

Cells were incubated in iso-osmotic (●) or hypo-osmotic (△) medium, or iso-osmotic medium in the presence of 10 mM glutamine (◆), or of 10 mM glutamine plus 2.5 mM leucine (○). Values for the fraction of total labelled [14 C]TAG that was secreted into the medium are shown. The number of cell preparations was as in Table 1.

[14 C]TAG secretion observed in the presence of both amino acids (Figure 1).

Hyperosmotic-medium-induced shrinkage of hepatocytes also resulted in the inhibition of the fractional rate of secretion of [14 C]TAG (Figure 3). The effect was evident at both 0.05 mM and 1 mM palmitate (experiments were not performed at 0.5 mM palmitate). The high degree of variability observed with this parameter is reflected in the large standard errors displayed.

Figure 3 Effects of hepatocyte shrinkage in the presence of hyperosmotic medium on the fractional rate of [14 C]TAG secretion in the presence of (a) 0.05 mM palmitate and (b) 1 mM palmitate

See the legend to Figure 2 for details: (●) iso-osmotic, (▲) hyperosmotic medium. The number of separate cell preparations was as indicated in Table 1.

Figure 4 Scheme of the possible pathways for the synthesis and secretion of TAG in the liver

The composite scheme is based on the proposals presented previously [31,32]. TAG synthesized on the ER is considered to be partitioned between association with apoB to form nascent VLDL (step 1) and formation of cytosolic TAG (step 2). The latter is hydrolysed (step 3) to form partial acylglycerols and fatty acyl-CoA. TAG is synthesized from the remodelled acylglycerols to give secretory TAG (step 4). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Figure 5 Hepatocyte shrinkage inhibits the secretion of preformed TAG (a), but not PL (b)

Intracellular lipids were prelabelled by incubation of hepatocytes with [^3H]glycerol for 45 min. They were then separated and washed and resuspended in iso-osmotic (●), hyperosmotic (■) or hypo-osmotic medium (△), or iso-osmotic medium in the presence of 10 mM glutamine and 2.5 mM leucine (○). Values (means $\pm$ S.E.M.) are expressed as a percentage of the amount secreted after 90 min by cells incubated under iso-osmotic conditions (control) for each of three separate preparations.

However, values for cells incubated under hyperosmotic conditions were significantly lower ($P < 0.02$) for each time point when data were analysed on a paired basis. Since cell shrinkage did not alter the phospholipid/TAG labelling ratio (Table 2), but nevertheless resulted in a significant decrease in overall [^{14}C]TAG secretion rate (Figure 1), it is likely that inhibition of the fraction of labelled TAG that was secreted (Figure 3) represented the main mechanism through which cell shrinkage mediated its overall effect.

Effects of cell hydration on secretion of preformed [^{14}C]TAG

It is evident from many previous studies (see, e.g., [25–27]) that the hepatocyte contains at least three TAG pools: one cytosolic and two in the endoplasmic reticulum (ER). Presumably most of the ER TAG is luminal and partly associated with apoB in nascent VLDL. Consequently, changes in the fraction of labelled

TAG that is secreted can be mediated either through altered partitioning of newly synthesized TAG between the cytosolic and ER pools, or through changes in the relative rates of formation and hydrolysis of the cytosolic pool (see Figure 4). In order to try to address this question, experiments were performed in which the intracellular TAG was prelabelled by incubation of hepatocytes in bulk, in iso-osmotic medium containing [^3H]glycerol for 45 min, followed by washing and resuspension in the media indicated.

The secretion of preformed [^3H]TAG into the medium was then monitored over the following 90 min. As shown in Figure 5(a), incubation of the washed cells in hyperosmotic medium, to induce shrinkage, resulted in a marked decrease in the rate of secretion of prelabelled TAG. This effect was specific to TAG secretion, as the secretion of [^3H]PL was unaffected (Figure 5b). Incubation of cells in either hypo-osmotic medium or iso-osmotic medium supplemented with 10 mM glutamine plus 2.5 mM leucine did not affect the rate of secretion of either prelabelled [^3H]TAG or [^3H]PL. This pattern was observed, irrespective of whether prelabelling of cellular glycerolipids with [^3H]glycerol was performed in the presence or absence of exogenous palmitate (1 mM). Therefore the combined data are presented in Figure 5.

The data indicate that, whereas both cell swelling and cell shrinkage result in the inhibition of secretion of newly synthesized [^{14}C]TAG when this is measured during exposure to [^{14}C]palmitate (Figures 1–3), only cell shrinkage inhibits the secretion of preformed TAG (Figure 5). The simplest explanation for this observation is that cell swelling and shrinkage affect the metabolism of different pools of TAG in mediating their respective effects. Swelling would appear to affect the partitioning of newly synthesized TAG at the ER, between association with apoB (whose co-translational insertion into the lumen gives rise to nascent VLDL at this site [28,29]) and diversion towards the cytosolic pool of TAG (i.e. between steps 1 and 2 in Figure 4). Because the effect of cell shrinkage on overall [^{14}C]TAG secretion is relatively small (Figure 1), whereas its effect on secretion of preformed TAG is substantial (Figure 5), it is suggested that hepatocyte shrinkage primarily affects the rate at which cytosolic TAG is mobilized (step 3 in Figure 4). The specificity of the effect of TAG (but not PL) secretion suggests that it may not be the secretory mechanism itself that is affected by shrinkage.

It is well established that cytosolic TAG has to be hydrolysed before its glyceryl backbone can be utilized for incorporation into secreted TAG [30–32]. Although previously it had been thought that this hydrolysis could be complete, recent evidence suggests that only partial hydrolysis may be involved [32]. These studies indicated that, after re-modelling of the acyl-chain composition of the DAG, it is used for resynthesis of secreted TAG [32]. The present data suggest that the two routes involved in the secretion of TAG may be differentially affected by opposing changes in the hydration state of the cell to achieve the same overall effect (i.e. inhibition of TAG secretion; Figure 1), although swelling is much more potent, especially at lower exogenous fatty acid concentrations.

Physiological considerations

As mentioned in the Introduction, the present studies were prompted by the observations that, *in vivo*, during the prandial/early-absorptive phase the incorporation of fatty acid into PL is transiently increased and that, concomitantly, the fractional rate of newly synthesized TAG is inhibited [1–6]. Although meal-induced insulin secretion could be involved in mediating these effects [14–16], we have shown that these responses are also evident (and, indeed, are more pronounced) in severely diabetic

rats during a meal [5], i.e. when the insulin-secretory response is markedly attenuated. We suggested, therefore, that other meal-induced stimuli may be involved in mediating these responses, one of which could be the increase in portal osmolyte amino acid concentrations during this period. It is evident from the present data that incubation of hepatocytes with the osmolyte amino acid glutamine (which is also the most abundant in portal blood [5,33]) results in the same pattern of DAG and TAG partitioning as observed *in vivo* during refeeding of starved rats. The overall effect is to decrease the rate of TAG secretion, and both the mechanisms involved could be mimicked by cell swelling induced by a hypo-osmotic medium. Interestingly, the increased diversion of fatty acid into PL rather than TAG synthesis induced by cell swelling was antagonized by increasing exogenous fatty acid in the medium. This suggests that, in the normal rat *in vivo*, any effect of cell swelling on DAG partitioning would become increasingly important as the NEFA concentrations decline during refeeding, due to the antilipolytic action of insulin in adipose tissue. Consequently, raised portal concentrations of Na⁺-cotransported amino acids, and lowered plasma NEFA concentrations, may act synergistically to mediate the diversion of acyl chains into PL at the expense of TAG synthesis. Cell swelling also decreases the proportion of newly synthesized, but not stored, cytosolic TAG that is secreted, suggesting that during a meal it is the partitioning of newly synthesized TAG that is acutely affected in conjunction with inhibition of TAG synthesis relative to that of PL. It is noteworthy that insulin itself causes hepatocyte swelling [34–36]. The present data suggest that its inhibition of TAG secretion by hepatocytes [14–16] could be partly mediated through its effect on cell hydration.

In addition, the apparently paradoxical observations that both insulin and glucagon can inhibit TAG secretion in cultured isolated hepatocytes [37–40] may be partly explained by the present findings. This is because, at physiological concentrations, glucagon decreases cell volume in the perfused rat liver [35] by about 12% at physiological concentrations. The present data show that this extent of hepatocyte shrinkage inhibits secretion of preformed TAG. Therefore it is suggested that insulin and glucagon mediate their inhibition of TAG secretion at least partly through their respective and opposite effects on cell hydration. Insulin-induced cell swelling would be expected to alter the partitioning of newly synthesized TAG between cytosolic storage and assembly into VLDL (Figure 4, steps 1 and 2), whereas glucagon-induced shrinkage would be expected to inhibit the utilization of cytosolic TAG for secretion (step 3). Such effects would complement those exerted by the hormones on other steps that determine VLDL-TAG secretion, e.g. apoB degradation [40,41].

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