The lipolysis/esterification cycle of hepatic triacylglycerol

Its role in the secretion of very-low-density lipoprotein and its response to hormones and sulphonylureas

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In hepatocyte cultures maintained in the absence of extracellular fatty acids, at least 70 % of the secreted very-low-density lipoprotein (VLDL) triacylglycerol was derived via lipolysis of intracellular triacylglycerol. This proportion was unchanged when the cells were exposed for 24 h to insulin or glucagon, hormones which decreased the overall secretion of intracellular triacylglycerol, or to chloroquine or tolbutamide, agents which inhibit lysosomal lipolysis. The rate of intracellular lipolysis was 2–3-fold greater than that required to maintain the observed rate of triacylglycerol secretion. Most of the fatty acids released were returned to the intracellular pool. Neither insulin nor glucagon had any significant effect on the overall lipolysis and re-esterification of intracellular triacylglycerol. In these cases a greater proportion of the released fatty acids re-entered the cellular pool, rather than being recruited for VLDL assembly. Tolbutamide inhibited intracellular lipolysis, but suppressed VLDL secretion to a greater extent. 3,5-Dimethylpyrazole did not affect lipolysis or VLDL secretion. The increased secretion of VLDL triacylglycerol observed after exposure of cells to insulin for 3 days was not accompanied by an increased rate of intracellular lipolysis. However, a larger proportion of the triacylglycerol secreted under these conditions may not have undergone prior lipolysis.

INTRODUCTION

Treatment of hepatocytes in culture with insulin for periods less than 24 h results in a decreased net rate of mobilization of cytosolic triacylglycerol [1] and a decrease in the rate of very-low-density lipoprotein (VLDL) lipid [1–5] and apoprotein B (apoB) secretion [6–10]. It has previously been suggested that at least some of the VLDL triacylglycerol is derived from the cytosolic storage pool via lipolysis, followed by re-esterification in the endoplasmic reticulum [11–14]. There has been no attempt to quantify this in mammalian liver. Glucagon and cyclic AMP appear to accelerate intracellular lipolysis [15], but in this case the mobilized fatty acids are, in rat liver at least, directed into the oxidative, rather than into the re-esterification/secretory, pathway [16]. This is in apparent contrast with the situation in avian liver, in which cyclic AMP derivatives induced the rate of triacylglycerol secretion [13]. Tolbutamik and chloroquine have been reported to inhibit intracellular lipolysis [17,18]. The aims of the present study were to estimate the proportion of secreted VLDL triacylglycerol which arose via lipolysis of intracellular material compared with that derived by secretion of intact triacylglycerol 'en bloc' and to test whether this proportion changed in the presence of agents known to affect VLDL secretion. Second, we wished to determine whether the inhibitory effect of insulin on the secretion of VLDL was associated with a decreased lipolysis of intracellular triacylglycerol in a manner similar to that of hormone-sensitive lipase in adipose tissue. In the present work, hepatocellular 'cycling' between fatty acids and triacylglycerol (i.e. lipolysis/esterification) was determined by comparing changes in the [3H] and [14C] specific radioactivities of cellular triacylglycerol pre-labelled with [14C]glycerol and [3H]oleate.

MATERIALS AND METHODS

Preparation of hepatocyte cultures

Animals were fed and housed as described previously [19]. Hepatocytes were prepared under sterile conditions and plated out into dishes as a suspension in Waymouth's medium MB752/1 containing serum and supplements [20]. After removal of the serum-containing medium, 3.0 ml of Waymouth's medium containing added amino acids, antibiotics [5], dexamethasone (1 μM), lactate (10 mM) and pyruvate (1 mM) (referred to as 'supplemented medium') was added. At this stage either a mixture of [3H]oleate (0.75 mM; 0.98×10^6 d.p.m./μmol) and [14C]glycerol (1.0 mM; 0.18×10^6 d.p.m./μmol) or non-labelled oleate (0.75 mM) was added to the cells (see the legend to Table 1). Oleate was added as a complex with BSA. Thus, whenever oleate was present in the cell medium, the concentration of albumin was 0.5 % (w/v). Insulin was added where appropriate. After 18 h, cells in the dishes containing the labelled substrates now contained intracellular triacyglycerol doubly labelled with 3H and 14C in the fatty acid and glycerol moieties respectively. The medium was removed, the cells were washed twice with warm Dulbecco's phosphate-buffered saline (PBS) and 3.0 ml of supplemented medium was added, together with additions as appropriate. Under these conditions, VLDL triacylglycerol secreted during the next 24 h was derived exclusively from intracellular pre-labelled triacylglycerol. At the end of this period the cells were harvested, and the medium VLDL triacylglycerol and cellular triacylglycerol fractions were isolated. These cultures are referred to as '24 h' cultures in Tables 1–4. We have previously shown that, in the absence of oleate, VLDL triacylglycerol secretion remains constant over a 24 h period [5]. Those dishes which originally contained unlabelled oleate only were used for

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the longer-term experiments. In this case, 16 h after addition of oleate, the medium was changed and the cells were labelled with [14C]glycerol and [3H]oleate as described in the Table legends. VLDL and cellular triacylglycerol metabolism was then studied during the final 24 h of a 66 h culture period in which insulin had been either present or absent for the whole period. During the last 24 h, VLDL triacylglycerol was derived exclusively from intracellular triacylglycerol pre-labelled with [14C]glycerol and [3H]oleate.

Isolation and measurement of cellular and VLDL triacylglycerol

At the end of the appropriate culture period, the medium was removed from the dish and the VLDL-containing fraction was isolated as described previously [19]. The cell monolayer was washed, scraped from the plate and pelleted by centrifugation [5]. Lipids were extracted from the VLDL and from the cellular pellet by the method of Folch et al. [21]. A portion of the total lipid extract was used for measurement of triacylglycerol [22] with a kit supplied by Boehringer Corp. (Triglycerides GPO-PAP; cat. no. 701904). Triacylglycerol was purified from the remaining extract by t.l.c. [23]. The fraction containing triacylglycerol was scraped from the plate and assayed for 14C and 3H by scintillation counting.

Calculations of the extent of intracellular triacylglycerol lipolysis and re-esterification

In hepatocytes cultured in the absence of exogenous fatty acids, the only source of new triacylglycerol is endogenous

Table 1. Changes in specific radioactivities of cellular and VLDL triacylglycerol labelled with [3H]oleate

Cultures in which additions were present for only 24 h were treated as follows. After removal of serum-containing medium (4 h after plating) the monolayers were washed with PBS twice and 3.0 ml of supplemented medium was added. To this was added [3H]oleate (0.75 mM; 0.98 × 10⁶ d.p.m./μmol) and [14C]glycerol (1.0 mM; 0.18 × 10⁶ d.p.m./μmol). Cells were cultured for a further 16 h to label the intracellular triacylglycerol. Cells were then washed twice with PBS. Two dishes were removed for determination of cellular triacylglycerol mass, 3H and 14C radioactivity. To the remaining dishes was added supplemented medium (without oleate and glycerol), and the cells, together with the appropriate addition, were cultured for a further 24 h. After this time the VLDL and cellular triacylglycerol were isolated. Cultures in which insulin was present for 66 h were treated similarly, except that insulin was added immediately after removal of serum and was present for the whole 66 h. In this case non-radioactive oleate (0.75 mM) was added immediately after removal of serum and was replaced with a mixture of [3H]oleate and [14C]glycerol (see above) after 16 h. Then 24 h later the medium was removed, and the monolayers were washed twice with PBS. Two dishes were removed from each of the control and insulin-treated groups for measurement of cellular triacylglycerol specific radioactivity. To the remaining dishes was added supplemented medium (without oleate or glycerol) in the presence or absence of insulin, and 24 h later the cellular and VLDL triacylglycerol fractions were isolated. The values below therefore represent the triacylglycerol secreted during the final 24 h of the 66 h culture period. Tolbutamide was contained in a solution of 1 M-NaOH (20 μl). Addition of 20 μl in 1 M-NaOH alone had no effect on the values for the control incubation. In no case was the final specific radioactivity of the cell or VLDL triacylglycerol significantly different from that of the initial cell triacylglycerol. The values below represent the means ± s.e.m. of between three and six independent experiments.

| Addition present for | Addition to medium | Sp. radioactivity of triacylglycerol (3H d.p.m./μg) | | |
		Initial cell	Final cell	VLDL
24 h	Control		1280 ± 156	1429 ± 142
	Insulin (78 nM)		1348 ± 362	1125 ± 177
	Glucagon (100 nM)		1350 ± 385	1106 ± 53
	Tolbutamide (5 mM)	1611 ± 219	1544 ± 207	1241 ± 100
	Chloroquine (10 μM)		1252 ± 226	1434 ± 107
	Glycodiazine (5 mM)		1118 ± 188	1289 ± 56
	Dimethylpyrazole (0.2 mM)		1089 ± 134	1272 ± 59
66 h	Control	1240 ± 190	1028 ± 126	1382 ± 85
	Insulin	1081 ± 102	886 ± 104	1023 ± 125

Table 2. Changes in specific radioactivities of cellular and VLDL triacylglycerol labelled with [14C]glycerol

Cells were treated as described in the legend of Table 1. In all cases the final specific radioactivities of the cell and VLDL triacylglycerol were significantly ($P < 0.05$ or less) lower than the corresponding initial specific radioactivities of the cell triacylglycerol. Values marked *, ** and *** are significantly different ($P < 0.05$, $P < 0.01$, $P < 0.001$ respectively) from their corresponding control values. The values below represent the mean ± s.e.m. of between four and nine independent experiments.

| Addition present for | Addition to medium | Sp. radioactivity of triacylglycerol (14C d.p.m./μg) | | | |
		Initial cell	Final cell	VLDL	VLDL (% of initial cell; maximum secretion 'en bloc')
24 h	Control		18.0 ± 1.7	9.2 ± 2.1	30.0 ± 5.2
	Insulin (78 nM)		11.0 ± 2.1***	8.0 ± 2.2	26.9 ± 3.7
	Glucagon (100 nM)		17.2 ± 4.8	9.5 ± 2.9	31.1 ± 3.5
	Tolbutamide (5 mM)	29.6 ± 3.8	14.8 ± 2.8	7.1 ± 1.2	27.1 ± 4.0
	Chloroquine (10 μM)		10.2 ± 1.6**	4.1 ± 1.0	19.6 ± 5.8
	Glycodiazine (5 mM)		21.4 ± 3.2	8.8 ± 0.9	24.4 ± 1.7
	Dimethylpyrazole (0.2 mM)		17.2 ± 3.5	8.2 ± 1.1	21.8 ± 1.3
66 h	Control	15.3 ± 2.3	7.1 ± 1.7	4.2 ± 0.6	27.6 ± 1.4
	Insulin	13.9 ± 0.7	7.4 ± 0.4	6.6 ± 0.6	51.4 ± 7.9*

Table 3. Turnover of cellular triacylglycerol (labelled from [14C]glycerol) during VLDL secretion

Cells were treated as described in the legend of Table 1. In all cases the final specific radioactivities of the total triacylglycerol (cell + VLDL) were significantly ($P < 0.05$ or less) lower than that of the initial cellular triacylglycerol. Values marked * and ** are significantly different ($P < 0.05$ and $P < 0.01$ respectively) from their corresponding controls. Values marked † are significantly different from the corresponding value at 24 h. Values in column 6 are derived by [(column 5)/column 5)−1]. Values in column 7 are derived by (column 4 × column 6). Values in column 8 are derived from the mass of VLDL triacylglycerol secreted (Table 4) divided by the total triacylglycerol hydrolysed (column 7). The values below represent means ± S.E.M. of between four and nine independent experiments.

Addition present for (1)	Addition to medium (2)	Initial cell triacylglycerol sp. radioactivity (d.p.m./μg) (3)	Final total triacylglycerol (VLDL+cell) Mass (μg/mg of protein) (4)	Sp. radioactivity (d.p.m./μg) (5)	Added pools (6)	Total triacylglycerol hydrolysed (μg/24 h per mg of protein) (7)	VLDL secretion (% of total hydrolysed) (8)
24 h	Control	29.6±3.8	211±21	11.5±1.4	1.40±0.17	318±46	42.8±4.3
	Insulin (78 nM)		210±30	11.0±2.7	1.79±0.20	368±85	11.4±1.8**
	Glucagon (100 nM)		199±28	12.1±3.1	1.21±0.09	223±66	34.8±5.5
	Tolbutamide (5 mM)		189±10	13.7±2.9*	1.00±0.10*	194±27*	13.9±3.8**
	Chloroquine (10 μM)		172±26	10.7±2.0	1.87±0.23	300±91	31.3±7.7
	Glycodiazine (5 mM)		196±22	14.6±2.0	1.50±0.10	294±53	29.3±2.3*
	Dimethylpyrazole (0.2 mM)		237±24	10.5±2.5	2.09±0.19	414±80	21.8±2.4**
66 h	Control	15.3±2.3	324±36†	6.4±1.6	1.96±0.36	546±152	23.7±2.2
	Insulin	13.9±0.7	613±76*†	6.8±0.5	1.43±0.44	732±214	33.4±10.1†

synthesis of fatty acids. Thus, if cellular triacylglycerol is pre-labelled with [14C]glycerol and [3H]oleate, in the absence of any other changes, a decrease in the specific radioactivity of cellular and secreted VLDL triacylglycerol should reflect the extent of fatty acid synthesis de novo. Further, the extent of the decline in the 14C and 3H specific radioactivities should be of the same magnitude. Any relative change in the specific radioactivities of the two radioisotopes requires an uncoupling of the [3H]oleate and [14C]glycerol moieties of the doubly labelled triacylglycerol, a process which must involve lipolysis followed by re-esterification. When cellular triacylglycerol was doubly labelled for 16 h, its 3H specific radioactivity was 1611 d.p.m./μg. This did not change significantly during a subsequent 24 h culture period in the absence of labelled substrates (1286 d.p.m./μg), indicating relatively little, if any, fatty acid synthesis de novo during this period. Neither was there any change in the 3H specific radioactivity of the secreted VLDL triacylglycerol (1429 d.p.m./μg) (Table 1). Despite this, under the same conditions, the specific radioactivity of the 14C-labelled material declined from 29.6 d.p.m./μg to 18.0 d.p.m./μg in the cellular and to 9.2 d.p.m./μg in the VLDL triacylglycerol (Table 2). In other words, there has been a 70% relative loss of the VLDL 14C-label, which must have arisen as a result of the dilution of the [14C]glycerol with unlabelled glycerol after lipolysis and before re-esterification. This suggests that at least 70% of the VLDL triacylglycerol must have been derived via a pathway which involved lipolysis of intracellular lipid. The remaining 30% could have been derived either by secretion of cellular triacylglycerol 'en bloc' (i.e. without lipolysis) or by lipolysis after re-esterification of the released fatty acid with labelled glycerol. Thus this value of 30% is the maximum upper limit for secretion of triacylglycerol 'en bloc'.

Cellular triacylglycerol pre-labelled with [14C]glycerol and [3H]oleate may either remain within the cell or be secreted as VLDL during a subsequent 24 h culture period. The extent to which the glycerol moiety of the pre-labelled triacylglycerol was diluted during this period may be calculated by comparing its initial 14C specific radioactivity with that of the total (cell + VLDL) triacylglycerol at the end of the culture period. This calculation is shown in Table 3, in which, in the control incubation, the 14C specific radioactivity decreased from an initial value of 29.6 d.p.m./μg to a final value of 11.5 d.p.m./μg 24 h later. To achieve this, the original triacylglycerol-glycerol pool must have been diluted by the addition of a further 1.40 pools of unlabelled glycerol. This did not result from synthesis of fatty acid de novo since the 3H specific radioactivity of VLDL and cellular triacylglycerol did not change significantly (Table 1). The unlabelled glycerol must therefore have entered the triacylglycerol pool by lipolysis followed by re-esterification. Since the total pool size amounted to 211 μg/mg protein (column 4, Table 3), entry of a further 1.40 pools must have required a lipolysis of 1.40 × 211 = 318 μg of triacylglycerol/mg of protein. It should be noted that this represents a minimum rate of lipolysis, since fatty acids released which are not re-esterified (e.g. those utilized for ketogenesis) would not contribute to a dilution of the pool.

Other methods

Cellular protein was measured by the method of Lowry et al. [24]. Oleate was bound to albumin (essentially fatty acid free) by the method of Van Harken et al. [25] and added to cells so that the initial concentration was 0.75 mM. This gave an initial albumin concentration of 0.5%.

Statistical methods

Each determination represents the average of two dishes of

cells. Each value represents the mean±s.e.m. of several in-dependent observations, each of which was the average of duplicate determinations. The degree of significance of observed differences was tested by a paired or unpaired Student's t test.

Materials

Radiochemicals were obtained from Amersham International (Little Chalfont, Bucks., U.K.). [³H]Oleate was bound to albumin as described above. [¹⁴C]Glycerol was supplied as a solution in water/ethanol (1:1, v/v). The solvent was evaporated under a stream of nitrogen gas at 35 °C, and non-radioactive glycerol was added. The mixture was dissolved in distilled water and added to the dishes to give an initial glycerol concentration of 1.0 mM. Tolbutamide and chloroquine were obtained from Sigma (Poole, Dorset, U.K.). The former was dissolved in 1 M-NaOH before use. Glycodiazine (sodium salt) was obtained from Schering A.G. (Berlin, Germany) and 3,5-dimethylpyrazole was from Fluka A.G., Buchs, Switzerland.

RESULTS AND DISCUSSION

Effects of hormones on triacylglycerol turnover and VLDL secretion rates

When added to the cell culture for 24 h only, insulin inhibited the secretion of VLDL triacylglycerol (Table 4), but had no effect on the maximum proportion secreted without lipolysis (i.e. 'en bloc'). This remained rather low (Table 2) and suggested that at least 70 % had undergone prior lipolysis. Neither did insulin affect the total mass of intracellular triacylglycerol hydrolysed, as judged by the extent of the [¹⁴C]glycerol dilution (Table 3, columns 5 and 6). However, the amount secreted as a percentage of the total triacylglycerol hydrolysed decreased from 42.8 to 11.4%. By contrast, the increased VLDL secretion observed during the final 24 h of a longer-term (66 h) exposure to insulin (Table 4) may have resulted, at least in part, from an increase in the 'en bloc' component of triacylglycerol secretion (Table 2). Again, despite this increase in triacylglycerol secretion, longer-term insulin had no significant effect on the total mass of intracellular triacylglycerol hydrolysed compared with the 66 h control (Table 3). Glucagon, present at a concentration of 0.1 µM for 24 h, also suppressed the secretion of VLDL triacylglycerol (Table 4), but had no effect on the proportion secreted as a result of lipolysis (Table 2). Neither did glucagon affect the extent of

dilution of the total ¹⁴C-labelled triacylglycerol (Table 3). Nevertheless, glucagon increases ketogenesis from endogenous triacylglycerol, a process which involves increased lipolysis [15]. The present results suggest that all the additional fatty acids released as a result of glucagon action are oxidized and that none are esterified and returned to the cell. This is evidenced by the unchanged specific radioactivity of the ¹⁴C-labelled triacylglycerol when glucagon was present, compared with the control. This raises the important question as to whether, in mammalian liver, the intracellular release of fatty acids for ketogenesis on the one hand, and for triacylglycerol secretion/recycling on the other, is independently controlled by different lipases.

It should be emphasized that dexamethasone was routinely present in the culture medium during all these experiments. This serves to enhance the secretion of VLDL [3] and contributes to the maintenance of VLDL output during long periods of cell culture [5,19]. There is also an improved visual appearance of the cells when examined under the light microscope. It is possible that dexamethasone, in combination with insulin, may have resulted in a change in fatty acid synthesis *de novo*. If this was the case, then we were unable to detect this, since addition of insulin to the medium already containing dexamethasone did not affect the ³H specific radioactivity of the cellular triacylglycerol, as might be expected from a changed influx of non-labelled fatty acids.

Effect of sulphonylureas and lipolysis inhibitors

Tolbutamide inhibited the secretion of VLDL triacylglycerol in a concentration-dependent manner and caused an increase in the amount of triacylglycerol remaining within the cell (Table 4 and Fig. 1). This result differs from that reported previously for a similar drug, chlorpropamide, which apparently stimulated the secretion of labelled triacylglycerol during a 60 min incubation of freshly prepared hepatocytes [17]. However, these latter measurements were carried out in the presence of exogenous [³H]oleate, and measurements of the secretion of the total mass of triacylglycerol derived exclusively from the endogenous source were not carried out. The present observation, that tolbutamide directly inhibits the secretion of VLDL triacylglycerol, may partly explain the decreased rate of VLDL triacylglycerol production in non-insulin-dependent diabetic subjects after sulphonylurea therapy [26].

In the present work, the proportion of the total triacylglycerol secreted which had undergone lipolysis remained unchanged in the presence of tolbutamide (Table 2). However, the drug inhibited the lipolysis/re-esterification cycle of triacylglycerol, as shown by the decreased extent of dilution of the ¹⁴C label (Table 3, columns 5–7). In addition to decreased lipolysis, a smaller proportion of the lipolysed pool was exported as VLDL triacylglycerol (Table 3). There are thus two components of the tolbutamide-mediated suppression of endogenous triacylglycerol secretion. One of these is decreased lipolysis. The other, which acts independently, is post-lipolytic and probably reflects defective VLDL assembly and/or secretion.

In contrast with tolbutamide, glycodiazine, a sulphonamide pyrimidine substance structurally related to the sulphonylureas [17], had no effect on VLDL secretion (Table 4) nor on the lipolysis/esterification cycle of triacylglycerol (Table 3).

The possible role of lysosomal lipase(s) on intracellular triacylglycerol turnover was studied by observing the effects of chloroquine (10 µM). Although this led to a decreased secretion of VLDL triacylglycerol (Table 4), there was no change in the overall mass of triacylglycerol hydrolysed and re-esterified (Table 3). Concentrations of chloroquine up to 100 µM progressively decreased the secretion of VLDL, but intracellular lipolysis remained unchanged (results not shown). Thus fatty acids

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Table 4. VLDL-triacylglycerol secretion from cultured hepatocytes: effects of sulphonylureas, chloroquine and pancreatic hormones

Cells were treated as described in the legend of Table 1. The values below represent means±s.e.m. of between four and nine independent experiments. Values marked ** and *** mean that they were significantly different from the corresponding controls ($P < 0.01$ and $P < 0.001$ respectively).

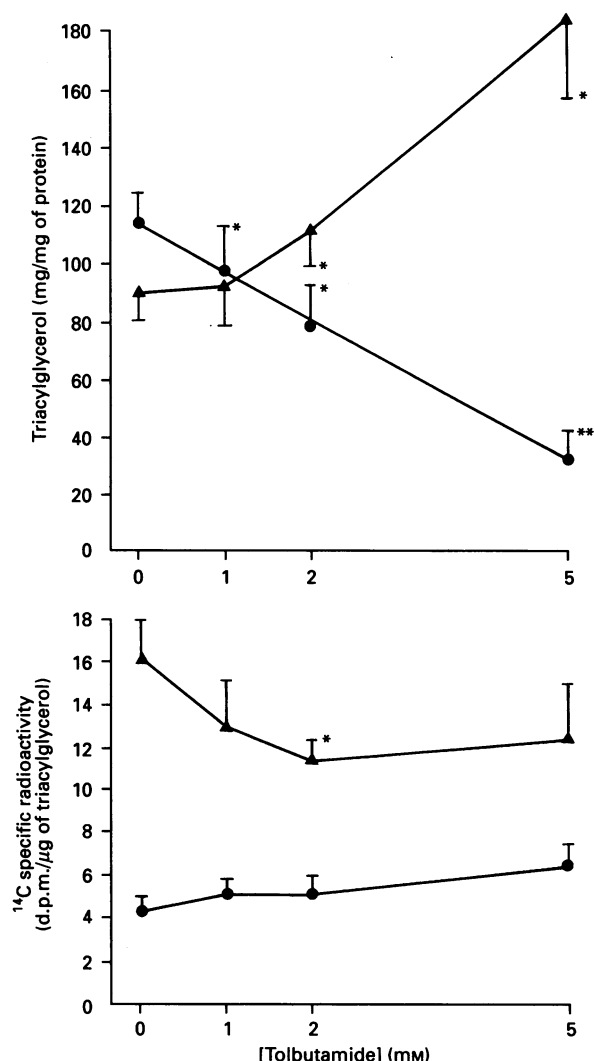| Addition present for	Addition to medium	Secretion of VLDL triacylglycerol (µg/24 h per mg of protein)
24 h	Control	106.5±13.5
	Insulin (78 nM)	39.9±7.2**
	Glucagon (100 nM)	66.3±12.0**
	Tolbutamide (5 mM)	26.4±8.5***
	Chloroquine (10 µm)	69.0±15.4**
	Glycodiazine (5 mM)	89.3±19.7
	Dimethylpyrazole (0.2 mM)	95.0±17.6
66 h	Control	128.2±36.1
	Insulin (78 nM)	204.5±39.1**

Fig. 1. Effect of tolbutamide on cell and VLDL triacylglycerol metabolism

Hepatocyte cultures were treated as described in the legend of Table 1. After prelabelling the cellular triacylglycerol with [^{14}C]glycerol and [^{3}H]oleate for 16 h, the medium was removed and the cell monolayers were washed twice with PBS. Supplemented Waymouth's medium (3.0 ml; without glycerol or oleate) was then added with or without tolbutamide to give initial concentrations of 0, 1, 2 and 5 mM. The cells were cultured for a further 24 h, after which the cellular and VLDL triacylglycerol fractions were isolated. Each point represents the mean ±s.e.m. of four independent experiments. Values marked * and ** are significantly different from the controls (0 mM) at $P < 0.05$ and $P < 0.01$ respectively. Key: ● VLDL triacylglycerol; ▲ cell triacylglycerol.

released under these conditions were not utilized for VLDL secretion, but were diverted back into the cellular triacylglycerol pool. This was evidenced by a decreased ^{14}C specific radioactivity of the intracellular triacylglycerol in the presence of chloroquine (Table 2). We therefore conclude that lysosomal lipolysis plays little, if any, role in the intracellular lipolysis/re-esterification cycle and that chloroquine does not block VLDL secretion by interfering with lysosomal enzyme activity.

3,5-Dimethylpyrazole inhibits adipose-tissue triacylglycerol lipolysis, as evidenced by the decreased plasma fatty acid levels in animals treated with the drug [27,28]. However, in liver this substance affected neither triacylglycerol turnover (Table 3) nor VLDL secretion rate (Table 4).

Throughout this work, the absence of extracellular fatty acid during the period over which measurements were made was an essential element of the experimental design. It thus remains possible that rates of intracellular lipolysis and secretion of VLDL are different when extracellular fatty acids are simultaneously present. However, we have some evidence that, irrespective of the presence or absence of extracellular fatty acids, the immediate precursor of VLDL triacylglycerol is stored, intracellular triacylglycerol (G. F. Gibbons, S. M. Bartlett, J. D. Sparks & C. E. Sparks, unpublished work). It therefore seems likely that this would be mobilized mainly by lipolysis, despite the simultaneous presence of fatty acids in the medium.

Significance of the hepatic triacylglycerol lipolysis/re-esterification cycle

Although previous work had implied that an unknown proportion of VLDL triacylglycerol was derived after lipolysis and re-esterification of intracellular triacylglycerol [11,12,14], there had been no attempt to quantify this in mammalian liver. The present results show that at least 70% of the secreted triacylglycerol is derived via this pathway and that this proportion remains remarkably constant in the presence of agents which provoke large changes in VLDL secretion (Tables 2 and 4). Under control conditions, the amount of intracellular triacylglycerol hydrolysed is about 2.5-fold greater than that required to meet the needs of VLDL assembly (Tables 3 and 4). The remaining fatty acids released are re-esterified to give triacylglycerol, which then re-enters the intracellular pool, resulting in a decreased ^{14}C specific radioactivity (Table 2). Thus, although intracellular lipolysis appears to be essential for VLDL triacylglycerol assembly, it does not appear to be rate-limiting. The inhibitory effect of insulin on VLDL secretion (Table 4) was not due to a decrease in intracellular triacylglycerol lipolysis, which remained unchanged (Table 3). Instead, the decreased VLDL secretion caused an increased diversion of re-esterified fatty acids back into the intracellular pool. This was evidenced by a decrease in the ^{14}C specific activity of the cellular triacylglycerol compared with the control value (Table 2). The increased recycled triacylglycerol in the presence of insulin was returned to the cytosolic storage pool rather than being sequestered in the secretory apparatus as incipient VLDL [1]. Thus the insulin-mediated suppression of lipid release from the liver does not result from a decreased rate of lipolysis, as in adipose tissue, but is secondary to some other rate-limiting factor, possibly the availability of functional apoB [6–8]. The steady-state concentration of apoB is itself dependent on a cycle of synthesis and intracellular degradation [8,29–32], which is regulated by insulin [8,33]. The large excess of intracellular triacylglycerol cycling capacity would ensure an instantaneous increase in VLDL secretion when more apoB became available for VLDL assembly.

Subtraction of the triacylglycerol leaving the cell as VLDL (Table 4) from the total triacylglycerol hydrolysed (Table 3) represents the amount of hydrolysed material which is re-esterified and returned to the cell. This amounts to approximately one pool per day. This is considerably higher than that observed in adipose tissue (approx. 10% of the pool size, as calculated from [34] and [35]) and may represent the capacity required to ensure a high potential for lipid secretion when the total steady-state pool size of triacylglycerol is relatively low, as is the case in liver, compared with that in adipose tissue.

Finally, an important question which remains to be answered relates to the nature and intracellular location of the triacylglycerol lipase(s) involved in the secretion/recycling pathway and its relationship to the assembly of VLDL. Based on evidence discussed above, we believe that a lysosomal lipase is not involved. A microsomal lipase has also been described and partially

characterized [36], and its intracellular location is well suited for a role in the assembly of VLDL.

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