Triacylglycerol accumulation and secretion in hepatocyte cultures

Effects of insulin, albumin and Triton WR 1339

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We have investigated the possibility that the apparent inhibition of very-low-density lipoprotein (VLDL)-triacylglycerol secretion by the addition of insulin to rat hepatocyte cultures may result from insulin-mediated enhancement of hepatic lipase secretion and, consequently, of extracellular triacylglycerol hydrolysis. We have, therefore, studied the effects of the inhibitor of lipase activity, Triton WR 1339, on the secretion of triacylglycerol by cultured rat hepatocytes. Incubation of hepatocyte cultures with increasing concentrations of Triton WR 1339 increased the accumulation of acylglycerol in the medium, suggesting that, in normal incubations, a substantial rate of degradation of secreted triacylglycerol does occur and that it results in an under-estimation of the rate of triacylglycerol secretion. However, Triton did not counteract the inhibitory effects of insulin, suggesting that the observed increased activity of hepatic lipase induced by the hormone cannot account for the inhibition of acylglycerol accumulation in the medium that occurred in the presence of insulin. BSA increased the accumulation of triacylglycerol in culture media by about 2-fold and also decreased the activity of hepatic lipase by 80%. A causative relationship between these two effects was supported by the further observation that Triton abolished the effects of BSA on triacylglycerol accumulation in the medium. The implications of these data for the validity of the use of Triton for the study of hepatic rates of triacylglycerol production *in vivo* and of secretion by hepatocytes *in vitro* are discussed.

INTRODUCTION

The secretion of triacylglycerol by hepatocyte cultures is generally determined from its accumulation in the culture medium [1–9]. These studies suggest that insulin suppresses the secretion of very-low-density-lipoprotein (VLDL)-triacylglycerol in hepatocyte cultures [1,3–8]. Paradoxically, however, hepatocyte cultures from insulin-deficient rats exhibit a lower rate of triacylglycerol secretion than do those from normal rats [9]. Using other *in vitro* techniques, such as the perfused liver [10–13] and freshly isolated rat hepatocyte suspensions [14], insulin was shown to increase [10,11], decrease [12,13] or have no effect [14] on VLDL secretion. These apparently conflicting observations raise the question as to whether these effects of insulin *in vitro* are due to artefacts of the system used.

Measurement of triacylglycerol secretion by hepatocyte cultures is based on the assumption that extracellular degradation is negligible [1]. However, hepatocyte cultures secrete hepatic lipase [15–19], which catalyses the hydrolysis of a variety of glycerolipids, including triacylglycerol [20–25]. Hepatic lipase deficiency [26,27] is associated with elevated circulating concentrations of VLDL. Both VLDL [28–32] and nascent VLDL [32] are good substrates for hepatic lipase. Consequently, the possibility that the accumulation of triacylglycerol in the medium of hepatocyte cultures is an under-estimate of the true rate of secretion because of extracellular hydrolysis by hepatic lipase requires serious consideration. As the secretion of hepatic lipase is increased by insulin [16,18], the inhibition by insulin of the accumulation of triacylglycerol in the medium of cultured hepatocytes may be due to enhanced extracellular hydrolysis by hepatic lipase.

Rates of secretion of triacylglycerol *in vivo* in the rat have been determined from the accumulation of triacylglycerol in the plasma after intravenous injection of the non-haemolytic detergent Triton WR 1339 [33–38], which inhibits the hydrolysis of

triacylglycerol by lipoprotein lipase [39]. The use of Triton WR 1339 in hepatocyte cultures could therefore help to quantify the state of triacylglycerol hydrolysis if this detergent inhibits hepatic lipase *in vitro*. This possibility was investigated in the present study. In addition, we have investigated whether the suppression by insulin of the accumulation of triacylglycerol in the medium of hepatocyte cultures could be due partly to enhanced extracellular hydrolysis by hepatic lipase.

MATERIALS AND METHODS

Materials

Triton WR 1339 (Tyloxapol) was from Sigma Chemical Co. (Poole, Dorset, U.K.). BSA (fraction V) was from either Boehringer (Mannheim, Germany) or Sigma, and was defatted [40] and dialysed [41] before use. Radiochemicals were from Amersham International (Amersham, Bucks., U.K.). Sources of other materials were as described previously [19,42].

Hepatocyte preparation

Parenchymal hepatocytes were isolated from male Wistar rats (body weight 200–350 g). Hepatocytes were isolated by the conventional collagenase perfusion technique from whole liver [43]. Periportal and perivenous hepatocytes were prepared by the digitonin–collagenase technique [44] with minor modifications [45]. The hepatocytes were cultured in monolayer in 12- or 24-multiwell plates in minimum essential medium (MEM) containing 5% (v/v) foetal bovine serum.

Determination of metabolic parameters

After cell attachment (6–8 h), the monolayers were pre-cultured (12–13 h) in serum-free MEM containing 10 nM-dexamethasone and either without or with 1 mM-palmitate

Abbreviations used: VLDL, very-low-density lipoprotein; MEM, minimal essential medium.

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complexed to BSA (12.5 mg/ml) and 0.3 mM-[U- 14 C]glycerol or [2- 3 H]glycerol. Other conditions were as described [42,45,46]. The cells were then exposed to fresh medium containing the additions indicated in the Tables. Additional monolayers were washed and extracted for the determination of cellular triacylglycerol and lipid-associated radioactivity at the start of the incubation. On termination of the incubation, the medium was collected for the determination of labelled lipid, triacylglycerol and other metabolites (see below), and the monolayers were washed and extracted as previously described [46].

The lipid content of the medium and hepatocytes was extracted by the method of Bligh & Dyer [47] as modified in [5], and the associated radioactivity was measured. For the determination of the secreted triacylglycerol, chloroform/methanol extracts were extensively washed with synthetic upper phase that contained no glycerol, and the lipid-containing lower phase was lyophilized and sonicated with extraction buffer [42] prior to determination of triacylglycerol by an enzymic method [48]. Palmitate, lactate dehydrogenase, acetoacetate, D-3-hydroxybutyrate and cellular triacylglycerol were determined enzymically as previously described [19,46]. Cellular protein was determined by an automated Lowry method [49].

Conditioned medium used in the experiments in Table 3 was prepared by incubation of hepatocyte monolayers (3×75 cm 2 flasks) for 12 h with MEM containing 1 mM-palmitate/albumin complex and 0.3 mM-glycerol. The media were then pooled and centrifuged at 50 *g* for 5 min to remove cellular debris, and supplemented with the additions indicated.

Secretion of hepatic lipase

After cell attachment, hepatocyte monolayers (in 12-well plates) were incubated for 10 h with 0.5 ml of MEM containing 10 nM-dexamethasone, 10 units of heparin (unless stated otherwise) and other additions as indicated. Determination of hepatic lipase activity was as described elsewhere [19].

Preparation of hepatic lipase from rat liver

Rat liver was first perfused with Hanks' Balanced Salts solution (200 ml) without recirculation to wash out the blood, and was then perfused with MEM (100 ml) containing 20 units of heparin/ml, recirculated at 37 °C (10 min). The resulting perfusate was used as a source of hepatic lipase.

RESULTS

Effects of Triton WR 1339 on hepatic lipase activity

Liver heparin perfusates containing hepatic lipase activity were assayed in the presence of Triton WR 1339 (Fig. 1). Inhibition of hepatic lipase was maximal (97%) at a concentration of 1 mg/ml (Fig. 1). Similar results were obtained when the effects of Triton WR 1339 were examined on hepatic lipase secreted by hepatocyte cultures in the presence of heparin (results not shown).

Effect of Triton WR 1339 on cellular integrity

Leakage of lactate dehydrogenase into the medium of hepatocyte cultures was determined in order to assess cellular integrity in response to a range of concentrations of Triton WR 1339 (0.1–10 mg/ml). During a 6 h incubation there was no detectable leakage of LDH at concentrations up to 1 mg/ml (Table 1). However, higher concentrations did cause leakage of the enzyme (Table 1).

Effect of Triton WR 1339 on fatty acid metabolism

To examine the validity of using Triton WR 1339 as a tool for studying the synthesis and secretion of triacylglycerol in hepato-

cyte cultures, the effects of a range of Triton WR 1339 concentrations (0.5–10 mg/ml) were examined using two protocols. (i) Hepatocytes that had been pre-cultured (12 h) with palmitate and [2- 3 H]glycerol were subsequently incubated with Triton WR 1339 in the absence of these substrates to measure the effects of the detergent on secretion of pre-formed glycerolipid. (ii) Hepatocytes that had been pre-cultured (12 h) without substrates were then incubated with Triton WR 1339 in the presence of palmitate and [2- 3 H]glycerol. This measured the effects on *de novo* synthesis and secretion of acylglycerides.

In hepatocytes that were pre-cultured with palmitate and [2- 3 H]glycerol, increasing concentrations of Triton WR 1339 caused a progressive increase in the accumulation of labelled acylglycerol in the medium in both control and insulin-treated cells (Table 2). In spite of the marked increase in the accumulation of labelled acylglycerol in the medium caused by Triton WR 1339, the tendency of insulin to suppress the accumulation of labelled acylglycerol in the medium was not abolished, although it was blunted (Table 2). In the insulin-treated cultures the intracellular triacylglycerol content at the end of the incubation was not affected by Triton WR 1339, whereas in control cultures the cellular triacylglycerol tended to be higher in the presence of Triton WR 1339 (Table 2).

Fig. 1. Effect of Triton WR 1339 on hepatic lipase activity

Heparin perfusate from the livers of two male rats containing hepatic lipase activity (25 nmol of glycerol released/h per ml) were incubated (2 h) with glycerol triolein emulsion in the presence of increasing concentrations of Triton WR 1339 as indicated. Results are expressed as a percentage of the original hepatic lipase activity and represent the means of triplicate incubations for each preparation. Error bars fall within the symbols.

Table 1. Effect of Triton WR 1339 on the release of lactate dehydrogenase (LDH) by hepatocyte cultures

* indicates that values are statistically significantly different ($P < 0.01$) from those obtained for incubations without Triton WR 1339.

Triton WR 1339 (mg/ml)	Medium LDH activity (munits/ml)
0	158 ± 14
0.5	133 ± 30
1.0	157 ± 33
2.5	412 ± 17*
5.0	461 ± 21*
10	638 ± 20*

Table 2. Effect of Triton WR 1339 on the secretion of endogenous [³H]acylglycerol

Hepatocytes were pre-cultured (12 h) with 1 mM-palmitate, 0.3 mM-[2-³H]glycerol (3 Ci/mol), dexamethasone (10 nM) and either without or with insulin as indicated. They were then incubated with fresh medium without substrates, but in the presence of Triton WR 1339 and insulin as indicated. Labelled acylglycerol (AG) (d.p.m./mg of protein) remaining in the cells and culture medium and the cellular triacylglycerol (TG; nmol/mg of protein) were determined at the end of the incubations (6 h). Values represent the means $\pm$ S.E.M. of duplicate incubations for four experiments. * $P < 0.05$, ** $P < 0.005$ relative to respective conditions without Triton WR 1339.

Hormone addition	Triton WR 1339 (mg/ml)	Cellular TG (nmol/mg)	10 ⁻² $\times$ Cellular [³ H]AG (d.p.m./mg)	10 ⁻² $\times$ Medium [³ H]AG (d.p.m./mg)	10 ⁻² $\times$ Medium + cellular [³ H]AG (d.p.m./mg)	Medium [³ H]AG (% of total)
Control	0	263 $\pm$ 46	2627 $\pm$ 391	200 $\pm$ 21	2853	7
	0.5	343 $\pm$ 47	3403 $\pm$ 377	269 $\pm$ 22*	3672	7.3
	1.0	353 $\pm$ 45	3507 $\pm$ 464	374 $\pm$ 47*	3881	9.8*
	2.5	366 $\pm$ 48	3535 $\pm$ 431	425 $\pm$ 50**	3960	10.7**
	5.0	376 $\pm$ 50	3711 $\pm$ 466	538 $\pm$ 37**	4249*	14.5**
	10.0	356 $\pm$ 86	3606 $\pm$ 738	612 $\pm$ 27**	4217*	14.5
100 nM-Insulin	0	384 $\pm$ 25	3821 $\pm$ 367	104 $\pm$ 13	3924	3.3
	0.5	409 $\pm$ 36	4005 $\pm$ 382	180 $\pm$ 13**	4185	4.3
	1.0	427 $\pm$ 30	3833 $\pm$ 471	261 $\pm$ 25*	4094	6.4*
	2.5	424 $\pm$ 36	4012 $\pm$ 379	358 $\pm$ 27**	4370	9.0**
	5.0	443 $\pm$ 42	3941 $\pm$ 385	413 $\pm$ 33**	4354	9.5**
	10.0	424 $\pm$ 57	3911 $\pm$ 550	518 $\pm$ 62**	4428	11.7**

Table 3. Effect of Triton WR 1339 on palmitate metabolism

Hepatocytes were pre-cultured (12 h) with MEM containing dexamethasone (10 nM) in the presence or absence of insulin as indicated, without substrates. They were then incubated (6 h) with medium containing 1 mM-palmitate, 0.3 mM-[2-³H]glycerol (3 Ci/mol), 0.5 mM-L-carnitine and the concentrations of insulin and Triton WR 1339 as shown. The rate of ketone body formation and uptake of palmitate are expressed as nmol/h per mg of protein. Labelled medium and cellular acylglycerol (AG) is expressed as d.p.m./mg of protein and cellular triacylglycerol (TG) as nmol/mg of protein. Values represent the means $\pm$ S.E.M. of duplicate incubations of four experiments. * $P < 0.05$, ** $P < 0.005$ relative to respective controls without Triton WR 1339.

Hormone addition	Triton WR 1339 (mg/ml)	Rate of ketone body formation (nmol/h per mg)	Rate of fatty acid uptake (nmol/h per mg)	Cellular TG (nmol/mg)	10 ⁻² $\times$ Cellular [³ H]AG (d.p.m./mg)	10 ⁻² $\times$ Medium [³ H]AG (d.p.m./mg)	10 ⁻² $\times$ Medium + cellular [³ H]AG (d.p.m./mg)	Medium [³ H]AG (% of total)
Control	0	197 $\pm$ 21	169 $\pm$ 31	181 $\pm$ 5	1493 $\pm$ 435	83 $\pm$ 8	1576	5.0
	0.5	191 $\pm$ 10	151 $\pm$ 23	173 $\pm$ 6	1162 $\pm$ 190	98 $\pm$ 19	1260	7.8
	1.0	144 $\pm$ 9*	138 $\pm$ 8	169 $\pm$ 9	1070 $\pm$ 132	93 $\pm$ 15	1162	8.0
	2.5	91 $\pm$ 10**	82 $\pm$ 19	168 $\pm$ 14	959 $\pm$ 98	108 $\pm$ 23	1067	10.1**
	5.0	65 $\pm$ 8**	60 $\pm$ 9*	151 $\pm$ 12*	933 $\pm$ 83	120 $\pm$ 13**	1053	12.9**
	10.0	37 $\pm$ 6**	24 $\pm$ 12**	130 $\pm$ 14**	807 $\pm$ 129	200 $\pm$ 50*	1007	19.8**
100 nM-Insulin	0	267 $\pm$ 23	285 $\pm$ 81	197 $\pm$ 6	1278 $\pm$ 277	67 $\pm$ 9	1344	5.0
	0.5	247 $\pm$ 25	249 $\pm$ 49	212 $\pm$ 6	1287 $\pm$ 230	103 $\pm$ 10*	1390	7.4
	1.0	193 $\pm$ 11*	240 $\pm$ 41	213 $\pm$ 9	1265 $\pm$ 209	83 $\pm$ 8	1349	6.2
	2.5	133 $\pm$ 5**	208 $\pm$ 33	202 $\pm$ 14	1209 $\pm$ 13	97 $\pm$ 20	1306	7.0
	5.0	93 $\pm$ 3**	157 $\pm$ 28	193 $\pm$ 20	1122 $\pm$ 96	135 $\pm$ 27*	1257	10.7*
	10.0	62 $\pm$ 4**	108 $\pm$ 25	178 $\pm$ 36	1142 $\pm$ 122	181 $\pm$ 46*	1324	13.6*

When hepatocytes were incubated with palmitate and [2-³H]glycerol, simultaneously with Triton WR 1339 (Table 3), the formation of ketone bodies, and to a lesser extent the rate of fatty acid uptake, were impaired with increasing Triton WR 1339 concentration in both the absence and the presence of insulin. The accumulation of intracellular triacylglycerol was suppressed to a lesser degree than the rate of ketogenesis, suggesting that Triton WR 1339 favoured increased partitioning of the palmitate towards glycerolipid, as opposed to ketone body formation (Table 3). Experiments in which the accumulation of labelled glycerolipids was measured in cells incubated with conditioned medium (containing BSA and unlabelled acylglyceride) showed that the effects of both insulin and Triton were unaffected by the presence of the conditioned medium (Table 4); nor did the medium by itself alter the rate of secretion of labelled acyl-

glycerols. These observations suggested that the presence, in the medium, of excess unlabelled acylglycerols did not compete with newly synthesized (labelled) acylglyceride for extracellular lipase-mediated hydrolysis of secreted triacylglycerol.

Effects of Triton WR 1339 on BSA-mediated accumulation of triacylglycerol in culture medium

Addition of BSA (12.5 mg/ml) increased [¹⁴C]acylglycerol accumulation in the medium about 2-fold (Table 4). In the presence of Triton WR 1339, the rate of [¹⁴C]acylglycerol secretion was much higher (see above, Table 4). However, whereas this resulted in the masking of any effect of albumin, the inhibitory effect of insulin was still evident (Table 4).

To establish whether the effects of albumin and Triton WR 1339 on the accumulation of labelled acylglycerol in the medium

Table 4. Effect of insulin, BSA and conditioned medium on the accumulation of labelled acylglycerols in the culture medium

Hepatocytes were pre-cultured (12 h) with 1 mM-palmitate, 0.3 mM-[U-¹⁴C]glycerol (0.4 Ci/mol) and insulin as indicated. They were then incubated (6 h) with fresh or conditioned medium (see the Materials and methods section) without substrates, but containing the additions shown. Labelled acylglycerols secreted into the culture medium are expressed as d.p.m./mg of protein. Values are means $\pm$ S.E.M. of triplicate determinations from at least two experiments. * $P < 0.05$, ** $P < 0.005$ relative to respective controls.

	Medium [¹⁴ C]acylglycerol (d.p.m./mg)		
	No additions	BSA (1.25%)	Conditioned medium
None	1680 $\pm$ 91	3499 $\pm$ 244	3727 $\pm$ 110
100 nM-Insulin	1289 $\pm$ 135*	2961 $\pm$ 356*	3268 $\pm$ 146**
Triton WR 1339 (1 mg/ml)	5955 $\pm$ 342**	6003 $\pm$ 397**	5920 $\pm$ 301**
100 nM-Insulin + Triton WR 1339 (1 mg/ml)	5074 $\pm$ 346**	4668 $\pm$ 600**	5162 $\pm$ 342**

Table 5. Comparison of the effects of BSA and Triton WR 1339 on labelled acylglycerol and triacylglycerol secretion by hepatocyte cultures

Hepatocytes (inoculated into 25 cm² flasks) were pre-cultured (12 h) with 1 mM-palmitate and 0.3 mM-[2-³H]glycerol (3 Ci/mol). The medium was then aspirated and replaced with medium containing no substrates and the additions indicated. After 6 h the media from triplicate flasks were pooled and assayed as described in the Materials and methods section. Values are expressed per flask and represent the means of two experiments.

Addition	[³ H]Acylglycerol (d.p.m.)	Triacylglycerol (nmol)
Control	6420	14.8
BSA (40 mg/ml)	8962	24.7
Triton WR 1339 (1 mg/ml)	13706	28.9

reflect changes in the mass of triacylglycerol in the medium, hepatocytes (cultured in 25 cm² flasks) were pre-cultured with 1 mM-palmitate and 0.3 mM-glycerol and the triacylglycerol in the medium was determined enzymically. Triacylglycerol was increased by Triton WR 1339 and albumin (Table 5). These

changes were similar to the changes in secreted labelled acylglycerol.

Effects of BSA and insulin on the secretion of hepatic lipase

To investigate whether the greater accumulation of labelled acylglycerol in the medium of cultures during incubation with BSA is associated with differences in the extracellular activity of hepatic lipase, the secretion of lipase activity was determined in hepatocyte cultures incubated with 10 units of heparin and different concentrations of albumin in the presence or absence of insulin (Table 6). These studies were performed with both periportal and perivenous hepatocytes, since secretion of hepatic lipase is higher in periportal than in perivenous hepatocyte suspensions [50]. Insulin increased the secretion of hepatic lipase by 43% and 57% in periportal and perivenous hepatocytes respectively (Table 6). With increasing BSA concentration there was a decline in hepatic lipase activity which was more marked in periportal hepatocytes (Table 6). At a BSA concentration of 12.5 mg/ml, the activity of hepatic lipase was decreased to 20% of the corresponding control values (Table 6). BSA had no effect on the activity of hepatic lipase in solution (in perfusates of livers treated with heparin; see the Materials and methods section) when incubated with the enzyme for 10 h at 37 °C (results not shown). This result suggests that albumin exerts its inhibitory effect on the secretion of hepatic lipase by hepatocyte cultures and not directly on the enzyme. Therefore, the use of high concentrations of BSA in experiments with hepatocyte cultures should minimize the under-estimation of triacylglycerol secretion.

DISCUSSION

The present data have implications for the measurement of the rates of triacylglycerol by hepatocytes *in vitro* and the liver *in vivo*. The use of Triton WR 1339 *in vivo* is based on the assumption that it inhibits extracellular degradation and that it does not, by itself, interfere with hepatic metabolism in general and triacylglycerol secretion in particular. Such studies generally necessitate the use of an effective plasma concentration of the detergent of 1%. The present data show that, although Triton inhibits hepatic lipase activity maximally at one-tenth of this concentration (Fig. 1), it also affects hepatocyte metabolism even at this relatively very low concentration. In particular, it increases the apparent secretion rate of ¹⁴C-labelled acylglyceride and decreases the rate of both hepatocyte fatty acid uptake and the formation of ketone bodies. The latter two effects are especially pronounced and are exacerbated at higher concentrations, such

Table 6. Effects of BSA, heparin and insulin on the release of hepatic lipase by periportal and perivenous hepatocyte cultures

Hepatocytes were incubated (10 h) with MEM containing dexamethasone (10 nM) in either the presence or the absence of insulin and heparin (10 units) except where indicated. Values of hepatic lipase activity are expressed as nmol of glycerol formed/h per mg of cell protein, and represent the means $\pm$ S.E.M. of duplicate incubations for the numbers of experiments shown in parentheses. * $P < 0.05$, ** $P < 0.005$ for paired observations relative to incubations without albumin.

Additions	Hepatic lipase activity in culture medium (nmol/h per mg)			
	Periportal hepatocytes (5)		Perivenous hepatocytes (4)	
	Controls	100 nM-Insulin	Controls	100 nM-Insulin
Control	18.3 $\pm$ 2.4	26.1 $\pm$ 3.6	4.8 $\pm$ 0.9	8.4 $\pm$ 0.9
BSA (1.0 mg/ml)	15.0 $\pm$ 3.6	22.8 $\pm$ 3.9	1.5 $\pm$ 0.6*	3.0 $\pm$ 0.6*
BSA (4.2 mg/ml)	4.5 $\pm$ 0.6**	9.3 $\pm$ 2.4**	1.8 $\pm$ 0.3**	1.8 $\pm$ 0.6**
BSA (12.5 mg/ml)	3.3 $\pm$ 0.3**	5.4 $\pm$ 0.6**	1.8 $\pm$ 0.6**	2.4 $\pm$ 0.6**
No heparin	1.8 $\pm$ 0.3**	2.1 $\pm$ 0.6**	0.9 $\pm$ 0.6**	1.2 $\pm$ 0.6**

that both parameters are inhibited by about 85% at a concentration of 1% Triton. Evidently, if the effective concentration of Triton WR 1339 as used in *in vivo* studies is about 1% [35], then serious reservations need to be expressed about the viability of the liver under these conditions, particularly with respect to fatty acid transport across the plasma membrane and mitochondrial function. It is possible, of course, that *in vivo* the effects of Triton on individual hepatocytes are mitigated by the fact that a much smaller proportion of their total plasma membrane surface area is exposed to the blood, and that other blood constituents may protect against any detergent effects of Triton (e.g. by binding the detergent).

With respect to the use of Triton in studies *in vitro*, our data suggest that it may inhibit, rather than stimulate, secretion of triacylglycerol, in spite of the increased accumulation of triacylglycerol (measured both by release of label and by lipid mass in the culture medium). This is because, in the presence of Triton, the accumulation of cellular acylglycerides was higher (Table 2). As assembly and secretion of VLDL-triacylglycerol is thought to involve lipolysis of endogenous triacylglycerol stores [51], it is possible that such increased accumulation of intracellular triacylglycerol reflects an inhibition of lysosomal lipolysis by Triton. However, the detergent also caused a substantial increase in the accumulation of triacylglycerol in the medium, which leads us to conclude that extracellular degradation of secreted lipid (which is inhibited by Triton) results in a significant under-estimate of the true rate of secretion. If Triton inhibits triacylglycerol secretion (see above), the extent of lipase-mediated hydrolysis of secreted triacylglycerol in hepatocyte culture media may even be underestimated. However, it is evident that the inhibitory effects of insulin on acylglycerol secretion were not abolished by inhibition of hepatic lipase activity, thus suggesting that insulin exerts a genuine inhibitory effect on secretion under these *in vitro* conditions. Whether this effect also occurs *in vivo* is still an open question. However, Vogelberg *et al.* [52] showed that insulin injected into the portal vein decreased hepatic triacylglycerol output in the absence of any change in fatty acid uptake. If Triton WR 1339 inhibits triacylglycerol secretion, secondary to inhibition of intracellular lipase activity, its use *in vivo* may lead to an under-estimation of the true rate of triacylglycerol secretion. It is noteworthy that rates of triacylglycerol secretion *in vivo* determined using Triton WR 1339 are, in general, substantially lower than values obtained from triacylglycerol turnover studies [53].

After completion of this work a report of an experiment in which a single concentration of Triton WR 1339 was found to increase both intracellular and secreted triacylglycerol in hepatocyte cultures appeared [54].

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