The intracellular triacylglycerol/fatty acid cycle: a comparison of its activity in hepatocytes which secrete exclusively apolipoprotein (apo) B\textsubscript{100} very-low-density lipoprotein (VLDL) and in those which secrete predominantly apoB\textsubscript{48} VLDL

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INTRODUCTION

Triacylglycerol (TAG) synthesized by the liver is transported into the plasma as particles of very-low-density lipoprotein (VLDL), the intracellular assembly of which is directed by apolipoprotein B (apoB). apoB provides the structural framework required for the addition of lipids in the secretory apparatus of the hepatocyte (for recent reviews, see [1–3]). Little is known of the molecular mechanisms involved in the transfer of lipid, particularly TAG, to the developing VLDL particle, and controversy remains as to whether the process involves a single discrete step [4,5] or a dual, or even a multi-step, cascade of lipid transfer [6–9]. What is clear, however, is that microsomal triacylglycerol transfer protein is obligatory for the transfer of at least some cellular lipid to apoB in the lumen of the secretory apparatus of the cell [10–13]. The metabolic route(s) by which hepatically synthesized TAG is made available for assimilation into VLDL is not yet been clearly defined [14]. In this respect, however, it seems likely that, at least in rat liver, TAG newly synthesized in the endoplasmic reticulum (ER) from fatty acids originating extracellularly is not immediately transferred to nascent VLDL, but passes initially into the cellular pool following re-esterification. Recycling in the hamster was somewhat greater than in the rat (66.1 ± 5.7% versus 53.7 ± 4.8% of TAG lipolysed respectively). Similarly, a larger proportion of newly synthesized TAG was retained within the cell, rather than secreted as VLDL, in the hamster compared with the rat (37.9 ± 2.8%, versus 20 ± 3.8%, P < 0.01). These factors may have contributed to the somewhat lower rate of VLDL TAG secretion in the hamster hepatocytes compared with those from the rat (43.3 ± 4.2 versus 96.4 ± 3.4 μg/24 h per mg of cell protein). Rat hepatocytes were more sensitive to inhibition of VLDL secretion by insulin than were those from hamster. In neither case did insulin affect total or fractional TAG turnover. The results suggest that assembly of both apoB\textsubscript{100} VLDL and apoB\textsubscript{48} VLDL is associated with efficient intracellular TAG lipolysis.

Abbreviations used: VLDL, very-low-density lipoprotein; apoB, apolipoprotein B; TAG, triacylglycerol; ER, endoplasmic reticulum.

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TAG) binding to apoB appears to protect the protein from intracellular degradation. It has been suggested that insulin inhibits the secretion of apoB, at least in part, by enhancing the susceptibility of apoB to degradation [1]. In this respect, in rat hepatocytes apoB<sub>apo</sub> is more vulnerable than apoB<sub>nas</sub> [1,32]. Differences in the mode of addition of lipid to apoB<sub>apo</sub> on one hand, and to apoB<sub>nas</sub> on the other [7,9,25,33], may underlie this differential susceptibility to insulin. A second objective of the present work therefore was to compare the susceptibility of hepatic VLDL secretion to inhibition by insulin in hamster hepatocytes, which secrete only apoB<sub>apo</sub>, with that in rat hepatocytes, which secrete mainly apoB<sub>nas</sub>.

MATERIALS AND METHODS

Materials

All reagents, including organic solvents, were of analytical grade. Materials for tissue culture (Waymouth’s medium, Dulbecco’s PBS, fetal calf serum, penicillin, streptomycin) were obtained from Gibco Ltd. (Paisley, Scotland, U.K.). Radiochemicals ([1-14C]oleate, [2-14H]glycerol) were obtained from Amersham International (Aylesbury, Bucks., U.K.). BSA (fatty acid free), dexamethasone, lactate, pyruvate, sodium oleate, glycerol and bovine insulin were from Sigma (Poole, Dorset, U.K.). The triacylglycerol assay kit (GPO-PAP) was obtained from Boehringer-Mannheim (Lewes, Sussex, U.K.).

Maintenance of animals

Male Wistar rats were maintained in an artificially lit room at 20 ± 2 °C as described previously [34]. Animals were acclimatized under these conditions for at least 2 weeks before hepatocyte preparation, at which time they weighed between 220 and 280 g. Male DSNI Golden Syrian hamsters (120–150 g) were maintained under similar conditions [55].

Hepatocyte preparation

Rat hepatocytes were prepared as described previously and were suspended (0.65 × 10<sup>6</sup> cells/ml) in Waymouth’s medium MB752/1 containing added amino acids, antibiotics and heat-inactivated fetal calf serum [36]. Hamster hepatocytes were prepared as described previously [37] and were suspended (0.92 × 10<sup>6</sup> cells/ml) in the above medium. Hepatocyte suspensions (3.0 ml) were plated into collagen-coated dishes [38] in the presence or absence of insulin (78 nM) for 4 h. During this period, cellular TAG was labelled by the addition of a mixture of [14C]oleate (0.375 mM; 1.96 × 10<sup>5</sup> d.p.m./nmol) bound to BSA (final concentration 0.5%), and [2-14H]glycerol (0.25 mM; 2.93 × 10<sup>5</sup> d.p.m./nmol). After 4 h, the medium containing the labelled substrates was removed and the VLDL fraction was isolated [34]. The cell monolayer was washed twice with PBS. Two dishes of cells from each experimental group (hamster + insulin, rat + insulin) were harvested. Each experimental manipulation with each hepatocyte preparation was carried out using duplicate sets of dishes and the values obtained were averaged. The 3H/14C ratios of the VLDL and cellular TAG were determined and the TAG mass of each was determined enzymically (see below).

Analytical methods

VLDL was isolated from the medium of the cells by ultracentrifugation at a density of 1.006 as described previously [34]. The protein content of the hepatocytes was determined using the method of Lowry et al. [39]. Lipids from cells and VLDL fractions were extracted using the method of Folch et al. [40]. Aliquots of these total-lipid fractions were removed for enzymic measurement of the mass of VLDL and cellular TAG using the TAG GPO-PAP assay kit. Individual lipids doubly labelled with 14C and 3H were separated from the remainder of the total-lipid fraction by TLC [41]. Bands containing these lipids were scraped from the plate and assayed for 14C and 3H by liquid-scintillation counting. Oleate was bound to BSA (essentially fatty acid-free) by the method of Van Harken et al. [42].

Calculations

When cellular TAG is pre-labelled with [3H]glycerol and [14C]-oleate, followed by culture of cells in the absence of extracellular fatty acid, de novo synthesis of unlabelled TAG might be expected to decrease the 3H specific radioactivity of VLDL and cellular TAG to exactly the same extent as that for 14C. Any differential change in the specific radioactivity of the two isotopes implies that the [3H]glycerol and [14C]oleate moieties must, at some stage, have become metabolically distinct during the subsequent culture period, a process which must have required lipolysis followed by re-esterification. For example, Table 1 shows that, in the rat, at the end of the 4 h pre-incubation period in the presence of the labelled TAG substrates, the 14C specific radioactivity of the cellular TAG was 1557 ± 175 d.p.m./µg (this value is subsequently referred to as the initial specific radioactivity). After removal of the labelled substrates, and further culture for 24 h, the 14C specific radioactivity of the cellular TAG was 951 ± 67 d.p.m./µg. That of the secreted VLDL TAG was 1470 ± 92 d.p.m./µg. The 14C specific radioactivity of the total (cell + VLDL) TAG after 24 h (final specific radioactivity) was 1347 ± 153 d.p.m./µg, which represents a small overall decrease of 18.6 ± 3.2% compared with the original cellular 14C TAG specific radioactivity. The initial specific radioactivity of the 3H-labelled cellular TAG was 2042 ± 248 d.p.m./µg. This declined to 655 ± 129 d.p.m./µg (cell) and 764 ± 43 d.p.m./µg (VLDL). The final specific radioactivity of the total (cell + VLDL) 3H TAG was 757 ± 64 d.p.m./µg, which represents 59.6 ± 6.3% decrease compared with the initial cellular 3H TAG from which it was derived. The latter change quite clearly represents a much larger decline than that observed for the specific radioactivity of the 14C TAG. Thus the excess decline in the 3H specific radioactivity compared with that of the 14C specific radioactivity is a measure of the extent to which the 3H glycerol moiety, specifically, was diluted compared with the 14C oleate moiety, an outcome which must have arisen by lipolysis of the original cellular TAG and re-esterification of the resultant labelled fatty acids with a glycerol-containing moiety of lower 3H specific radioactivity. The overall effect is a net influx of unlabelled glycerol into the cellular TAG pool, the extent of which may be measured by the relative loss of 3H compared with 14C from the TAG pool, i.e. a decrease in the 3H/14C specific-activity ratio. Thus it may be calculated from the above example that the 3H/14C specific-activity ratio declined from an initial value of 1.36 ± 0.13 immediately after pre-labelling to 0.61 ± 0.11 after 24 h culture in the absence of...
Table 1 Relative decline in [3H]TAG specific radioactivity

<table>
<thead>
<tr>
<th>Rat</th>
<th>Hamster</th>
</tr>
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<tbody>
<tr>
<td><strong>Initial cell TAG</strong></td>
<td></td>
</tr>
<tr>
<td>Specific activity [3H] (d.p.m./µg)</td>
<td>2042±247</td>
</tr>
<tr>
<td>Specific activity [14C] (d.p.m./µg)</td>
<td>1557±175</td>
</tr>
<tr>
<td>[3H]/[14C] ratio</td>
<td>1.36±0.13</td>
</tr>
<tr>
<td><strong>Final cell TAG</strong></td>
<td></td>
</tr>
<tr>
<td>Mass (µg/mg of cell protein)</td>
<td>38.6±6.8</td>
</tr>
<tr>
<td>Specific activity [3H] (d.p.m./µg)</td>
<td>655±129</td>
</tr>
<tr>
<td>Specific activity [14C] (d.p.m./µg)</td>
<td>951±67</td>
</tr>
<tr>
<td>[3H]/[14C] ratio</td>
<td>0.77±0.13***</td>
</tr>
<tr>
<td>[3H]/[Initial cell ratio]</td>
<td>57.1±8.9</td>
</tr>
<tr>
<td><strong>Final VLDL TAG</strong></td>
<td></td>
</tr>
<tr>
<td>Mass (µg/mg of cell protein)</td>
<td>96.4±3.4</td>
</tr>
<tr>
<td>Specific activity [3H] (d.p.m./µg)</td>
<td>764±43</td>
</tr>
<tr>
<td>Specific activity [14C] (d.p.m./µg)</td>
<td>1470±92</td>
</tr>
<tr>
<td>[3H]/[14C] ratio</td>
<td>0.55±0.09***</td>
</tr>
<tr>
<td>[3H]/[Initial cell ratio]</td>
<td>39.4±2.5†</td>
</tr>
<tr>
<td><strong>Final total (cell + VLDL) TAG</strong></td>
<td></td>
</tr>
<tr>
<td>Mass (µg/mg of cell protein)</td>
<td>136±4</td>
</tr>
<tr>
<td>Specific activity [3H]</td>
<td>757±64</td>
</tr>
<tr>
<td>Specific activity [14C]</td>
<td>1347±153</td>
</tr>
<tr>
<td>[3H]/[14C] ratio</td>
<td>0.61±0.11***</td>
</tr>
<tr>
<td>[3H]/[Initial cell ratio]</td>
<td>44±5</td>
</tr>
<tr>
<td>Fractional turnover of TAG (pools/24 h)</td>
<td>1.39±0.23</td>
</tr>
</tbody>
</table>

labelled and unlabelled exogenous glycerol and oleate. This latter value was 44±5% of the initial value. The excess net dilution of the original TAG glycerol pool by unlabelled glycerol thus amounts to (100/44)−1 pools. In general therefore, if the final [3H]:[14C] specific-activity ratio is X%, the net amount of an unlabelled glycerol-containing moiety which has entered the TAG glycerol pool to achieve this dilution is (100/X)−1 pools. This value is equivalent to the fractional rate of lipolysis (or turnover). As the total mass of the cellular TAG pool is known, this calculation may be used to estimate the total mass of TAG that has undergone lipolysis followed by re-esterification (i.e. fractional turnover multiplied by pool size). The validity of these calculations depends partly upon the assumption that there is little or no unesterified [14C]oleate present in the cell during the chase period. That this assumption was justified is clear from measurements of [14C]oleate at the beginning and end of the chase period. These amounted to 6.5±1.7% and 9.8±1.2% of the corresponding [14C]TAG label respectively for the rat. For the hamster, the corresponding values were 14.5±6.7% and 10.1±1.4%.

**Statistical analysis**

All values are presented as the mean±S.E.M. of several independent experiments. Significant differences were obtained using a paired Student’s t-test.

RESULTS

**VLDL and cellular TAG turnover**

Table 1 shows the changes in the [3H] and [14C] specific radioactivities of VLDL and cellular TAG that occurred during a 24 h culture of hamster and rat hepatocytes in which the original cellular TAG was pre-labelled with [3H]glycerol and [14C]oleate. In both species the decline in the [3H] specific radioactivity was greater than that observed for the [14C] specific radioactivity. During the 24 h culture period, some of the original dual-labelled cellular TAG was secreted as VLDL and some remained within the cell. Summation of the specific radioactivity of TAG in each of these pools for both [3H] and [14C] and comparison with the specific radioactivity of the original cell TAG permits calculation of the relative decline in [3H] specific radioactivity and thus of the net entry of an unlabelled glycerol-containing moiety into the TAG pool (see the Materials and methods section). These calculations show that, in the rat hepatocytes, the [3H]:[14C] specific-activity ratio of the total TAG at the end of the culture period was only 44±5% of that of the initial cellular TAG. This is equivalent to the entry of 1.39±0.23 pools of an unlabelled glycerol-containing moiety into the original TAG pool. A similar relative loss of [3H] TAG glycerol also occurred during 24 h culture of hamster hepatocytes. In this case the [3H]:[14C] specific-activity ratio declined to 38±4% of the original value, which represents an entry of 1.83±0.28 pools of unlabelled glycerol. These values represent the fractional turnover of TAG (lipolysis and re-esterification) in rat and hamster hepatocytes respectively. Table 1 also shows that the relative decline in the [3H] specific radioactivity of the VLDL TAG was greater than that in the cellular TAG at the end of the 24 h culture period. This was true for both the rat and hamster hepatocytes. Thus, in the former, the relative specific radioactivity of the [3H] VLDL TAG declined to 0.55 compared with 0.77 in the remaining cell TAG. Since the relative [3H] specific radioactivity of the initial cell TAG was 1.36, the value for VLDL (39.4% of initial) suggested a relative loss of 66.6% of the VLDL [3H] TAG label and this represents the minimum proportion of VLDL TAG that was secreted following lipolysis and re-esterification. The relative decline in the [3H] specific radioactivity of VLDL TAG in the hamster (30.9%, of original) suggested that at least 69.1% of this material had, in this case, been secreted after lipolysis. It is possible that the relative loss of labelled glycerol was a result of extracellular lipolysis of VLDL TAG followed by re-uptake and cellular re-esterification of the resulting fatty acids. This alternative explanation was ruled out by culturing hepatocytes with added extracellular VLDL in which the TAG had been labelled with [3H]oleate (150 µg of VLDL TAG; 1.07×10^6 d.p.m.). Over a 24 h period there was a cellular uptake of only 8.3±1.2% of the label. This corresponds to a maximum re-uptake of 12.4 µg of TAG compared with a total cellular TAG turnover of 186 µg during this period (Table 2).

**Effects of insulin**

To determine the effects of insulin on TAG turnover and VLDL output in a model secreting exclusively apoB_48 VLDL, hamster hepatocytes containing TAG, pre-labelled with [3H]glycerol and [14C]oleate for 4 h, were cultured for 24 h in the presence or absence of insulin. Because, in rat hepatocytes at least, the secretion of VLDL is affected by pre-exposure of the cells to insulin [34,36,43], the hormone was also present or absent during the pre-labelling period. The results of this experiment are shown in Table 3. In general, rates of VLDL TAG secretion in the rat hepatocytes were considerably higher than those in hamster
Table 2  TAG lipolysis and re-esterification in rat and hamster hepatocytes: effects of insulin

Hepatocytes from rat and hamster were cultured for consecutive periods of 4 h and 24 h in the presence or absence of insulin as described in the Materials and methods section. VLDL TAG output was measured during the final 24 h period. Values are the mean ± S.E.M. of five animals in each group. The presence or absence of insulin is denoted by ‘−’ and ‘+’ respectively. Values marked *** in the rat are significantly different (P < 0.001) from the corresponding values in the hamster.

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Fractional turnover (pools TAG/24 h)</th>
<th>Total TAG pool size (µg/mg of protein)</th>
<th>Absolute TAG turnover (µg/24 h per mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–4 h</td>
<td>4–28 h</td>
<td>Hamster</td>
</tr>
<tr>
<td>−I −I</td>
<td>1.83 ± 0.28</td>
<td>1.39 ± 0.23</td>
<td>77 ± 5</td>
</tr>
<tr>
<td>−I +I</td>
<td>1.81 ± 0.13</td>
<td>1.49 ± 0.27</td>
<td>80 ± 6</td>
</tr>
<tr>
<td>+I −I</td>
<td>1.78 ± 0.16</td>
<td>1.39 ± 0.22</td>
<td>87 ± 8</td>
</tr>
<tr>
<td>+I +I</td>
<td>1.99 ± 0.18</td>
<td>1.48 ± 0.20</td>
<td>87 ± 6</td>
</tr>
</tbody>
</table>

Table 3  Effects of insulin on VLDL TAG output in rat and hamster hepatocytes

Hamster and rat hepatocytes were cultured under the conditions described in the legend to Table 2. The values below represent the mean ± S.E.M. of five hepatocyte preparations from each species. The presence or absence of insulin is denoted by ‘−’ and ‘+’ respectively. ‘∗∗’ and ‘***’ are significantly different from the corresponding values for the hamster (P < 0.01 and P < 0.001 respectively). Values marked ‘±’ and ‘††’ are significantly different (P < 0.01 and P < 0.001 respectively) from the corresponding values obtained in the absence of insulin.

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>VLDL TAG output (µg/mg of cell protein)</th>
<th>Relative effect of insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–4 h</td>
<td>4–28 h</td>
</tr>
<tr>
<td>−I −I</td>
<td>43.3 ± 4.2</td>
<td>96.4 ± 3.4***</td>
</tr>
<tr>
<td>−I +I</td>
<td>36.6 ± 4.1</td>
<td>60.4 ± 4.0***††</td>
</tr>
<tr>
<td>+I −I</td>
<td>47.8 ± 6.1</td>
<td>102.6 ± 10.9**</td>
</tr>
<tr>
<td>+I +I</td>
<td>38.0 ± 4.4</td>
<td>75.2 ± 6.5**</td>
</tr>
</tbody>
</table>

hepatocytes cultured under identical conditions. Insulin, present after the pre-labelling period only, suppressed VLDL TAG output to a greater extent in the rat hepatocytes compared with the hamster cells. The presence of insulin during the pre-labelling period had little subsequent effect on the secretion of VLDL in hepatocytes of either species. However, in the rat hepatocytes, as observed previously [34,36,43], pre-treatment with insulin attenuated the subsequent inhibitory effect of insulin on the secretion of VLDL TAG.

The effects of the various regimes of insulin treatment on TAG turnover were compared in the rat and hamster hepatocytes (Table 2). The presence of insulin during either the pre-incubation or the subsequent culture periods had no effect on either the fractional or total turnover of TAG. This was the case both in the rat and in the hamster. However, in the rat, the absolute turnover of TAG was somewhat higher than that in the hamster, an effect which was due to the significantly larger TAG pool size in the former species. The increase in the TAG pool size in the rat resulted, at least in part, from a higher rate of TAG synthesis from extracellular oleate during the initial 4 h culture period compared with that observed in the hamster hepatocytes. During this period, rat and hamster hepatocytes incorporated 130 ± 22 and 70 ± 8 nmol [14C]oleate respectively into the sum of the cellular and VLDL TAG per mg cell protein. This gave rise to a larger intracellular TAG pool in the rat cells at the start of the subsequent 24 h culture period compared with that in the hamster cells. Insulin had little effect on TAG synthesis during the 4 h labelling period (158 ± 22 and 79 ± 8 nmol [14C]oleate incorporated in the rat and hamster hepatocytes respectively).

We have previously shown that, in rat hepatocytes, a large proportion of the fatty acids released from the cytosolic pool by lipolysis do not enter the TAG secretory pathway but are re-esterified and recycled, by some as yet unidentified pathway, back to the cell cytosol. We therefore compared, in hamster and rat hepatocytes, the proportion of re-esterified fatty acids which entered the secretory pathway on the one hand, and that which was recycled back to the intracellular pool on the other. To do this, the mass of TAG secreted as VLDL (Table 3) was subtracted from the value determined for absolute TAG turnover (Table 2). This calculation assumes that all the VLDL TAG was synthesized after lipolysis, an assumption which probably over-estimates the quantity of TAG secreted via this route. Thus the values shown in Table 4 for the proportion of TAG turnover estimates the quantity of TAG secreted as VLDL. We also determined (Table 2) the proportion of fatty acids secreted and a larger proportion recycled, compared with rat hepatocytes. In both species, treatment of hepatocytes with insulin during the 24 h period decreased the proportion of fatty acids, released by lipolysis, that entered the secretory pathway after re-esterification. A similar pattern of distribution of newly synthesized TAG between the cellular and VLDL secretory pools.

Table 4  VLDL TAG output and intracellular TAG recycling

Absolute values for cellular TAG turnover were calculated as in Table 2 and values for VLDL TAG output are given in Table 3. TAG recycling was calculated as the difference between total TAG turnover and VLDL TAG output. Values are the mean ± S.E.M. for five animals in each group.

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>VLDL output (% of TAG turnover)</th>
<th>TAG recycling (% of TAG turnover)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–4 h</td>
<td>4–28 h</td>
</tr>
<tr>
<td>−I −I</td>
<td>33.9 ± 5.7</td>
<td>46.3 ± 4.8</td>
</tr>
<tr>
<td>−I +I</td>
<td>25.0 ± 2.6</td>
<td>27.0 ± 4.6</td>
</tr>
<tr>
<td>+I −I</td>
<td>31.7 ± 3.4</td>
<td>40.1 ± 5.0</td>
</tr>
<tr>
<td>+I +I</td>
<td>21.8 ± 2.2</td>
<td>28.8 ± 2.4</td>
</tr>
</tbody>
</table>
was also observed in hamster and rat hepatocytes (Table 5). In this case, in the absence of insulin, hamster hepatocytes secreted a smaller proportion of the newly synthesized TAG and retained a greater proportion within the cell. In both species, but especially in the rat, insulin decreased the proportion that was secreted.

**DISCUSSION**

Rat hepatocytes secrete TAG-rich particles in association either with apoB_{100} or with apoB_{100} [28]. Several previous studies have provided evidence that the mechanism(s) of bulk lipid recruitment for the synthesis of particles containing apoB_{100} differs from that involved in the synthesis of particles containing apoB_{100} [7,9, 25,33]. It is possible that this difference may provide the mechanistic basis for the differential metabolism of these two types of particle following dietary manipulation [44,45].

In rat hepatocytes, the major direct source of VLDL TAG is the intracellular storage pool of cytosolic TAG [15,16,46]. The assembly of VLDL requires transfer of this TAG pool from the cytosol across the phospholipid bilayer of the ER membrane, a process which involves a large degree of TAG lipolysis and re-esterification [16-20]. A similar mechanism also appears to be involved in the recruitment of TAG for chylomicron assembly in enterocytes [26]. In view of the different mechanisms for assembly of apoB-[1] and apoB-[2] containing lipoproteins, it might be argued that the lipolytic pathway of TAG recruitment is associated only with those cells (e.g. hepatocytes of rat, and enterocytes) which utilize mainly, or predominantly, apoB,[3] since the existence of a similar pathway has not yet been demonstrated in an apoB-[4] lipoprotein-secreting hepatocyte. Indeed, the human hepatoma cell line HepG2, which secretes exclusively apoB,[5], is relatively inactive in TAG lipolysis [22]. The present results show that there is no such inherent difference between apoB-[6] and apoB-[7], secreting hepatocytes, since hamster hepatocytes, which utilize exclusively apoB,[8] for the assembly of VLDL, contain an active lipolysis and re-esterification pathway. If, as proposed earlier in a rat hepatoma cell line, TAG recruitment for apoB,[9] VLDL assembly occurs exclusively and simultaneously with apoB,[10] translation/translocation [7,9], and assuming that the mechanism of assembly of apoB,[11]-VLDL is similar in rat hepatoma and hamster hepatocytes, it follows that cytosolic TAG lipolysis and re-esterification must be intimately associated with the apoB,[12] translocation step in hamster liver. However, in HepG2, even when the intracellular TAG concentration is not limiting for VLDL assembly, apoB,[13] is not translocated into the secretory lumen as a lipid-rich particle and this abnormality may be linked to a defective lipolysis/re-esterification cycle [22].

The proportion of VLDL TAG secreted after lipolysis, and the extent of fractional turnover (lipolysis/re-esterification) of TAG in hamster hepatocytes, were similar to that observed in the rat (Tables 1 and 2). The lower rate of VLDL TAG output in the former cells (Table 3) was not therefore a consequence of defective TAG turnover per se, but most probably resulted from a combination of a smaller intracellular pool size (Table 2), which was itself a consequence of a lower rate of TAG synthesis, and a redistribution of the products of lipolysis/re-esterification in favour of the recycling pathway rather than the secretory pathway (Table 4). The latter calculation was also consistent with similar observed differences in the distribution of newly synthesized TAG into the secretory and storage pathways in the hamster and rat (Table 5). It has previously been reported that the perfused liver of the hamster secreted more VLDL TAG than that of the rat [47]. That study, however, utilized Holtzmann rats, the livers of which secreted considerably less VLDL than those of the Wistar rats used in the present work.

A major question concerning the relevance of intracellular TAG lipolysis to the process of lipid recruitment for VLDL assembly is whether some of the fatty acids released by lipolysis are immediately sequestered and targeted for secretion. The other alternative is that all the fatty acids produced by lipolysis are re-esterified and returned to the cytosol. This alternative would also result in a relative decline in the specific radioactivity of [3H]TAG, but no further lipolysis would be required for VLDL secretion. The latter alternative would imply the presence of a constitutive intracellular fatty acid/TAG cycle which was not linked to VLDL TAG recruitment specifically. This latter model predicts that the cellular and VLDL TAG pools are identical and in simple equilibrium with each other. If this was the case then the radioactivity of the VLDL TAG would be identical with that of the cellular TAG after a further 24 h of culture. This pattern was not observed (Table 1). The significantly greater decline in the [3H]:[14C] specific activity ratios of the VLDL TAG compared with that of the final cell TAG in both rat and hamster hepatocytes suggested that these two pools are not in simple equilibrium. Similar differences were also observed during the various regimes of insulin treatment (results not shown). These results suggest that the recruitment of cellular TAG for VLDL assembly involves a process in which fatty acids released by lipolysis are transferred to a cellular re-esterification site(s) at which at least some of the resultant TAG is targeted exclusively into the secretory pool. A recent report which documents the presence of a luminal form of diacylglycerol acyltransferase [48] supports this interpretation.

The possibility remains, of course, that cytosolic TAG is itself present in metabolically distinct pools, each with a different initial [3H]:[14C] ratio at the end of the labelling period, and that preferential secretion of one pool would result in a change in the overall TAG label. We cannot completely exclude this possibility.
by direct measurement. On the other hand, if this were substantially the case, then it might be expected that a decline in the $^3$H:$^{14}$C specific activity ratio in the cell TAG would be mirrored by a reciprocal increase in the ratio of the VLDL TAG. This was not observed. Nevertheless, it should be pointed out that our results do not negate that a portion of the cytosolic TAG is sequestered into a specific pool prior to lipolysis.

It has previously been shown that, in rat hepatocytes, insulin enhances the degradation of newly synthesized apoBapoB $^{[22]}$ is not therefore an inherent consequence of the presence of

apoBapoB $^{[22]}$. It might be expected therefore that VLDL assembly in the hamster, a process which utilizes exclusively apoBapoB $^{[32]}$. It might be expected therefore that VLDL assembly in the hamster, a process which utilizes exclusively apoBapoB $^{[32]}$, but results from some fundamental defect in intracellular TAG turnover and transport into the secretory apparatus of the cell.

In conclusion, the recruitment of cellular TAG for the assembly of VLDL appears to occur predominantly via a process of lipolysis and re-esterification, irrespective of whether VLDL particles are assembled with apoBapoB $^{[100}$ or apoBapoB $^{[18]}$. The decreased contribution of the lipolytic pathway to lipid secretion in HepG2 $^{[22]}$ is not therefore an inherent consequence of the presence ofapoBapoB $^{[32]}$ but results from some fundamental defect in intracellular TAG turnover and transport into the secretory apparatus of the cell.

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