Use of cytosolic triacylglycerol hydrolysis products and of exogenous fatty acid for the synthesis of triacylglycerol secreted by cultured rat hepatocytes

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Abstract **The fatty acyl moieties incorporated into triacylglycerol (TAG) secreted by rat hepatocytes are derived from diacylglycerol (DAG) that is synthesized de novo through the phosphatidate pathway or derived from endogenous, cytosolic TAG after hydrolysis to DAG and re-esterification. We have used a dual-labeling technique (overnight labeling of cell TAG with [^{3}H]oleate, followed by 3 h incubation with [^{14}C]oleate) to quantify the contributions of the two sources towards TAG secretion by cultured rat hepatocytes. A wide range of TAG secretion rates was achieved by short-term incubation of the cells under a variety of conditions. There was no correlation between the overall amount of exogenous ^{14}C-labeled fatty acid metabolized and the rate of either [^{14}C]- or [^{3}H]TAG secretion. By contrast, there was a strong positive correlation between the fraction of newly synthesized [^{14}C]TAG that was secreted (the fractional secretion rate, FSR) and the absolute rate of TAG secretion. This suggests that the partitioning of DAG between (re)synthesis of cytosolic TAG and synthesis of secreted TAG is an important locus for the control of the rate of TAG secretion. Comparison of the ratio: oxidation/TAG secretion for ^{3}H- and ^{14}C-labeled acyl moieties indicated that, for all conditions studied, approximately half the acyl moieties already esterified to the glyceroyl backbone within cytosolic TAG remain unavailable for oxidation when this pool of TAG is mobilized for the synthesis of secreted TAG. The data provide evidence that hydrolysis of cytosolic triacylglycerol (TAG) does not proceed fully to the constituent fatty acids and glycerol, but only to the level of diacylglycerol, followed by re-modelling of approximately half of its acyl chains, before re-esterification to form secretory TAG.—** Lankester, D. J., A. M. Brown, and V. A. Zammit. **Use of cytosolic triacylglycerol hydrolysis products and of exogenous fatty acid for the synthesis of triacylglycerol secreted by cultured rat hepatocytes. *J. Lipid Res.* 1998.** 39: **1889–1895.**

Supplementary key words very low density lipoprotein • secretion • liver • hepatocytes • diacylglycerol • diacylglycerol acyltransferase (DGAT)

The liver contains several pools of triacylglycerol (TAG; see ref. 1) one of which is cytosolic droplet TAG. It is well established that cytosolic TAG contributes significantly to secreted TAG. However, it is not incorporated en bloc across the endoplasmic reticular (ER) membrane (2) but first has to be hydrolyzed. Theoretically, hydrolysis of cytosolic TAG could be complete to the component fatty acyl chains and glycerol (3–5) necessitating the duplication of the phosphatidate pathway enzymes at a second (secretion-dedicated) site. However, recent observations on the positional distribution of specific acyl chains of liver TAG and very low density lipoprotein (VLDL)-TAG have indicated that cytosolic TAG may be only partially hydrolyzed (6). Thus, whereas there is almost complete identity between the distribution of fatty acyl chains in the *sn*-1 and *sn*-2 positions, there are differences between the stereospecific distribution of the acyl chains in the *sn*-3 position (6). This has led to the suggestion, therefore, that hydrolysis proceeds primarily to *sn*-1, 2 diacylglycerol (DAG) which, after re-modelling, is re-esterified to form a secretory pool of TAG (6).

Teleologically, there is a distinct advantage to the hepatocyte of hydrolyzing cytosolic TAG only to DAG rather than all the way to fatty acyl-CoA and glycerol. Thus, instead of having to duplicate all the enzymes of the phosphatidate pathway at a secretion-dedicated site, only one TAG-synthesizing enzyme would need to be duplicated, namely diacylglycerol acyltransferase (DGAT). Such a second DGAT would need to have a subcellular location at which its product can be targeted towards secretion. We have previously shown that rat liver microsomes contain overt and latent DGAT activities (7) and have suggested that the lumen-facing activity may be specialized for the synthesis of TAG destined for secretion. The existence of this lumen-facing DGAT implies the transfer of DAG (and acyl moieties, presumably as acylcarnitines, see refs. 1, 3) across the ER membrane. This makes the branch-point

Abbreviations: TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; PL, phospholipid; VLDL, very low density lipoprotein; FSR, fractional rate of secretion of triacylglycerol; apoB, apolipoprotein B; FA, fatty acid; DGAT, diacylglycerol acyltransferase.

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represented by the partitioning of DAG, between the (re)synthesis of cytosolic droplet TAG and the formation of secretory TAG, a potentially important locus for the control of the rate of TAG secretion. Evidence in favor of this suggestion emerged from in vivo studies in which a strong correlation was observed between the fraction of newly synthesized TAG that was secreted by the liver (fractional secretion rate, FSR) and the rate of appearance of VLDL-TAG in the circulation i.e., hepatic TAG secretion (8). Moreover, several in vivo studies have shown that the partitioning of newly synthesized TAG between retention in the liver and secretion can be acutely modulated (e.g., during the prandial period (9–13)). It would be anticipated, therefore, that this partitioning should also be capable of modulation by factors that affect the rate of TAG secretion in isolated hepatocytes (e.g., insulin (14) and aniso-osmotic conditions (15)).

In the present studies we have used acute exposure of cultured rat hepatocytes to several of these factors, singly or in combination, to obtain a 2-fold range of TAG secretion rates. We used a dual-labeling technique to differentiate between the incorporation of acyl moieties from exogenous (added fatty acid) and endogenous (cytosolic TAG) sources into secreted TAG and other major products of fatty acid metabolism. The results indicate *i*) that a common regulatory step in determining the rate of TAG secretion resides at the partitioning of DAG between (re)synthesis of cytosolic-TAG and utilization for VLDL-TAG synthesis, and *ii*) that cytosolic TAG is not hydrolyzed fully to its component fatty acids, but only to DAG, approximately half of which is turned over, before re-synthesis of secretory TAG.

MATERIALS AND METHODS

Preparation and culture of hepatocytes

Rat hepatocytes were prepared as previously described (15) except that aseptic conditions were used. Cells were washed in Earl's Balanced Salts Solution (EBSS) containing 116 mm NaCl, 5.4 mm KCl, 1.8 mm $CaCl_2$, 0.8 mm $MgSO_4$, 26 mm $NaHCO_3$, 1 mm NaH_2PO_4, and 5.5 mm d-glucose. They were finally resuspended in cell attachment medium containing, in addition, 7% newborn calf serum, 10 mm HEPES (pH 7.4), non-essential amino acids (NEAA; 1:400 dilution of stock solution 11140-035, Gibco BRL, Life Technologies, Paisley, UK), essential amino acids (1:200 dilution of stock solution 11130-306, Gibco BRL), 25 μm glutamine, and 1:100 dilutions of vitamin and antibiotic solutions (Gibco BRL). They were seeded on 60-mm diameter tissue culture-treated plastic dishes (Costar UK Ltd., High Wycombe, UK) at a density of approximately 2.4×10^6 cells (1.5 mg protein) per plate. After 3 h, when cell attachment had occurred, the medium was discarded and Waymouth's medium (Gibco catalogue no. 31220-023) supplemented with 0.75 mm 9,10-[^{3}H]oleate (0.40 μCi/μmol) complexed to 1% fatty acid-poor bovine serum albumin, 10 mm lactate, and 1 mm pyruvate was added (3 ml per plate). Hepatocytes were cultured overnight with [^{3}H]oleate in order to label the intracellular (cytosolic) TAG. After 18 h, the medium was removed and the cells were washed and cultured for a further 3 h in medium in which the [^{3}H]oleate was replaced by 0.75 mm [1-^{14}C]oleate. Secretion of ^{3}H- and ^{14}C-labeled TAG was measured over this 3-h period. Measurements of cellular TAG concentrations at the beginning and at the end of the 3-h incubations were used to calculate the mean specific activity of oleoyl moieties in cytosolic TAG during the 3-h period. This value and the known specific radioactivity of the [^{14}C]oleate added exogenously were used to quantify the rate of incorporation of acyl chains into secreted TAG from endogenous and exogenous sources, respectively. In addition, the partitioning of ^{14}C-labeled fatty acid between secreted TAG and incorporation into intracellular (cytosolic droplet) TAG could also be calculated.

Two types of media were used for the 3-h experimental period during which secretion was measured. In the first, the overnight medium was changed to fresh Waymouth's medium (1.0 ml) supplemented with the same additions used for overnight culture, except that [1-^{14}C]oleate (0.36 μCi/μmol) was substituted for [9,10-^{3}H]oleate (see above). The second type of medium consisted of EBSS containing [1-^{14}C]oleate, lactate and pyruvate (see above), but no amino acids. The latter medium was used in those experiments in which the effects of either hypo-osmotic swelling or of addition of glutamine plus leucine were studied. The short-term omission of amino acids was necessary to avoid the osmolyte effects of a concentrated, complete amino acid mixture that would obscure the experimentally imposed cell volume changes. This approach has been described in detail elsewhere (16). Controls, in which cells were incubated for this 3-h period in iso-osmotic medium in the absence of insulin, were run concurrently for each type of medium. Incubations were restricted to 3 h in order to minimize equilibration of specific activities of exogenously added [^{14}C]oleate and cellular TAG (see below).

Analyses

These were performed for the zero-time and 3-h time points. Cells and portions of the medium were extracted separately for total lipids using chloroform–methanol 2:1 (vol/vol). The incorporation of ^{3}H and ^{14}C labels into different lipid classes was quantified after separation by thin-layer chromatography (17). Preliminary experiments established that incorporation into cellular and secreted TAG and cellular phospholipids accounted for >95% of overall [^{14}C]oleate metabolism into complex lipids. Therefore, routinely only incorporation into these products was quantified. The rate of fatty acid oxidation was measured as described previously (15) by measuring the rate of ^3H_2O and ^{14}C-labeled acid-soluble product (ketone bodies, acylcarnitines) formation. No collection of $^{14}CO_2$ was attempted as, under the conditions used, the formation of $^{14}CO_2$ is a very minor proportion (<5%) of overall [^{14}C]oleate oxidation (16). Secretion of labeled PL was minimal (not shown). Triacylglycerol and protein contents of the cells were measured in duplicate plates as described previously (15).

Calculations

In normal rats fed a chow diet the contribution made by fatty acids derived from de novo synthesis is very minor (5, 18). Secreted TAG is largely derived either from products of the cytosolic droplet TAG hydrolysis pathway (6, 19, 20) or from DAG newly synthesized through the phosphatidate pathway. Therefore, a dual-labeling protocol was used to quantify secretion of acyl moieties that were either *i*) mobilized from cytosolic TAG, or *ii*) newly incorporated into glycerolipid from exogenous fatty acid. The specific activity of [^{14}C]oleoyl moieties within cellular TAG was still <1.5% of that of added oleate even after 3 h. Therefore, [^{14}C]oleoyl moieties recycled through the cytosolic pool did not make any significant contribution to secreted [^{14}C]TAG, as they were diluted to a very low specific activity by the endogenous cytosolic TAG, relative to that of the [^{14}C]oleate supplied exogenously (see Table 3). Consequently, our measurements quanti-

fied exclusively the flux of [^{14}C]acyl moieties incorporated directly into secreted TAG through either the phosphatidate pathway or the esterification of DAG by DGAT. The net flux of acyl moieties from cytosolic TAG into secreted TAG was quantified by ^{3}H-label incorporation into the latter. The same considerations apply to the labeling of oxidation products. The respective fluxes were calculated from the specific radioactivities of cellular triacylglycerol (measured experimentally) and exogenously added oleate (see Results section). As the specific radioactivity of cellular ^{3}H-labeled TAG decreased during the 3-h period by approximately 20% (see Results section), routinely the mean of the 0-h and 3-h values was taken for the calculation of the average specific activity during the 3-h incubation period. All fluxes were calculated in terms of the rate of oleoyl moieties (nmol fatty acid) metabolized per 3-h incubation period per mg cell protein. Statistical analysis was by ANOVA.

Materials

Culture media were obtained from Gibco BRL (Life Technologies, Paisley, UK). Other inorganic chemicals were obtained from BDH-Merck (Poole, UK). Radiochemicals were obtained from Amersham plc (Amersham, Bucks, UK). TAG-measuring kit (GPO-Trinder) was obtained from Sigma (Poole, Dorset, UK).

RESULTS

Modulation of the rate of TAG secretion

The mass of TAG secreted could not be determined directly, due to the short duration of the experiments (3 h). However, the rates of TAG secretion calculated from the specific activities of exogenously added oleate and endogenous, TAG-associated acyl moieties (**Table 1**) were very similar to those reported by other workers (approximately 20 nmol TAG/3 h per mg cell protein) for rat hepatocytes cultured under comparable conditions (2, 5, 19, 21). In agreement with our previous observations on freshly prepared hepatocytes, exposure of cultured hepatocytes to hypo-osmotic media did not affect the rate of TAG secretion (not shown). However, contrary to what we had observed with fresh hepatocytes, exposure of cultured cells to glutamine plus leucine increased TAG secretion by approximately 30% (**Table 2**). Inhibition of TAG secretion (by approximately 25%, see Tables 1 and 2) was achieved by exposure of the cells either to insulin (cf. refs. 14, 22, 23) or to hyperosmotic media (cf ref. 15). When cells were incubated with insulin in hyperosmotic medium the effects were additive (Table 1). The use of these various conditions singly and in combination resulted in >200% variation in the rate of TAG secretion. **Table 3** shows that there was no significant net accumulation of cellular TAG during the 3-h experimental period, i.e., synthesis and secretion were approximately equal. However, as expected, the ^{3}H-specific activity of the acyl moieties in cytosolic TAG declined over the same period due to the turnover of cytosolic TAG (Table 3). Although [^{14}C]oleate was incorporated into cytosolic TAG, the specific activity of the latter never exceeded 1.5% of that of the added [^{14}C]oleate, even after 3 h (Table 3). Consequently, recycling of [^{14}C]oleoyl moieties through the cytosolic TAG pool did not interfere with the measurement of flux from exogenous oleate to secreted TAG or oxidation products.

Exogenous and endogenous sources of acyl moieties utilized for TAG secretion

There was no significant correlation between the overall amount of exogenous [^{14}C]oleate metabolized and the rate of TAG secretion, from either exogenous or endogenous sources (Tables 1 and 2). The contribution of exogenous ^{14}C-labeled fatty acid towards the overall flux of acyl chains into secreted TAG was considerably lower (approximately 17% of the total) than that made by acyl chains derived from cytosolic ^{3}H-labeled TAG hydrolysis (Tables 1 and 2). When the rate of TAG secretion was varied over a 2-fold range, this proportion remained constant, resulting in a highly positive correlation between the individual contributions of ^{14}C- and ^{3}H-labeled acyl moieties towards TAG secretion (**Fig. 1**).

The only parameter that changed in parallel with the rate of TAG secretion was the FSR which ranged from 7.1 ± 0.6% to 2.6 ± 0.5%, as the overall rate of TAG secretion was decreased from 111.1 to 52.6 nmol FA/3 h per mg protein (Tables 1 and 2). The changes in the value of the FSR were statistically significant for paired observations ($P < 0.01$) and were highly and positively correlated with the rate of either ^{14}C- or ^{3}H-labeled TAG secretion and, consequently, of total TAG secretion (**Fig. 2**).

TABLE 1. Effects of insulin and hyperosmolar medium on the metabolism of [^{14}C]oleate (exogenous) and pre-labeled (endogenous) ^{3}H-labeled acyl moieties in cytosolic TAG of rat cultured hepatocytes

	Rate of Incorporation						
	[^{14}C]Oleate					[^{3}H]Oleate	
	Secreted TAG	Cellular TAG	Cellular PL	Acid-Soluble Products	Total Metabolized	Secreted TAG	$^{3}H_2O$
	nmol fatty acid/3 h/mg protein						
Control	16.1 ± 1.1	257.1 ± 9.5	25.2 ± 2.2	51.2 ± 4.1	348.5 ± 9.6	86.2 ± 5.7	115.1 ± 10.4
Insulin	11.5 ± 0.9[a]	298.4 ± 11.8[a]	32.8 ± 2.6[a]	37.8 ± 3.1[a]	380.5 ± 13.6	64.5 ± 4.2[a]	87.7 ± 8.7[a]
Hyperosmotic	9.4 ± 2.4[a]	246.1 ± 8.4	23.2 ± 2.1	55.6 ± 11.1	342.4 ± 10.8	59.3 ± 4.6[a]	132.6 ± 10.5[a]
Hyperosmotic + insulin	7.0 ± 0.7[a]	265.5 ± 9.7	27.7 ± 2.8	44.9 ± 4.3	345.1 ± 0.9	45.6 ± 3.5[a]	113.6 ± 12.9

See Methods for details of culture conditions. Values are expressed as nmol of fatty acid incorporated per 3 h per mg cell protein. Values are means ± SEM for six separate hepatocyte preparations; TAG, triacylglycerol; PL, phospholipid.

[a] $P < 0.01$, significantly different from controls.

TABLE 2. Effects of insulin and glutamine plus leucine on the metabolism of [^{14}C]oleate (exogenous) and pre-labeled (endogenous) ^{3}H-labeled acyl moieties in cytosolic TAG of rat cultured hepatocytes

	Rate of Incorporation						
	[^{14}C]Oleate					[^{3}H]Oleate	
	Secreted TAG	Cellular TAG	Cellular PL	Acid-Soluble Products	Total Metabolized	Secreted TAG	$^{3}H_2O$
	nmol fatty acid/3 h/mg protein						
Control	12.6 ± 0.5	206.6 ± 11.8	21.1 ± 2.7	60.4 ± 5.6	300.5 ± 17.7	76.6 ± 6.7	166.9 ± 11.7
Insulin	9.7 ± 0.6[a]	243.4 ± 14.4[a]	23.2 ± 3.1	50.9 ± 4.5[a]	333.2 ± 16.6	57.6 ± 4.1[a]	157.2 ± 10.9
Glutamine + leucine	16.5 ± 1.1[a]	221.3 ± 8.4	24.8 ± 3.3	53.5 ± 4.2[a]	316.1 ± 10.7	94.6 ± 8.3[a]	152.1 ± 12.9
Glutamine + leucine + insulin	8.5 ± 0.5[a]	261.8 ± 15.3[a]	31.5 ± 3.3[a]	43.2 ± 5.0[a]	344.0 ± 20.4	56.9 ± 3.9[a]	128.4 ± 13.1

See Methods for details of culture conditions. Values are expressed as nmol of fatty acid incorporated per 3 h per mg cell protein. Values are means ± SEM of six separate hepatocyte preparations; TAG, triacylglycerol; PL, phospholipid.

[a] $P < 0.01$, significantly different from controls.

Use of endogenous and exogenous sources of acyl moieties for oxidation

As in the case of ^{14}C and ^{3}H incorporation into secreted TAG, the ^{14}C/^{3}H ratio of label incorporated into soluble oxidation products was constant throughout the range of values observed (**Fig. 3**). This occurred in spite of the fact that the different conditions studied did not always affect the rates of TAG secretion and fatty acid oxidation in the same direction. Thus, whereas both insulin and hyperosmotic medium inhibited TAG secretion, the former inhibited oxidation whereas the latter did not (Table 1). Insulin and hypo-osmotic media inhibited fatty acid oxidation (cf refs. 24, 25) whereas hyperosmolar medium had no significant effects (Tables 1 and 2). The combined effects of insulin and hypo-osmotic media were additive (Table 2).

DISCUSSION

DAG partitioning as a determinant of TAG secretion rate

In this study we concentrated on the contributions of exogenously added ^{14}C-labeled fatty acid and of cytosolic droplet TAG (pre-labeled with [^{3}H]oleate) towards TAG secretion and formation of oxidation products, under conditions characterized by a steady state between TAG synthesis and TAG secretion (Table 3). The metabolic routes of acyl moieties derived exogenously (i.e., via the phosphatidate pathway) and endogenously (i.e., after cytosolic TAG hydrolysis) are expected to share two common substrates: acyl-CoA and DAG. These are utilized for four major pathways: *i*) (re)synthesis of cytosolic TAG, *ii*) incorporation into cellular phospholipids, *iii*) β-oxidation, accompanied by the release of acid-soluble products, and *iv*) synthesis of secreted TAG. The partitioning of ^{3}H- and ^{14}C-labeled acyl chains was identical for both TAG secretion and formation of oxidation products (Figs. 1 and 3). Such a constant relationship can only arise if the two sources of acyl moieties contribute to common pools of intermediates. DAG and acyl-CoA are the only intermediates common to the synthesis of TAG either directly through the phosphatidate pathway or after partial hydrolysis of cytosolic TAG. Thus, the data acquired for the flux of [^{14}C]oleate into cytosolic and secreted TAG should reflect the overall pattern of DAG and acyl-CoA partitioning.

TABLE 3. Cellular TAG content and its ^{3}H and ^{14}C specific activities at the end of the 3-h experimental period

	Intracellular TAG after 3 h Incubation		
	Content	^{3}H Specific Activity	^{14}C Specific Activity
	μmol/mg protein	*μCi/μmol TAG*	*nCi/μmol fatty acid*
Waymouth's medium			
Iso-osmotic control	0.76 ± 0.02	0.154 ± 0.015	4.0 ± 0.1
Iso-osmotic + insulin	0.74 ± 0.02	0.159 ± 0.013	4.7 ± 0.2
Hyperosmotic	0.72 ± 0.03	0.137 ± 0.026	4.0 ± 0.2
Hyperosmotic + insulin	0.67 ± 0.03	0.162 ± 0.015	3.8 ± 0.1
EBSS medium			
Iso-osmotic control	0.67 ± 0.02	0.166 ± 0.013	3.5 ± 0.3
Iso-osmotic + insulin	0.69 ± 0.01	0.171 ± 0.016	3.9 ± 0.3
Glutamine + leucine	0.70 ± 0.02	0.161 ± 0.014	3.6 ± 0.2
Glutamine + leucine + insulin	0.71 ± 0.02	0.166 ± 0.013	4.1 ± 0.3

The initial TAG content at 0 h was 0.68 ± 0.03 μmol TAG/mg cell protein and was not significantly different from any of the other values. The initial ^{3}H specific activity of cell TAG was 0.198 ± 0.015 μCi/μmol. The specific activity of the added [^{14}C]oleate was 360 nCi/μmol (i.e., 0.36 μCi/μmol, see text). Values are for the same cell preparations used to generate the data in Tables 1 and 2.

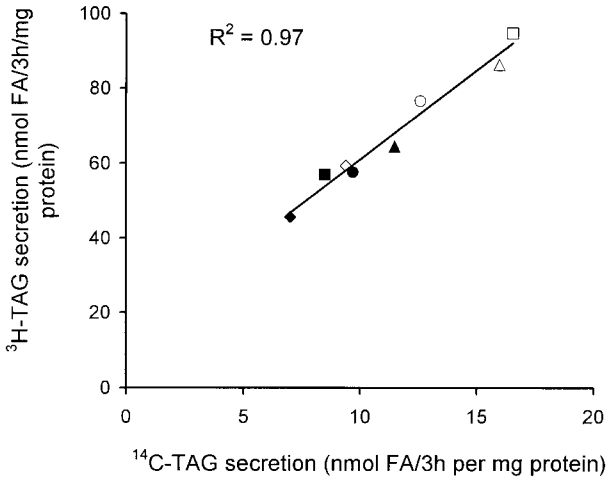

Fig. 1. Relationship between the rates of incorporation of endogenously (^{3}H-labeled) and exogenously derived (^{14}C-labeled) fatty acyl moieties into TAG secreted by rat cultured hepatocytes. Cells were cultured for 18 h in supplemented Waymouth's medium containing 0.75 mm [^{3}H]oleate and then exposed for 3 h to 0.75 mm [^{14}C]-oleate in either Waymouth's (triangles, diamonds) or EBSS (circles, squares) media, in the absence (hollow symbols) or presence (filled symbols) of insulin (100 nm). The ^{3}H and ^{14}C radioactivity associated with secreted TAG was quantified. Key: iso-osmotic EBSS (○,●); iso-osmotic EBSS plus glutamine and leucine (□,■); iso-osmotic Waymouth's (△,▲); hyper-osmotic Waymouth's (◇,◆). The values (expressed as nmol fatty acyl (FA) moiety/3 h/mg cell protein) are means for the six individual experiments recorded in Tables 1 and 2.

The low values for the FSR of newly synthesized ^{14}C-labeled TAG indicate that incorporation of DAG into cytosolic TAG occurs at a much higher rate (approximately 20-fold) than their incorporation into secreted TAG (Tables 1 and 2) i.e., the rate of substrate cycling between cytosolic TAG and DAG is much greater than the net flux of

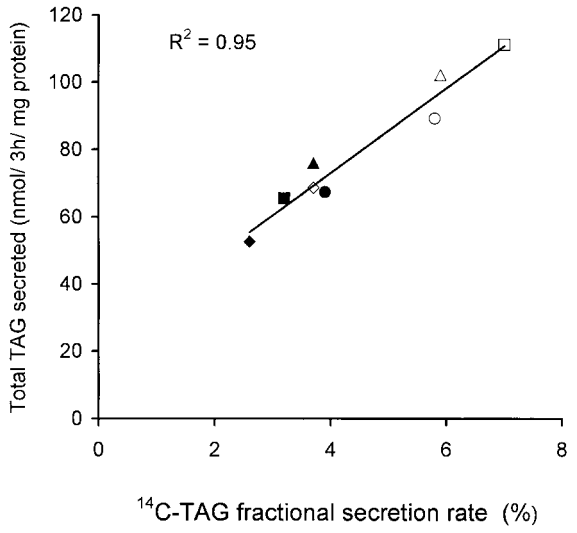

Fig. 2. Correlation between the total rate of TAG secretion (^{3}H plus ^{14}C-labeled) and the fractional secretion rate (FSR) of newly synthesized ^{14}C-labeled TAG. See legend to Fig. 1 for key to symbols. The values are means for the six individual experiments recorded in Tables 1 and 2.

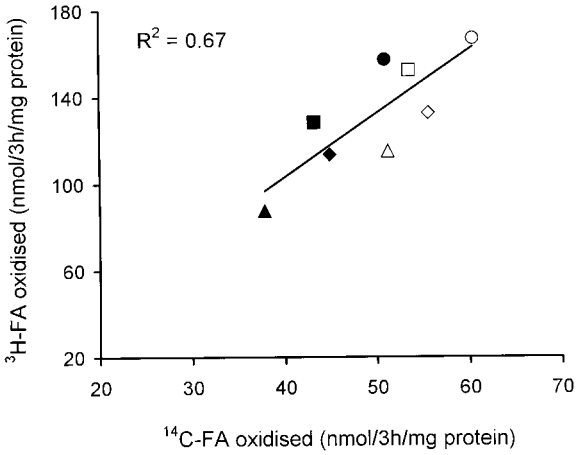

Fig. 3. Relationship between the rates of incorporation of endogenously (^{3}H-labeled) and exogenously derived (^{14}C-labeled) fatty acyl moieties into oxidation products released by rat cultured hepatocytes. See legend to Fig. 1 for key to symbols. The values are means for the six individual experiments recorded in Tables 1 and 2.

TAG into the secretory pathway (see scheme in **Fig. 4**). Although the FSR values were always low, in agreement with previous studies on cultured hepatocytes; see e.g. ref. 21, they could be acutely modulated and were highly and positively correlated with the rate of TAG secretion (Fig. 2). This suggests that the branch-point represented by the partitioning of DAG between (re)synthesis of cytosolic TAG and synthesis of secretory TAG (see Fig. 4) is a major determinant of the rate of TAG secretion, in agreement

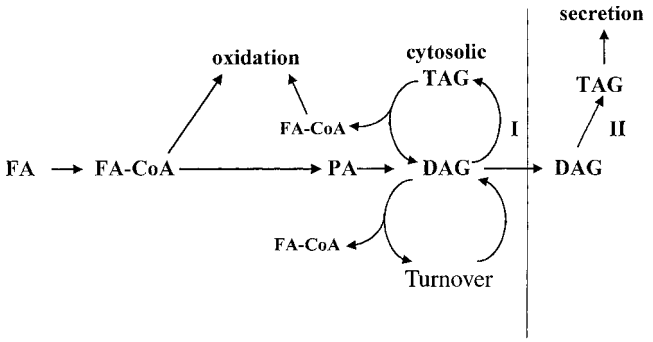

Fig. 4. Schematic representation of the partitioning of acyl-CoA between oxidation and esterification, and of DAG between formation of either cytosolic or secreted TAG. Comparison of the oxidation/secretion ratio for exogenously and endogenously derived acyl chains provides information about the degree of hydrolysis of cytosolic TAG. Acyl-CoA released during cytosolic TAG hydrolysis becomes available for either oxidation or esterification, whereas acyl chains that remain esterified to the glyceroyl moiety are only available for incorporation into glycerolipids. By contrast, all acyl-CoA chains derived from exogenous fatty acids are available for either oxidation or esterification. Note that only DAG and acyl-CoA are common substrates for the phosphatidate pathway and the resynthesis of TAG after partial hydrolysis. Partitioning of DAG between the formation of cytosolic and ER-lumen pools of TAG is proposed to be dependent on the relative activities of overt (I) and latent (II) DGAT. Phospholipid synthesis has been omitted for clarity. PA, phosphatidic acid.

with the model presented in ref. 7. Acute modulation of the FSR in vivo, in response to food intake in awake, unrestrained rats, has been observed for several physiological perturbations (8–11, 13, 26, 27). In the present study insulin was shown to decrease acutely the value of the FSR in parallel with inhibition of TAG secretion (Tables 1 and 2). Therefore, the hormone may be involved in mediating this acute response to food intake in starved-refed rats in vivo (13).

The strong correlation between the rate of hepatic TAG secretion and the FSR is also observed in vivo (8). However, the value of the FSR is much higher (approximately 60%) when TAG secretion is studied in vivo (8–13) or in the intact perfused rat liver (18, 28). In these experimental systems the flux of exogenous fatty acids to secreted TAG is also much higher than in cultured hepatocytes (about 6-fold) and there is no equilibration of exogenous fatty acid label with cytosolic TAG prior to incorporation into secreted TAG. The disparity between the FSR values observed between the intact liver (in vivo or isolated perfused) and isolated hepatocytes suggests that in the latter the recycling between DAG and cytosolic TAG is much more extensive. In view of the strong positive correlation between the magnitude of the FSR and the absolute rate of TAG secretion (Fig. 2 and ref. 8), its much lower value in isolated hepatocytes may be directly related to their poor capacity to secrete TAG. Wu and co-workers (29) have recently suggested that in HepG2 cells (in which TAG secretion is even lower than in rat hepatocytes) low rates of apoB secretion result from a lack of "secretion-coupling" of the cytosolic TAG pool. The present results indicate that such "uncoupling" could be due to the extensive recycling between DAG and cytosolic TAG. The regulatory functions of such substrate cycles, involving an intermediate (DAG) which occurs just before the flux is committed to a specific pathway (secretory TAG synthesis) are well recognized (30). The function of such a substrate cycle between DAG and cytosolic TAG (see Fig. 4) may be to enable the more precise regulation of the supply of DAG for VLDL-TAG synthesis.

Degree of hydrolysis of cytosolic TAG

It has been suggested that cytosolic TAG is not hydrolyzed to completion when mobilized (6). If this is valid, the ^{3}H-labeled acyl moieties within cytosolic TAG that remain esterified to the glyceroyl moiety would not be available for β-oxidation, whereas those that are released as acyl-CoA (either through hydrolysis (2, 31) or during transacylation (32, 33) would be available for both β-oxidation and (re)esterification. Similarly, all the acyl-CoA formed from exogenous fatty acid would be available for competition between the two pathways. The data in **Fig. 5** provide a quantitative estimate of the relative proportion of ^{3}H-labeled acyl moieties that become available for oxidation during partial lipolysis of cytosolic TAG. The ratio: [rate of oxidation/rate of TAG secretion] for ^{3}H-labeled acyl moieties is plotted against the same ratio for ^{14}C-labeled fatty acyl chains. The resulting plot shows that, throughout the range of TAG secretion rates achieved,

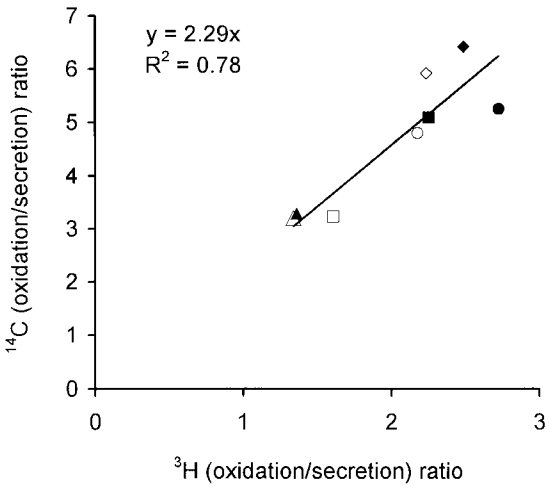

Fig. 5. The relationship between the values for the oxidation/TAG secretion ratio for ^{3}H- and ^{14}C-labeled acyl moieties. See legend to Fig. 1 for key to symbols. The values are means for the six individual experiments recorded in Tables 1 and 2.

there was a 2.3-fold greater partitioning of ^{14}C-labeled than of ^{3}H-labeled acyl moieties towards oxidation. We interpret this observation as follows. If cytosolic TAG lipolysis were to proceed completely to acyl-CoA and glycerol, then both endogenously and exogenously derived acyl-CoA would have equal access to oxidation and the slope in Fig. 5 would be expected to be close to unity. Similarly, if lipolysis/transacylation were to involve only one of the three acyl chains per molecule of TAG, the value of the slope in Fig. 5 would be approximately equal to 3.0. Involvement of two acyl chains per TAG molecule would result in a slope of 1.5. The fact that the experimentally determined value (2.29) falls between these two theoretical values indicates that cytosolic TAG hydrolysis proceeds to DAG (with the generation of one acyl-CoA) and that approximately half of the DAG is turned over (with the generation of the equivalent of a further 0.5 acyl-CoA per DAG molecule) before re-esterification to form secretory TAG. This re-modelling of DAG may result from transacylation (which is known to occur in hepatic microsomes (32, 33)) rather than from hydrolysis to MAG and re-esterification, because the activity of monoacylglycerol acyltransferase (MGAT) in the adult rat liver is relatively low (34).

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