Distinct ontogenic patterns of overt and latent DGAT activities of rat liver microsomes

Ian J. Waterman, Nigel T. Price, and Victor A. Zammit

Hannah Research Institute, Ayr, Scotland, United Kingdom

Abstract  We have studied the ontogeny of the two functional diacylglycerol acyltransferase (DGAT) activities (overt and latent) during postnatal development in rat liver. We find that the ontogenic patterns of the two are highly distinct. Overt DGAT shows a transient rise in activity up to day 4 postnatally, after which it declines until weaning; thereafter, it increases steadily to reach high adult values that may contribute to the high rates of turnover of cytosolic triacylglycerol (TAG). By contrast, latent DGAT activity increases continuously during the suckling period but falls sharply upon weaning onto chow but not onto a high-fat diet. Rates of TAG secretion by hepatocytes are higher than in the adult during the first 7 days after birth, and are largely dependent on the mobilization of the abundant intrahepatocyte TAG as a source of acyl moieties. When the hepatic steatosis is cleared (after day 7) the TAG secretion rate declines by 80% to reach adult values. Quantification of the content of mRNA for the DGAT1 and DGAT2 genes does not show correlation with either of the DGAT activities. We conclude that post-translational modification may play an important role in the overt and latent distribution of DGAT activity in the liver microsomal membrane.

Supplementary key words  diacylglycerol acyltransferase • triglyceride • very low density lipoproteins • secretion • development

Diacylglycerol acyltransferases (DGATs) catalyze the final and dedicated step in the synthesis of triacylglycerol (TAG). In the liver, TAG is either retained within cytosolic droplets or secreted within VLDL particles. These are assembled within the lumen of the endoplasmic reticulum (ER) in a two-step lipidation of apolipoprotein B (apoB), of which each VLDL particle contains one molecule. We have previously identified two functional DGAT activities in rat liver microsomes: an overt DGAT activity that is associated with the cytosolic aspect of the endoplasmic reticular membrane and is accessible to palmitoyl-CoA in intact microsomes and a latent DGAT activity that is accessible to palmitoyl-CoA only in permeabilized microsomes, i.e., it is latent and displays its catalytic activity only when the endoplasmic reticular membrane is made permeable to acyl-CoA substrate (1, 2). Overt DGAT activity was suggested (1, 3, 4) to be involved in cytosolic droplet TAG synthesis, whereas latent DGAT activity was suggested to function in the synthesis of TAG destined for assembly into nascent VLDL particles within the rough ER, and that of non-apoB-associated TAG droplets within the lumen of the smooth ER (5). The role of the two DGAT activities is likely to be highly complementary, as cytosolic droplet TAG turns over rapidly (6) at a rate that can be calculated to be 20-fold higher than the rate of TAG secretion in cultured rat hepatocytes (7). Hydrolysis of cytosolic TAG provides substrates not only for oxidation (acyl-CoA), but also for the synthesis of secreted TAG in the form of both acyl-CoA and partial glycerides (7, 8). The use of acylcarnitines for the intraluminal synthesis of triglycerides and acyl-CoA has been demonstrated experimentally in reconstituted cell-free systems (9, 10).

To test the ability of the two DGAT activities to be modulated independently, we have studied their respective ontogeny during rat development, which is characterized by marked changes in dietary and hormonal conditions that provide a good model for rapidly changing rates of hepatic TAG synthesis and secretion (11, 12). Moreover, within the first 7 days postpartum, the neonatal liver undergoes a cycle of marked accumulation of intracellular TAG (resulting in steatosis) and subsequent rapid mobilization (13, 14). We have also tested whether the hepatic levels of mRNA of two genes that have recently been shown to code for proteins that express DGAT activity in vitro (15, 16) are related to the overt and latent DGAT activities observed enzymatically.

Abbreviations: apoB, apolipoprotein B; DGAT, diacylglycerol acyltransferase; EAAT, ethanol:acyl-CoA acyltransferase; ER, endoplasmic reticulum; TAG, triacylglycerol.
MATERIALS AND METHODS

Animals

Wistar rats were maintained under a 12 h light/12 h dark lighting regime and a controlled temperature environment, in accordance with local animal welfare regulations. They were fed ad libitum on a chow diet (RPMI; Special Diet Services, Edinburgh, United Kingdom) unless otherwise indicated. The litter size of lactating dams was adjusted to 10 pups at birth. Only female pups were used for liver sampling throughout the developmental stages. Pups were weaned by separation from the dams on day 20 postpartum. When pups were to be weaned onto a high-fat diet, this was made available as a powdered diet to the mother from day 15 of lactation onwards, because it is known that pups start nibbling solid diet from this age. Control animals were given powdered chow. The composition of the standard chow diet (5% fat) was as described previously (17). The isocaloric high-fat diet contained, by weight, 30% lard, 32% casein, 6% sucrose, 17% cornstarch, 9.7% cellulose, 0.3% methionine, and 5% vitamin and mineral mixture (Special Diet Services).

Preparation of liver microsomes and DGAT assays

Animals were killed 1 h into the light phase by cervical dislocation, and after excision of a lobe for quantification of tissue triglyceride content, livers were homogenized in ice-cold medium containing 300 mM sucrose, 1 mM EGTA, 5 mM Tris-HCl (pH 7.4). Microsomal membranes were then prepared by differential centrifugation as described previously (1). The final microsomal membrane suspension was divided into aliquots and stored at −70°C until used. Permeabilization of microsomes with alamethacin was carried out as previously described immediately before the assay of DGAT activities in intact and permeabilized microsomes, respectively (1). Nonpermeabilized microsomes were treated with carrier (0.1% ethanol) only. DGAT and ethanol:acyl-CoA acyltransferase (EAAT) activities were measured simultaneously in the same assay mixture, as previously described (1). The liposomal substrate mixture for assay of DGAT activities was also prepared as described in (1). Briefly, to assay medium containing 300 mM sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EGTA, 10 mM MgCl2, and 1 mg defatted BSA/ml, were added 3.35 mM dipalmitoylglycerol and 2.67 mM phosphatidylglycerol. The mixture was warmed to 65°C and sonicated using a 2.5 mm microprobe sonicator (Kontes, Burkaard Scientific, Ukberidge, Middlesex, UNITED KINGDOM) operated at 20 µm and at 80% of maximal power, for one 15 s period every 2 min, over 40 min. The mixture was then diluted with assay buffer to give final lipid concentrations of 1 mM and 0.8 mM, respectively, and palmitoyl-CoA substrate was required to achieve maximal activity.

Hepatocyte isolation and incubation

Hepatocytes were prepared by collagenase digestion, using a two-step Ca2+ perfusion method, as described previously (7) except that, when using suckling rat pups up to 10 days old, livers were digested using a single pass perfusion, rather than recirculation of the medium. Hepatocytes were sedimented by centrifugation at 50 g for 90 s and washed once in Krebs medium containing 12 mM glucose. Routinely, cells were killed >90% viable, as determined by trypan blue exclusion. Quantification of the contributions of endogenous and exogenous acyl moieties toward TAG secretion and fatty acid oxidation by freshly prepared hepatocytes was performed using the dual-labeling method described in (7). Briefly, hepatocytes (6.6 × 10⁶ cells) were incubated in 3 ml Krebs medium containing 12 mM glucose in stopped 25 ml flasks that were continuously gassed with 95% O2/5% CO2. Prelabeling of the endogenous pool of lipids was performed by adding 5 µCi of 1-¹⁴C]oleate (55 µCi/µmol) as tracer, for 15 min. Longer prelabeling did not alter the results qualitatively. They were then washed twice in Krebs medium containing 1% BSA, and incubated for a further 30 or 60 min in Krebs medium containing 12 mM glucose, 1 mM pyruvate, 10 mM lactate, and 0.75 mM 9,10-[³H]ololate (containing 0.36 µCi). Media and cells were harvested, total lipid extracts were prepared using chloroform-methanol, and the radioactivity associated with TAG quantified. Incorporation of radioactivity into acid-soluble products (as a measure of fatty acid oxidation) (7) was quantified in separate aliquots of media acidified with perchloric acid, from which acid-insoluble material was removed by centrifugation. The mean specific activity of cellular [¹⁴C]TAG before and after incubation with [³H]oleate was calculated by measurement of the initial and final TAG content of cells and associated radioactivity (7).

Isolation of rat liver RNA and cDNA production

Livers from rats at selected developmental stages were removed and immediately placed into 20 ml ice-cold RNAlater (Ambion, Cambridgeshire, United Kingdom) and stored as recommended by the manufacturer. Total RNA was isolated (Ambion RNAqueous kit) and its integrity verified by electrophoresis on ethidium bromide-stained agarose gels. Template cDNA synthesis was primed with oligo(dT)₁₅ (1st Strand cDNA Synthesis Kit (AMV), Roche Diagnostics, East Sussex, United Kingdom). After RNase-free DNase treatment, 1 µg total RNA (as measured in duplicate by Ribogreen RNA Quantitation kit, Molecular Probes, The Netherlands) was used for cDNA synthesis.
Real-time PCR quantification of mRNA

The LightCycler system (Roche Diagnostics) was used for fluorescent real-time PCR using SYBR Green for quantification of the target transcripts, DGAT1 and DGAT2 (15, 16), with β-actin and glyceraldehyde-phosphate dehydrogenase (GAPDH) as reference transcripts. For β-actin, a primer pair known to work across a wide range of species was used (GTGCAACCGGTCT-GGSCATGTG and CTGTCRGRATGCCWGGTACAT). GAPDH primers (ACCGGCGTTTCAACGGCAGTCAA and GCCTTTCCAGAGGGCACTCACA) were designed using the published sequence (Accession number AF108680). For DGAT2, no rat-specific cDNA sequence was available. In the case of rat DGAT1, the single available sequence (AF296131) showed a large number of differences from rat expressed sequence tag sequences, and was thus not considered reliable. Hence, for both rat DGAT1 and DGAT2, sequences from rat expressed sequence tag clones were identified and assembled. PCR primers were designed to regions with no discrepancies, and were used to amplify regions of the DGAT cDNAs from rat liver cDNA (not shown). Products were cloned and sequenced from three independent cDNA syntheses and PCR reactions. These sequences allowed design of the primers for the quantitative PCR. The partial rat DGAT1 sequence has been deposited in the public databases with accession number AJ345014. For each of the four transcripts, primers were chosen in exons separated by one or more introns (as judged by available mammalian gene sequences). The forward and reverse primers for DGAT1 were: TTTCTGCG-TACCGGGGCTCTTGTAG and ACCGGGTGCCCAATGATGACGGCGGGTTCTTGAG and ACCGGTTGCCCAATGATGAGTGTC, respectively. For DGAT2, the primers were: GGAGG-CCACCGAAGTACACCCGAGAAA and ACCCCCCAGGTTGTCAGAG-GAGAAG, respectively.

Optimal MgCl₂ and cDNA template concentrations were determined empirically. The PCR reaction mix (18 µl) was transferred to LightCycler capillary tubes and 2 µl of appropriately diluted cDNA added. The protocol used for the experimental run consisted of three segments followed by a melting curve analysis (60–95°C with a heating rate of 0.1°C/s and continuous fluorescence measurement) to verify the specificity of the products formed [Segment 1: 95°C for 10 min (denaturation and enzyme activation); Segment 2: (amplification and quantification) 30 cycles (95°C for 15 s, followed by 60–62°C for 5 s, then 72°C for n/25 s, where n is the product length, with a single fluorescence measurement)]. For DGAT1 and DGAT2, an additional step was included prior to the fluorescence whereby the temperature was increased to 87°C or 85°C, respectively. This step eliminated “signal noise” identified in preliminary melting curve experiments by the presence of a small fluorescence peak around 5°C below the product melt curve peak. Examination of the product of the DGAT1 and DGAT2 reactions by agarose gel electrophoresis with ethidium bromide staining showed that only a single band with the expected appropriate product size was present. The quantification of the target DGAT1 and DGAT2 transcripts relative to reference GAPDH and β-actin mRNA was determined as previously described (19). The efficiency for each transcript was determined using a dilution series of a standard cDNA sample, where a linear relationship was found over a wide range of concentrations. Crossing point (CP) values were also determined to verify that a linear relationship with amount of RNA used in the cDNA synthesis reaction existed over a wide range. The “Fit Point Method” of CP determination using LightCycler software 3.5 (Roche Diagnostics) was employed, and CP values were determined at a constant fluorescence level (20).

Materials

Radiolabeled 1-[14C]palmitoyl-CoA (50 µCi/µmol) was obtained from Amersham Life Sciences (Amersham, United Kingdom). 9,10-3H(N) triolein (1 µCi) was obtained from Dupont NEN Research Products (Hounslow, United Kingdom). Mannose-6-phosphate, alamethacin, phosphatidylglycerol, and the Infinity Reagent TAG detection kit were purchased from Sigma, Poole, United Kingdom. Palmitoyl-CoA was from Lipid Products (Surrey, United Kingdom). Silica-gel 60 TLC plates were from Merck.

RESULTS

Differences in the ontogenic patterns of overt and latent DGAT activities in rat liver microsomes

The values of both DGAT activities were extremely low on day 1 postnatally. By day 3 they had both increased several-fold to reach similar values (approximately 1 nmol TAG synthesized/min per mg microsomal protein, at 37°C). However, thereafter they followed distinct developmental patterns (Fig. 1A, B). Whereas the initial rise in overt DGAT activity was reversed by day 10, that of latent DGAT continued to increase until the end of the suckling period (day 20). Consequently, the latent/overt DGAT activity ratio increased to a value of 4.5 ± 1.1 at day 20.

The divergent developmental pattern of overt and latent DGAT activities was also apparent upon weaning of
the pups onto a chow diet. Weaning resulted in a 60% decline in latent DGAT activity to reach adult values by day 30 postpartum, whereas weaning had no significant effect on overt DGAT activity (Fig. 1). Moreover, after day 30, the activity of overt DGAT started a steep increase such that it was 3.5-fold higher in the microsomes isolated from 100-day-old rats than in those from 30-day-old ones. There was no significant increase in latent DGAT activity over the same period. These different developmental patterns for overt and latent DGAT caused the activity ratio to be restored to near unity in the adult (1).

**Ontogeny of the pattern of fatty acid partitioning in hepatocytes**

The dual-labeling method used in this work enabled us to determine the partitioning of fatty acyl moieties of endogenous and exogenous origin between fatty acid oxidation and TAG synthesis and secretion. Freshly prepared hepatocytes were used throughout to avoid possible complicating factors arising from cell culture (21). Preliminary experiments established that TAG secreted into the media could be recovered exclusively in the d < 1.006 fraction. The data in Fig. 2 show that endogenous TAG was always the main source of acyl moieties for secreted TAG (7), especially in the first days after birth, when endogenously derived acyl moieties constituted >96% of those in secreted TAG (Fig. 2). TAG secretion rates were already high one day after birth, and remained so until day 7, after which there was a decline of about 80% toward adult values (Fig. 2). This pattern paralleled the triglyceride content of the cells, which was very similar to that observed in the liver in vivo (Fig. 3). The magnitude of TAG secretion achieved at the end of the suckling period was similar to that observed in hepatocytes isolated from adult rats (Fig. 2).

The data in Fig. 4 show that endogenous TAG mobilization also accounted for most of the high rate of fatty acid oxidation until day 7 postnatally. Thereafter, exogenously added fatty acid became the main source of acyl moieties for oxidation. There was a 60% decline in the overall rate of fatty acid oxidation after weaning, which persisted in cells isolated from adult rats (Fig. 4).
Effects of weaning onto a high-fat diet on DGAT activities

In view of the distinctive response of latent DGAT to weaning from a high-fat (milk) to a chow (high-carbohydrate) diet (Fig. 1), we wanted to ascertain whether weaning to a high-fat diet was able to prevent this marked drop in activity. As shown in Table 1, weaning of 20-day-old pups onto a high-fat diet resulted in the prevention of the large decrease in latent DGAT activity. The activity of overt DGAT tended to be increased after weaning onto a high-fat diet, but the effect did not reach statistical significance.

Measurement of mRNA levels for DGAT1 and DGAT2

The cDNAs that code for proteins that exhibit DGAT activity have recently been described (15, 16) and termed DGAT1 and DGAT2, in the order in which they were described. Therefore, we tested whether either of the respective mRNA levels changed in parallel with either of the overt or latent DGAT activities (Fig. 5). Both DGAT1 and DGAT2 mRNA levels were highest immediately after birth but rapidly declined such that by day 2 they reached low levels that were maintained throughout the suckling period and through to adulthood. Qualitatively similar results were obtained irrespective of whether GAPDH or β-actin was used as the reference gene. Therefore, transcriptional control of these two genes appears to be minimal after day 1, assuming that mRNA stability is unaltered throughout.

DISCUSSION

This study was designed primarily to investigate the effect of a succession of physiological perturbations, which are known to be accompanied by rapid changes in the rate of hepatic triglyceride secretion, on the two DGAT activities previously described in rat liver microsomes (1). The present data illustrate that the ontogenic patterns of overt and latent DGATs during the suckling period are highly different: latent DGAT expression increased throughout the suckling period, whereas overt DGAT activity increased only transiently during the early postnatal phase (Fig. 1).

There were also marked differences in the response of the two activities to weaning and further growth of the rats into adulthood. Weaning of the pups onto a normal chow diet (3% fat) resulted in a marked decline only of latent DGAT activity. Moreover, weaning onto a high-fat diet prevented this decline. By contrast, there was a steady increase in overt DGAT activity from its low value at the time of weaning to that observed in adult rat liver microsomes. These observations suggest that overt and latent DGAT activities respond differently to developmental and associated nutritional changes. Recently, two unrelated genes, the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activities, have been described and termed DGAT1 and DGAT2, reflecting the products of which express DGAT activi...
Significance of ontogenic pattern of hepatic TAG secretion

Although the hepatic secretion of TAG by the liver of the developing rat has been studied extensively (21, 26) the changes that occur during the first 7 days postnatally have been poorly described, except for the demonstration that hepatic TAG content increases rapidly after birth (13, 14). This paucity of data has largely been due to the experimental difficulties encountered in measuring rates of TAG secretion in small yields of hepatocytes obtained from rat neonates. Consequently, inferences have been drawn by extrapolation from data obtained on pups later in development (21, 26–29), which, as shown in Fig. 2, have a much lower rate of TAG secretion. In addition, the methods used previously to quantify TAG secretion radiochemically have greatly underestimated the contribution of the endogenous triglyceride within the cytosolic droplets of the hepatocytes as a source of fatty acyl moieties for secreted TAG. Measurements were performed using either radiolabeled acetate, which measures secretion only of de novo synthesized fatty acid, or radiolabeled glycerol (21), which would be adequate only if secreted TAG are synthesized exclusively from acyl-CoA and glycerol within the liver. However, it is now known (7, 8) that partial glycerides, the glycerol moiety of which does not equilibrate with exogenously added glycerol, are the major precursors of secreted TAG. This is expected to hold true particularly in the neonatal rat liver, in which the high activity of monoacylglycerol acyltransferase induced at birth (30) would ensure that complete hydrolysis of cytosolic TAG to its constituent fatty acyl groups is minimized. Consequently, the underestimation of the contribution of endogenously derived acyl moieties is especially important in neonates up to 7 days postpartum, when hepatic cytosolic TAG is still high and is mobilized.

Our results indicate that the rates of TAG secretion in the suckling rat before day 7 postnatally are higher than those in adult rat hepatocytes. This agrees with the high levels of apoB and VLDL in rat plasma observed in the first days postpartum (12, 31) and the ability of the liver to secrete apoB in pups immediately after birth (14). The rapid accumulation of TAG within the liver in the early neonatal period is indicative of high rates of TAG synthesis, and has previously been assumed to reflect an inability of the liver to secrete TAG during this phase (21). The current data show that this assumption needs to be re-evaluated, as the ontogeny of TAG accumulation in the liver is paralleled by high rates of TAG secretion (Figs. 2, 3). We have also failed to observe any increase in the rate of secretion of TAG by hepatocytes after weaning. This agrees with recent observations that when hepatocytes isolated from 15-day-old pups are supplemented with exogenous fatty acids and dexamethasone in culture, their rate of VLDL secretion is as high as that in cells isolated from adult rats (28).

The role of DGAT activities in postnatal hepatic acylglyceride metabolism

The rapid increase in overt DGAT activity observed on day 4 postpartum parallels those of acyl-CoA synthase and lysophosphatidate acyltransferase (21). Indeed a postnatal increase in DGAT activity was observed in (21), although this is likely to have been an indeterminate mixture of both overt and latent DGAT activities, depending on the degree of microsomal integrity. The postnatal rise in overt DGAT activity observed in the present study was transient, although at day 10 it did not return to the very low levels observed immediately after birth (Fig. 1). In this early phase, the pattern of overt DGAT activity paralleled that of hepatic TAG content, re-enforcing our previous conclusion (1) that the primary product of overt DGAT is cytosolic TAG. The fact that latent DGAT activity continued to increase by several-fold after the decline in overt DGAT activity (Fig. 1) and the extensive mobilization of cytosolic TAG after day 4 (Fig. 2) supports the suggestion that the function of the latent DGAT activity is to maintain TAG secretion, even when the liver content of the main source of DAG and acyl moieties, namely cytosolic TAG, is diminished. It is of interest that the activities of both monoacylglycerol acyltransferase (30) and latent DGAT are both at their highest in the liver of suckling rats, indicating that they act synergistically to divert partial glycerides toward TAG secretion by minimizing the extent of the complete hydrolysis of endogenous TAG to acyl-CoA and glycerol under conditions characterized by much higher rates of fatty acid oxidation, which competes for acyl-CoA esters (11, 32).

The dependence of the overall rates of TAG secretion on cytosolic TAG mobilization during early development is of interest in view of the recent description of candidate proteins for the role of cytosolic TAG hydrolase in the liver (29, 33–36). The reversal of the hepatic steatosis of the early neonate after day 4 (Fig. 3) (13) requires the presence of hydrolase activity, irrespective of whether the resulting products are used for the re-synthesis of TAG or, in the case of acyl-CoA esters, for oxidation, which in the
early stages is also heavily dependent on mobilization of endogenous TAG (Fig. 4). It has been argued previously (34) that the large postweaning (>27 day) induction of triglyceride hydrolase (TGH) coincides with a large putative increase in TAG secretion by the liver. Our data do not support this inference, as we observed no difference in the capacity of hepatocytes to secrete TAG between pups 10 days postpartum and the adult (Fig. 2). More recent data from the same laboratory (29) indicate that substantial TGH expression already exists immediately after birth. This early expression of TGH is potentially important in mobilizing TAG during the first 7 days postnatally and reversing the steatosis induced at birth. However, the postweaning induction of TGH does not correlate with either the rate of TAG secretion or the utilization of endogenous TAG as a source of acyl moieties for oxidation (Figs. 1, 4). It is interesting that this postweaning induction of TGH expression is matched by a large increase in overt DGAT activity that occurs over the same period as the activity reaches adult values (Fig. 1A). The parallel increase in these two activities may account for the high rate of cytosolic TAG turnover observed in adult rat hepatocytes (6, 7). A diagram of the suggested interactions between cytosolic TAG hydrolysis and TAG secretion is shown in Fig. 6.

In conclusion, we have shown that overt and latent DGAT activities of hepatic ER exhibit very different ontogenic patterns in response to normal physiological perturbations that involve large changes in the rates of hepatic TAG synthesis and secretion. Moreover, the postweaning decline in latent DGAT activity, which in the normal animals occurs upon switching to a high-carbohydrate chow diet, was prevented by weaning the animals onto a low-insulin conditions that prevail during the suckling period (37).

This work was supported by the British Heart Foundation and by the Scottish Executive Environment and Rural Affairs Department. The authors thank Anna Brown and Nicola Britton for excellent assistance.

REFERENCES


