Ontogeny of microsomal activities of triacylglycerol synthesis in guinea pig liver

Rosalind A. Coleman, Elaine B. Haynes, and C. Denise Coats

Departments of Pediatrics and Biochemistry, Duke University Medical Center, Durham, NC 27710

Abstract Because the onset of triacylglycerol-rich lipoprotein synthesis occurs in guinea pig liver during fetal life, we investigated the microsomal enzyme activities of triacylglycerol synthesis in fetal and postnatal guinea pig liver. Hepatic monoacylglycerol acyltransferase specific and total microsomal activities peaked by the 50th day of gestation and declined rapidly after birth to levels that were virtually unmeasurable in the adult. Peak fetal specific activity was more than 75-fold higher than observed in the adult. The specific activities of fatty acid CoA ligase and lysophosphatidic acid acyltransferase increased 2- to 3-fold before birth; lysophosphatidic acid acyltransferase increased a further 2.6-fold during the first week of life. Specific activities of phosphatidic acid phosphatase, microsomal glycerophosphate acyltransferase, and diacylglycerol acyltransferase varied minimally over the time course investigated. These data demonstrate that selective changes occur in guinea pig hepatic microsomal activities of triacylglycerol synthesis before birth. Because of an approximate 11-fold increase in hepatic microsomal protein between birth and the adult, however, major increases in total microsomal activity of all the triacylglycerol synthetic activities occurred after birth. The pattern of monoacylglycerol acyltransferase specific and total microsomal activities differs from that of the rat in occurring primarily during the last third of gestation instead of during the suckling period. This pattern provides evidence that hepatic monoacylglycerol acyltransferase activity probably does not function to acylate 2-monocacylglycerols derived from partial hydrolysis of diet-derived triacylglycerol. — Coleman, R. A., E. B. Haynes, and C. D. Coats. Ontogeny of microsomal activities of triacylglycerol synthesis in guinea pig liver. J. Lipid Res. 1987. 28: 320—325.

Supplementary key words maternal—fetal exchange • monoacylglycerol acyltransferase • glycerolipid biosynthesis • ontogeny • guinea pigs

Triacylglycerol, the major form of stored energy in mammals, helps the newborn withstand intermittent fasts after birth by lessening its requirement for glucose. The liver plays a major role in triacylglycerol metabolism and energy homeostasis by synthesizing and secreting very low density lipoprotein (VLDL). Stored triacylglycerol within hepatocytes may provide energy for liver metabolism and may also provide a repository of long-chain fatty acids that can be used to synthesize phospholipid for bile and membrane biogenesis. In the rat, the specific activities of hepatic microsomal enzymes of triacylglycerol biosynthesis increase 4- to 70-fold perinatally; the major increases occur during the week after birth (1). These increases in enzyme activity are probably required to handle the large influx of fatty acids provided by the milk diet of the suckling rat (2, 3). In addition to increases in the activities of the glycerophosphate pathway of glycerolipid biosynthesis, suckling rat liver contains monoacylglycerol acyltransferase whose activity is 700-fold higher than that observed in the adult rat (4). As yet, the role of the monoacylglycerol pathway during the suckling period remains unclear; the presence of high liver-associated lipase activities postnatally (5) would seem to preclude diet-derived triacylglycerol as a source of monoacylglycerol. Instead, the monoacylglycerol pathway may facilitate the recycling of triacylglycerol stored in hepatic lipid droplets and may help regulate cellular diacylglycerol pools.

The program of hepatic differentiation differs in the guinea pig which, unlike the rat, is relatively mature at birth (6, 7). The fetal guinea pig receives a large obligatory influx of fatty acids that are transported across the placenta during the last 20 days of its 65- to 72-day gestation (8). At this time, the fetal guinea pig develops a fatty liver with triacylglycerol contributing as much as 25% of the dry liver weight (9). Fetal plasma becomes hyperlipidemic with a mean triacylglycerol content of 600 mg/dl after the 65th gestational day (9).

Thus, the guinea pig develops a fatty liver during fetal life, in contrast to the rat in which the liver becomes fatty after birth (10). We wondered whether the hepatic microsomal activities of triacylglycerol biosynthesis and the monoacylglycerol acyltransferase activity would increase during prenatal exposure to large amounts of fatty acid or whether the activities would develop with suckling as in the rat. An increase in hepatic monoacylglycerol acyltransferase activity during one or the other specific time-frame would, in addition, suggest whether its role was related to the reacylation of monoacylglycerols derived...
from partially hydrolyzed dietary triacylglycerol. Therefore, we examined the ontogeny of the microsomal activities of triacylglycerol biosynthesis in preparations of guinea pig liver during the final 30 days of gestation and at selected intervals after birth.

EXPERIMENTAL PROCEDURES

Materials

DL-α-Glycerophosphate, Triton X-100, egg lecithin phosphatidic acid, N-ethylmaleimide, bovine serum albumin (essentially fatty acid-free), and 5,5′-dithiobis(2-nitrobenzoic acid) were purchased from Sigma. 1-Oleoylglycerol-3-phosphate and 1,2-sn-dioleoylglycerol were from Serdary. Palmitoyl-CoA, ATP, and CoA were from P-L Biochemicals. Sodium dodecyl sulfate was from BDH Biochemicals. Aquasol-2 and [3H]palmitic acid were from New England Nuclear. [3H]Palmitoyl-CoA (11) and [3H]glycerol-3-phosphate (12) were synthesized by previously reported methods.

Methods

Timed pregnant Dunkin Hartley guinea pigs (Hazleton Dutchland, Denver, PA) ate Purina guinea pig chow ad libitum. Postnataally, the young animals were kept with their mothers and allowed to suckle or eat chow ad libitum. Between 8 and 10 AM on selected days, the pregnant or neonatal animals were anesthetized with ether. The fetal, neonatal, and adult livers were rapidly removed and placed in ice-cold Medium 1 (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4). Litter size varied from two to seven fetuses. The length of the guinea pig gestation varies between 65 and 72 days. Animals killed on the day of birth were less than 12 hr old.

Livers were weighed, minced, and homogenized with five up-and-down strokes in a motor-driven Teflon-glass homogenizer at moderate speed in ice-cold Medium 1. To obtain a microsomal preparation, the homogenate was centrifuged at 1000 g for 10 min and the pellet was discarded. The supernatant was centrifuged at 25,000 g for 10 min. This pellet was discarded and the supernatant was centrifuged at 100,000 g for 1 hr to obtain a microsomal fraction. Aliquots of microsomes were resuspended in Medium 1 and stored at −70°C. Once thawed and assayed, each aliquot was discarded. Total particulate or microsomal fractions (1.0 mg of protein/ml) were incubated with N-ethylmaleimide (1.0 mM) for 15 min at 4°C and aliquots were removed for assay of microsomal (NEM-sensitive) and mitochondrial (NEM-resistant) glycerophosphate acyltransferase activity (17).

Enzyme assays

Fatty acid CoA ligase activity was measured using 50 μM [3H]palmitate and 5 mM ATP as previously described (16). Glycerophosphate acyltransferase activity was assayed using 112.5 μM palmitoyl-CoA and 300 μM [3H]glycerol-3-phosphate (17). Lysophosphatidic acid acyltransferase activity was determined spectrophotometrically (18) using 55 μM oleoylglycerophosphate and 50 μM oleoyl-CoA. Phosphatidic acid phosphatase activity was determined by monitoring orthophosphate release (19) using 1.0 mM phosphatidic acid. Diacylglycerol acyltransferase activity was assayed using 30 μM [3H]palmitoyl-CoA and 200 μM 1,2-sn-dioleoylglycerol dispersed in acetone (15). Monoacylglycerol acyltransferase activity was assayed using 25 μM [3H]palmitoyl-CoA and 50 μM m-2-monoleoylglycerol dispersed in acetone by a previously reported method (4) except that MgCl2 was omitted.

All assays were performed at 25°C except for phosphatidic acid phosphatase which was performed at 37°C. All assays were proportional to the time and the amount of protein employed. The substrate concentrations employed gave maximal activities in both fetal and postnatal preparations. Activities are reported as the mean ± SD. Specific activities are expressed as nmol of product/min per mg protein. Total microsomal activities are expressed as μmol of product/min × microsomal protein recovered.

Inhibition by N-ethylmaleimide

Total particulate or microsomal fractions (1.0 mg of protein/ml) were incubated with N-ethylmaleimide (1.0 mM) for 15 min at 4°C and aliquots were removed for assay of microsomal (NEM-sensitive) and mitochondrial (NEM-resistant) glycerophosphate acyltransferase activity (17).

RESULTS

The monoacylglycerol acyltransferase activity was studied in microsomal preparations of guinea pig liver during late gestation when placental fatty acid transport increases markedly (8), during the first postnatal week when sucking occurs, and in the adult. Monoacylglycerol acyltransferase specific activity peaked by day 50 of the 65- to 72-day guinea pig gestation after a 3.6-fold rise from day 40 (Fig. 1A). Specific activity remained elevated through late gestation, then declined rapidly to low levels by the 8th postnatal day, and was virtually unmeasurable in the adult (<0.5 nmol/min per mg). Total microsomal activity had a similar pattern over the course of late gestation (Fig. 1B). This pattern is similar to that observed in the rat but is displaced in time. In the rat, hepatic monoacylglycerol acyltransferase specific activity rises
after birth, peaks 6 to 8 days after birth, and declines steadily over the following 3 weeks. Rat liver specific activity on the 8th postnatal day is 700-fold higher than that observed in adult microsomes (4). In guinea pigs, the average monoacylglycerol acyltransferase specific activity during the peak in late gestation was more than 75-fold higher than in adult guinea pig liver microsomes.

Hepatic microsomal fatty acid CoA ligase activity increased 3.3-fold during the final third of gestation. These specific activity levels persisted through the first postnatal week and were about 60% higher than observed in adult animals (Fig. 2A). Since fatty acid CoA ligase is present in mitochondrial outer membrane and in peroxisomes as well as in microsomes, altered activity measured in a "microsomal" fraction may reflect one or both of the other ligases if differential centrifugation allowed varying amounts of other membranes to remain during the pre- and postnatal preparations. Lack of significant contribution by the mitochondrial fatty acid CoA ligase is suggested by the presence of low amounts of another outer membrane activity, mitochondrial (NEM-resistant) glycerophosphate acyltransferase, which comprised less than 4% of total microsomal activity in all preparations (see below) and by the different ontological pattern previously reported for fatty acid CoA ligase activity from total particulate fractions (20). Although the specific activity of fatty acid CoA ligase did not change perinatally, total microsomal activity rose markedly after birth (Fig. 1B). Total microsomal activity in the adult rat liver was about 12-fold higher than at birth.

The specific activity of the microsomal (NEM-sensitive) glycerophosphate acyltransferase, the committed step of the glycerophosphate pathway, varied minimally between the final third of gestation and the first postnatal week. Specific activities before and after birth were 6.5 ± 3.7 and 8.1 ± 3.3, respectively. Like fatty acid CoA ligase, the average postnatal specific activity was 56% higher than adult specific activity. Total microsomal activity rose throughout late gestation and was 10-fold higher in the adult than at birth (Fig. 3). Mitochondrial (NEM-resistant) glycerophosphate acyltransferase activity was examined in total particulate preparations. Specific activity remained constant at 0.29 ± 0.1 nmol/min per mg protein in fetal, postnatal, and adult liver.
Lysophosphatic acid acyltransferase specific activity doubled during the final third of gestation and, unlike the other microsomal activities, continued to rise another 2.6-fold during the first postnatal week before declining to adult levels (Fig. 4A). Total microsomal activity in the adult was 15.5-fold higher than at birth (Fig. 4B). The specific activity of phosphatidic acid phosphatase varied minimally in microsomal preparations during the late fetal and postnatal period. Between days 40 and 50 of gestation, specific activity was 4.7 ± 2.2 and during the first week after birth, activity was 6.2 ± 1.8. Total microsomal activity, however, increased 11-fold between newborn and adult (Fig. 5).

Surprisingly, the specific activity of diacylglycerol acyltransferase, the enzyme unique to triacylglycerol biosynthesis, did not vary during late gestation and postnatal life (Fig. 6A). Guinea pig specific activities throughout gestation were 2.1 ± 0.8 nmol/min per mg compared to adult values of 2.0 ± 0.7. These activities are relatively low compared with activities of 8 to 12 nmol/min per mg observed in microsomes from rat livers of comparable developmental ages (1). The rise in total microsomal activity between birth and adult was about 11-fold (Fig. 6B).

**DISCUSSION**

In guinea pig liver, the activities of fatty acid synthesis are highest between days 40 and 60 of gestation before declining to low postnatal levels (21). Placental transport provides a second major source of fatty acid to the fetus during the last third of gestation (8). Increased fatty acid availability is associated with the onset of lipoprotein production. Small lipoprotein particles can be observed in hepatic Golgi from the 52-day-old guinea pig fetus, liver from the 68-day-old fetus contains numerous large lipoprotein particles in Golgi, secretory vesicles, and the space of Disse, and mean fetal plasma triacylglycerol content after day 65 is 600 mg/dl (9). We found that the specific activities of fatty acid CoA ligase and lysophosphatic acid acyltransferase rose and peaked between the 50th and 60th days of gestation, whereas diacylglycerol acyltransferase specific activity remained relatively low and constant. Increases in liver content of microsomal pro-

---

1Interpretation of the relative specific activities of these microsomal enzymes and the rate-limiting steps of the pathways must remain guarded because of the complexity of the in vitro assay methods. Incubation mixtures contain variable amounts of microsomal proteins and phospholipids, salts, bovine serum albumin, and hydrophobic substrates that have been dispersed in detergents or solvents. Specific activities that are measured in vitro may not be strictly comparable to in vivo rates.
3.0L PHOSPHATIDIC ACID PHOSPHATASE

Fig. 5. Time course showing changes in microsomal activity of phosphatidic acid phosphatase in liver microsomes from fetal, neonatal, and adult guinea pigs. Each point represents a determination from an entire fetal litter, from two fetal littermates, or from a single postnatal animal. Adult values are the mean ± SD from five adult pregnant guinea pigs.

tein, however, result in large increases in total microsomal activity for all of the glycerophosphate pathway enzymes examined. In each instance, the major increase in total microsomal activity occurred after birth. The total microsomal activities presented underestimate by 70 to 80% the true total activity per liver, since substantial, though consistent, losses of endoplasmic reticulum protein occurred during the differential centrifugations.

Despite this evidence for active synthesis, assembly, and secretion of triacylglycerol-rich lipoproteins, the fetal guinea pig liver also accumulates prominent cytoplasmic lipid droplets, and hepatic triacylglycerol content increases dramatically to contribute to as much as 25% of the liver's weight (9). These findings suggest that the late fetal liver can readily synthesize triacylglycerol from fatty acids that are endogenously synthesized or placentally transported, but that the liver's capacity to assemble and secrete triacylglycerol-rich lipoprotein may be relatively insufficient, making the liver become a triacylglycerol storage organ.

The situation is similar to that in the newborn rat which develops a fatty liver during its first postnatal week (10). Evidence has been presented that the rat liver does not secrete triacylglycerol-rich lipoproteins until after the 14th day of life (22, 23). Thus, both guinea pig and rat develop fatty livers although the developmental timing differs for each species.

Hepatic monoacylglycerol acyltransferase activity is present in both rat and guinea pig in association with the development of a fatty liver. In both animals, the decline in monoacylglycerol acyltransferase specific activity and total microsomal activity parallels the decline in hepatic triacylglycerol content. Since the term fetal guinea pig liver contains 1 g of triacylglycerol which declines rapidly after birth, Bohmer, Havel, and Long (9) suggested that hepatic triacylglycerol stores are available for postnatal energy requirements. The stored triacylglycerol in rat pup hepatocytes may function similarly. Rat hepatic lipase is present and lipoprotein lipase in liver is high postnatally (5), so it is unlikely that monoacylglycerol substrate originates from outside the hepatocyte. The monoacylglycerol acyltransferase may aid in the recycling of hepatic triacylglycerol thereby providing a pathway that reacylates 2-monocacylglycerols that have been released from hepatic triacylglycerol storage droplets by intracellular lipases. Additionally, the hepatic monoacylglycerol acyltransferase may regulate diacylglycerol pools and, as has been hypothesized for the intestinal isoenzyme (24), it may channel monoacylglycerol specifically toward triacylglycerol rather than phospholipid biosynthesis. Reacylation in the outer monolayer of the endoplasmic reticulum (25) would provide the diacylglycerol substrate for triacylglycerol and phospholipid biosynthesis.

The patterns of change occurring in each of the micro-
somal activities of the glycerophosphate and monoacylglycerol pathways of triacylglycerol synthesis suggest that the activities are independently regulated. Independent regulation has been inferred for the activities of glycerolipid biosynthesis in differentiating 3T3-L1 adipocytes (26) and in developing rat liver (1). In the guinea pig it is likely that these changes are hormonally controlled, perhaps by epinephrine and cortisol whose concentrations rise 5- and 7.5-fold, respectively, between the 50th and 60th days of gestation (7, 27), coinciding with the increases observed in the specific activities of the glycerolipid synthetic enzymes. This work was supported in part by grants HD19068 from the National Institutes of Health and 1-850 from The March of Dimes Birth Defects Foundation.

REFERENCES


