Ontogeny of glycerolipid biosynthetic enzymes in swine liver and adipose tissue

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Abstract Enzymes associated with glycerolipid biosynthesis were examined in microsomal fractions of liver and adipose tissue obtained from swine of various ages. Generally, liver glycerophosphate acyltransferase, phosphatidate phosphohydrolase, diglyceride acyltransferase, and choline phosphotransferase activities were substantial at birth but increased 2- to 3-fold by day 14 postpartum, decreased at day 25, then increased at the oldest ages studied (up to 155 days postpartum). In adipose tissue, enzyme activities were low at birth and developed through day 25 in a pattern generally similar to that observed in liver. In contrast to liver, the adipose enzymes were depressed immediately postweaning (day 32) with subsequent recovery. The observed decline in adipose tissue enzyme activities expressed on a tissue basis at older ages was primarily the result of increased adipocyte size, since the activities expressed on a cell basis did not decline as rapidly. In both liver and adipose tissue, phosphatidate was the major glycerolipid synthesized by the microsomal glycerophosphate acyltransferase enzymes at all ages (generally >75%). The ratio of neutral lipids to phospholipids produced by acylation of glycerophosphate was increased when a microsomal–cytosolic preparation was used as a source of enzyme in contrast to a microsomal preparation.—**Steffen, D. G., G. Phinney, L. J. Brown, and H. J. Mersmann.** Ontogeny of glycerolipid biosynthetic enzymes in swine liver and adipose tissue. *J. Lipid Res.* 1979. **20:** 246–253.

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Adipose tissue and liver fatty acid biosynthetic activities have been extensively investigated in neonatal and growing swine (1–5). In general, glucose incorporation into adipose tissue slice lipid increased dramatically after weaning, whereas liver slice lipogenesis was marginal at all ages studied. Furthermore, in swine adipose tissue the neutral lipid fraction (>95% triglyceride) increased rapidly after birth and then more gradually after weaning (3). Such variations in the lipid composition of adipose tissue suggest age-related changes in the synthesis of glycerolipids.

The major triglyceride biosynthetic route in swine adipose tissue appears to be the glycerophosphate pathway (6). Although this pathway is active in swine liver (6), a monoglyceride pathway has also been implicated in triglyceride formation (7). Since our primary interest is in swine adipose tissue metabolism, we have investigated the age-related changes in several key enzymes of glycerolipid synthesis in adipose tissue and, for comparison, in liver.[3] The sequential acylation by activated fatty acids (acyl-CoA) of *sn*-glycerol-3-phosphate to yield phosphatidate (GPAT[4] activity) is followed by hydrolysis of the phosphatidate to yield diglyceride (PPH activity) and, finally, by acylation of the diglyceride to yield triglyceride (DGAT activity). An alternate fate of the diglyceride is phospholipid production completed by base transfer reactions as exemplified by CPT activity, the product of which is phosphatidylcholine (6, 8–11). The *sn*-glycerol-3-phosphate may result from glycolysis, gluconeogenesis, or from plasma glycerol, whereas fatty acyl-CoA may be synthesized de novo in adipose tissue, derived from plasma free fatty acids or plasma triglyceride via lipoprotein lipase, or produced by lipolytic activity within the tissue (9, 10, 12).

METHODS

Animals

Purebred Yorkshire piglets were raised according to usual husbandry procedures (castration of males at 10 days of age) but with access to only sow's milk until weaning at day 28, i.e., no solid food. They were weaned to a pelleted diet containing 22% protein and

Abbreviations: GPAT, glycerophosphate acyltransferase; PPH, phosphatidate phosphohydrolase; DGAT, diglyceride acyltransferase; CPT, choline phosphotransferase; NL, neutral lipid; PL, phosphatidate.

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[3] A preliminary account of a portion of this work was presented (*Federation Proc.* **36:** 1156. 1977).

[4] Enzymes: Acyl-CoA *sn*-glycerol-3-phosphate *O*-acyltransferase, EC 2.3.1 (GPAT); acyl-CoA:1,2-diglyceride-*O*-acyltransferase, EC 2.3.1.20 (DGAT); CDP-choline: 1,2-diglyceride-choline phosphotransferase, EC 2.7.8.2 (CPT); phosphatidate phosphohydrolase, EC 3.1.3.4 (PPH).

3% fat. At days 35 and 80 postpartum the diet was changed to 18 and 15% protein, respectively. Pigs had ad libitum access to feed. In a second experiment, crossbred animals (Yorkshire, Hampshire, Duroc) were utilized with similar husbandry conditions except that the preweaned pigs had access to solid feed.

In experiment 1 (Tables 1 and 2), animals were killed by a cephalic blow coupled with exsanguination at the following postpartum ages: day 0 (<12 hr), day 3 (2–4 days), day 14 (13–14 days), day 25 (22–26 days), day 32 (30–33 days), day 45 (42–46 days), day 75 (73–77 days), and day 155 (152–163 days). At days 0, 3, and 14, equal amounts of adipose tissue from 6, 4, and 2 animals, respectively, were pooled to obtain sufficient tissue for the assays. Equal amounts of liver from each animal were also pooled, and the data obtained from each pooled sample were considered to be from one animal. Each age was represented by five animals from at least three different litters except day 0 where only two litters were used. In experiment 2 (Table 3), four animals were used at each age (similar to those in experiment 1); however, pooled tissue samples were not necessary since only one enzyme activity was assayed.

Tissue preparation

Subcutaneous adipose tissue from the dorsal neck region and liver were chilled in cold 0.9% NaCl. An adipose homogenate (1 g of tissue + 2 ml of buffer) and a liver homogenate (1 g of tissue + 7 ml of buffer) were prepared with 0.15 M KCl–10 mM HEPES (i.e., *N* - 2 - hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid)–1 mM ethylenediaminetetraacetic acid—1 mM dithiothreitol (pH 7.0), as previously described (13). The microsomal fractions (13) obtained from 1 g of tissue were reconstituted in 4 ml (liver) or 1 ml (adipose) of cold homogenization medium.

The 10,000 *g* supernatant fraction (experiment 2) was prepared by homogenization of 1 g of tissue with 3.5 ml or 1 ml of homogenization medium for liver and adipose tissue, respectively. Centrifugation was at 10,000 *g* for 15 min, and the supernatant fraction was decanted and used as the enzyme source.

Substrate preparation

Diolein suspended in benzene was dried under N_2 and diluted *a*) with Tween 20 (2.4 mg/ml) to diolein concentrations (μmol/ml) of 36 (for liver DGAT), 24 (for adipose DGAT), and 10 (for adipose CPT); or *b*) with Tween 20 (10 mg/ml) to give 20 μmol diolein/ml (for liver CPT). The mixtures were sonicated on ice for 5 min (Bronwill Biosonick III, microtip, maximum energy level) and used immediately for enzyme assays. An aqueous dispersion of 3 mM phosphatidic acid was prepared by dissolving phosphatidic acid in diethyl ether (ca. 3 ml), adding the appropriate amount of water, and sonicating on ice for 1 min. The ether was evaporated, with N_2, until no detectable trace remained, and the aqueous mixture was subjected to a short sonication (10 sec) immediately prior to use. All emulsions were prepared fresh daily. All fatty acids were used as the potassium salts.

Enzyme assays

Enzyme rates were measured using optimal conditions, determined by preliminary experiments, and in a range where activity was proportional to time and enzyme concentration as indicated. All activities were corrected for recovery of the reaction product and for background activity in the absence of enzyme. Reactions were started by addition of the appropriate microsomal or 10,000 *g* supernatant fraction to bring the final volume to 0.4 ml in experiment 1 or to 0.6 or 0.8 ml for liver and adipose tissue, respectively, in experiment 2. The assay tubes were incubated at 37°C with shaking at 120 strokes/min. Enzyme activities were expressed on a recovered microsomal or 10,000 *g* supernatant protein basis (SAp), on a cell basis in adipose tissue (SAc), and on a tissue basis (SAw). The latter calculation was an extrapolation from the recovered microsomal or 10,000 *g* supernatant fraction for experiments 1 and 2, respectively, and assumed constant recovery.

Liver microsomal GPAT activity (experiment 1) was assayed as suggested by Raju and Six (8) in a mixture containing 150 mM HEPES (pH 7.4), 20 mM potassium phosphate (pH 7.4), 1 mM dithiothreitol, 10 mM $MgCl_2$, 6 mM ATP, 80 μM CoA, 0.6 mM palmitate, 0.2 mg albumin, 20 mM glycerol-3-phosphate (0.3 μCi per flask), and 0.05 ml of enzyme; the adipose enzyme assay was similar but contained 3 mM ATP, 17.5 mM glycerol-3-phosphate, and 0.1 ml of enzyme. The 10,000 *g* supernatant GPAT assay, in experiment 2, contained the same components at 67 and 50% of the concentrations indicated for the liver and adipose tissue microsomal assays, respectively. The enzyme was added as 0.05 or 0.25 ml of 10,000 *g* supernatant fraction for liver and adipose tissue, respectively. The microsomal GPAT enzyme activity was linear for 15 min up to 450 μg of protein and for 15 min up to 205 μg of protein for liver and adipose tissue, respectively, whereas the 10,000 *g* supernatant GPAT enzyme activity was linear for 10 min up to 8 mg or 7 mg of protein for liver and adipose tissue, respectively.

After a 10-min or 8-min incubation for microsomal or 10,000 *g* supernatant fraction, respectively, reactions were terminated. The products were extracted

and separated by thin-layer chromatography, and the radioactivity was determined by liquid scintillation counting, as previously indicated (13). Radioactivity was incorporated into phosphatidate (phosphatidic plus lysophosphatidic acids) and into neutral lipids. The primary neutral lipid products of the reaction were triglyceride and diglyceride; the small amount of radioactivity in the monoglyceride fraction (<7%) was ignored. The activity of GPAT was calculated from the incorporation of [U-^{14}C]glycerol-3-phosphate into phosphatidate plus neutral lipids. Recovery of neutral lipid and phosphatidate by this procedure was 90 and 82%, respectively. The neutral lipid/phosphatidate ratio (NL/PL) represents the radioactivity incorporated into (diglyceride + triglyceride)/(lysophosphatidate + phosphatidate) × 100.

A modification of the method of Young and Lynen (14) was used to follow liver DGAT activity in a medium containing 150 mM HEPES (pH 7.4), 1 mM dithiothreitol, 20 mM $MgCl_2$, 6 mM ATP, 80 μM CoA, 0.2 mg of albumin, 0.8 mM oleate (0.05 μCi per flask), 9 mM diolein in 240 μg of Tween 20, and 0.05 ml of enzyme; adipose assay conditions were similar but with 30 mM $MgCl_2$, 3 mM ATP, 100 μM CoA, 0.4 mM oleate, 6 mM diolein, and 0.1 ml of enzyme. The enzyme activity was linear for 20 min up to 225 μg and for 15 min up to 130 μg of microsomal protein for liver and adipose tissue, respectively. After a 10-min incubation, the reactions were terminated. The triglyceride product was extracted and isolated by thin-layer chromatography, and the radioactivity was determined by liquid scintillation counting, as previously described (13). The extraction and chromatography procedure recovered 88% of the triglyceride product.

The GPAT and the DGAT activities were both assayed using an acyl-CoA generating system that was optimized for all components for each enzyme in both liver and adipose tissue. Although Carey (15) suggests that such a fatty acyl-CoA synthetase system may be rate-limiting, other evidence (6, 8, 9, 16) indicates that fatty acid activation is more physiological than other systems of fatty acid presentation.

The procedure of Skurdal and Cornatzer (17) was used to assess the activity of liver CPT in a medium of 100 mM HEPES (pH 7.4), 25 mM $MgCl_2$, 1 mM dithiothreitol, 0.2 mg of albumin, 0.25 mM CDP-choline (0.05 μCi per flask), 3 mM diolein, 600 μg Tween 20, and 0.05 ml of enzyme. The modified medium for the adipose enzyme contained 1.25 mM diolein, 120 μg Tween 20, and 0.1 ml enzyme. The enzyme activity was linear for 15 min up to 450 μg and 205 μg microsomal protein for liver and adipose tissue, respectively. After a 7-min incubation, the phosphatidylcholine product was extracted, and the radioactivity was determined by liquid scintillation counting (13). This extraction method recovered 92% of the newly formed phosphatidylcholine.

Microsomal PPH activity of swine liver and adipose tissue was assessed according to Lamb and Fallon (18) by measuring the release of inorganic phosphate (19) from 1.5 ml of phosphatidic acid in the presence of 100 mM HEPES (pH 7.4). There was 0.1 ml of enzyme in a total volume of 1 ml with incubation for 20 min. The enzyme activity was linear for 30 min up to 900 μg and 260 μg of microsomal protein for liver and adipose tissue, respectively.

Other procedures

Protein determinations were on trichloroacetic acid precipitates using a biuret method with bovine serum albumin as standard (20). Adipocyte size and number were determined on cells isolated by a collagenase method using optical sizing coupled with tissue triglyceride determination (21). Data were analyzed by one-way (age) analysis of variance (22). The analysis of variance error estimate was used to calculate a standard error (SE) as estimate of the variation of the group means. Heterogeneity of variance did not appear to be a problem. Age patterns in the group means, where not obvious, were evaluated by the least significant difference technique.

Materials

Most special chemicals were obtained from Sigma chemical Company (St. Louis, MO) including bovine serum albumin (fatty acid free, fraction V), L-α-phosphatidic acid (Grade 1 from egg yolk), and glycerol-3-phosphate (DL-α-glycerophosphate, Grade X). Palmitate, oleate, and 1,2-diolein were obtained from Nu-Chek Prep (Elysian, MN), triolein was from Applied Science Laboratories, Inc. (State College, PA), and CoA was from PL Biochemicals, Inc. (Milwaukee, WI). Cytidine diphospho-[methyl-^{14}C]-choline, L[U-^{14}C]glycerol-3-phosphate, and Omnifluor were purchased from New England Nuclear (Boston, MA), and [1-^{14}C]oleic acid was from Amersham/Searle (Arlington Heights, IL). Preactivated TLC plates (linear Q), coated with 0.25 mm silica gel, were obtained from Kontes Glass Company (San Leandro, CA).

RESULTS

Adipose tissue (Fig. 1 and Table 1)

The activity of microsomal GPAT, expressed on a tissue basis (SAw), increased in the early postnatal

period, decreased sharply in the late postnatal and weaning period, increased by 2 weeks postweaning (day 45), and then gradually declined. The data expressed on a protein basis (SAp) generally followed a similar pattern except that the decline after day 45 was more gradual. The expression of GPAT activity on a cell basis (SAc) also followed a pattern similar to that of SAw activity except that the increase between days 3 and 14 was more abrupt and the day 45 level was perhaps maintained longer before declining to day 155 levels. This microsomal enzyme system produced more neutral lipid (greater NL/PL ratio) during the early preweaning ages and after weaning at day 45 and 70 than at other periods.

The pattern of DGAT activity was generally similar to that for GPAT activity until day 45 regardless of the tissue base used for data expression. The DGAT activity, expressed on a tissue basis, at day 70 did not decline as did GPAT and, when expressed on a cell basis (SAc), increased compared to day 45. The developmental patterns (SAw, SAp, and SAc) for CPT activity were all similar to the GPAT patterns. The microsomal PPH activity (SAw) increased markedly between birth and day 3, declined throughout the rest of the preweaning period and immediate postweaning period, possibly increased at day 45, and then again declined at the oldest ages. The protein-based activity (SAp) followed a somewhat similar pattern but only doubled between day 0 and 3 and then remained at the same level until day 25. The data expressed on a cell basis (SAc) increased to a maximum at day 14, declined to day 32, and then increased and remained elevated.

Liver (Fig. 2 and Table 2)

The microsomal GPAT activity, expressed on a wet weight basis (SAw), increased during the early postnatal period, decreased in the late postnatal period, and then gradually increased after weaning. In the early postnatal period, more neutral lipid was produced by this system than at older ages, i.e., the NL/PL ratio decreased with age. The microsomal PPH activity (SAw) increased between birth and day 14, decreased in the late postnatal period (day 25), and increased again after weaning. The microsomal DGAT activity followed a pattern similar to PPH activity but with a greater postweaning increase in activity. For all three enzymes the preweaning developmental pattern expressed on a protein basis (SAp) was similar to the SAw pattern. After weaning, there was much less increase in SAp compared to SAw activities due to the concomitant increase in isolated microsomal protein. The incorporation of diglyceride into phospholipids (CPT activity) gradually increased throughout development.

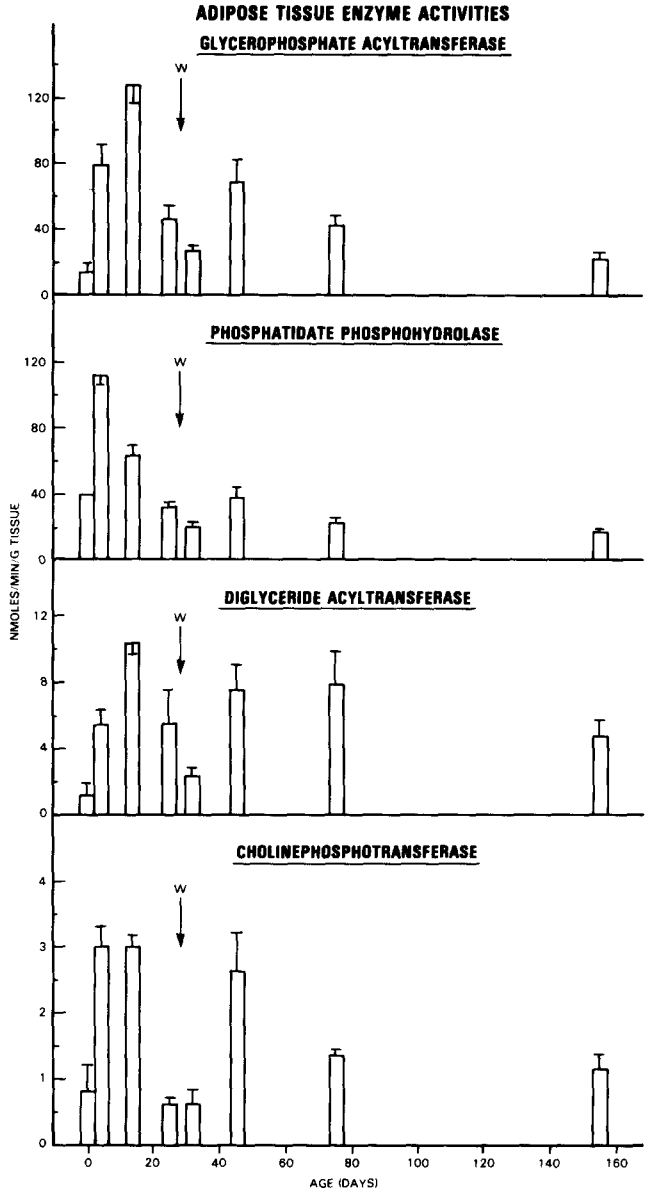

Fig. 1. Swine adipose tissue complex lipid synthetic enzyme activity. Data are indicated as nmol/min/g tissue with the standard error indicated by the vertical bars. The age of weaning is indicated (W).

Microsomal—cytosolic activity (Table 3)

The use of a microsomal–cytosolic preparation, i.e., 10,000 *g* supernatant fraction, as enzyme source for liver GPAT activity produced a different ontogenic pattern in liver than did the microsomal preparation (Table 2). The activity (SAw and SAp) increased abruptly between day 0 and 3, declined at day 10, increased at day 25, and gradually declined there-

TABLE 1. Adipose tissue microsomal enzyme activity

	Age (Days)								
	0	3	14	25	32	45	75	155	SE[i]
N[a]	2	5	5	5	5	5	5	5	
Triglyceride[b]	284	297	427	494	502	544	621	791	56, 35
Cell size[c]	13.3	24.7	50.4	48.2	45.0	53.5	66.3	79.6	2.1, 1.3
Protein[d]	2.2	3.2	1.7	0.9	1.0	1.3	0.9	0.8	0.2, 0.1
GPAT[e,f]									
SAw	14	79	127	46	27	68	41	22	14, 9
SAp	6	25	74	49	29	52	43	28	9, 6
SAc	<0.5	4	31	11	5	17	18	11	4, 2
NL/PL[g]	29	50	30	17	16	30	30	13	5, 3
PPH[e,h]									
SAw	40	112	63	32	20	38	22	16	7, 5
SAp	18	35	36	34	21	29	24	20	5, 3
SAc	<0.5	4.7	14.9	7.6	4.1	9.5	9.8	7.8	1.7, 1.1
DGAT[e,h]									
SAw	1.3	5.5	10.3	5.5	2.4	7.5	7.8	4.5	2.1, 1.3
SAp	0.6	1.7	6.0	6.0	2.6	5.7	8.4	5.4	1.9, 1.2
SAc	<0.05	0.2	2.5	1.4	0.5	1.8	3.4	2.2	0.7, 0.4
CPT[e,h]									
SAw	0.8	3.0	3.0	0.6	0.6	2.6	1.3	1.1	0.4, 0.3
SAp	0.4	1.0	1.7	0.6	0.6	1.9	1.4	1.4	0.3, 0.2
SAc	<0.05	0.1	0.7	0.1	0.1	0.6	0.6	0.5	0.09, 0.06

[a] N, number of animals. Weaning was at ca. day 28.

[b] mg triglyceride per g tissue.

[c] Average adipocyte diameter in μm.

[d] mg recovered microsomal protein per g tissue.

[e] GPAT, glycerophosphate acyltransferase; PPH, phosphatidate phosphohydrolase; DGAT, diglyceride acyltransferase; CPT, choline phosphotransferase.

[f] Incorporation of [^{14}C]glycerolphosphate into phosphatidate + diglyceride + triglyceride as determined by thin-layer chromatography. The activity is expressed as nmol per min per g tissue (SAw) or per mg recovered microsomal protein (SAp) or per 10^6 cells (SAc).

[g] Radioactivity recovered in (1,2- + 1,3-diglyceride) + triglyceride/(lysophosphatidate + phosphatidate) × 100. The diglyceride fractions represent about 40–50% of the radioactivity in the neutral lipid fractions at all ages except days 32 and 45 (54 and 30%, respectively).

[h] nmol substrate incorporated or product formed per min per g tissue (SAw) or per mg recovered microsomal protein (SAp) or per 10^6 cells (SAc).

[i] Standard errors for age 0 and other age means, respectively.

after. The microsomal enzyme alone synthesized less neutral lipid than the 10,000 *g* supernatant system, i.e., the NL/PL ratios were less (compare Tables 2 and 3).

The adipose tissue microsomal–cytosolic GPAT activity (SAw and SAp) had developmental patterns similar to those for the microsomal GPAT activities. As for liver, the amount of neutral lipid produced (as indicated by the NL/PL ratio) was greater than with the microsomal enzyme alone. There was also a relatively large amount of diglyceride produced by the adipose tissue microsomal–cytosolic preparation compared to the microsomal system (compare Tables 1 and 3).

DISCUSSION

Measurement of enzyme activities in subcellular fractions isolated from homogenates of adipose tissue represents the activity of not only adipocytes but also other cell types such as fibroblasts, endothelial cells, and reticulocytes (21). The use of isolated adipocytes as starting material for subcellular fractionation would eliminate the contribution of nonadipocyte cells to the measured activity. However, Jamdar (23) has recently demonstrated considerably less activity in isolated cells because these preparations result in loss of many large, more metabolically active adipocytes. It has also been shown that nonadipocytes contribute little to adipose tissue GPAT activity (24). Consequently, all enzyme activities were measured in the microsomal pellet or the microsomal–cytosolic fraction recovered from adipose tissue or liver homogenates.

The ratio of neutral lipid to phosphatidate produced by acylation of glycerophosphate may be increased with increased microsomal protein as in the system of Raju and Six (8) or by the use of a microsomal–cytosolic preparation as studied by Stokes, Poteat, and Tove (6). In our studies, the major product of microsomal GPAT activity, at all ages, for both liver and adipose tissue was phosphatidate. Ad-

dition of a cytosolic fraction increased the proportion of neutral lipid formed in both tissues (compare the NL/PL ratios in Tables 1 and 2 with Table 3). The cytosol may contain specific factors that stimulate neutral glyceride synthesis (25) or a soluble PPH enzyme that drives neutral lipid synthesis (11, 18, 24).

Since enzyme activities for both swine liver and adipose tissue were optimized for reaction components, they represent an estimate of the capacity to catalyze the indicated reaction rather than in vivo function. The activity measured as GPAT was optimized for incorporation of radioactivity into phosphatidate plus neutral lipid and not for individual products of the reaction. As indicated above, the products of the GPAT activities can easily be changed by the reaction conditions including the enzyme source or amount. Consequently, physiological meaning must be cautiously derived from enzyme activities or from the amount of neutral lipid synthesized in the GPAT systems.

Ontogeny

The developmental patterns for hepatic microsomal enzyme activities are most readily observed in Fig. 2 for the SAw data with the SAp data presented in Table 2. The patterns for GPAT, PPH, and DGAT activities were generally similar throughout the neonatal and growing period and were different from the gradual increase in CPT activity. The capacity for hepatic glyceride synthesis, as measured by enzyme activities, was rather large throughout development and was coupled with high rates of glycerophosphate dehydrogenase (3, 26), a potential major source of glyceride-glycerol. The associated marginal hepatic capacity for fatty acid biosynthesis at any age (3, 26, 27) seems to indicate that the role of the liver in swine lipid metabolism at all stages of development is directed toward complex lipid synthesis rather than de novo synthesis of fatty acids. The microsomal–cytosolic enzyme preparation yielded a different developmental pattern for GPAT activity (Table 3) than did the microsomal enzyme preparation, suggesting the influence of factors added with the cytosol. Nutritional influence is not precluded since the animals in experiment 2 had access to solid feed of low-fat content before weaning.

The rapid enhancement of fat depots in the neonatal piglet was accompanied by a marked increase in glycerolipid enzyme activities. The developmental patterns are readily observed in Fig. 1 for the microsomal SAw data with the SAp and SAc data presented in Table 1. The microsomal–cytosolic enzyme data for GPAT is presented in Table 3. At this early stage of development, the rates of adipose tissue fatty acid

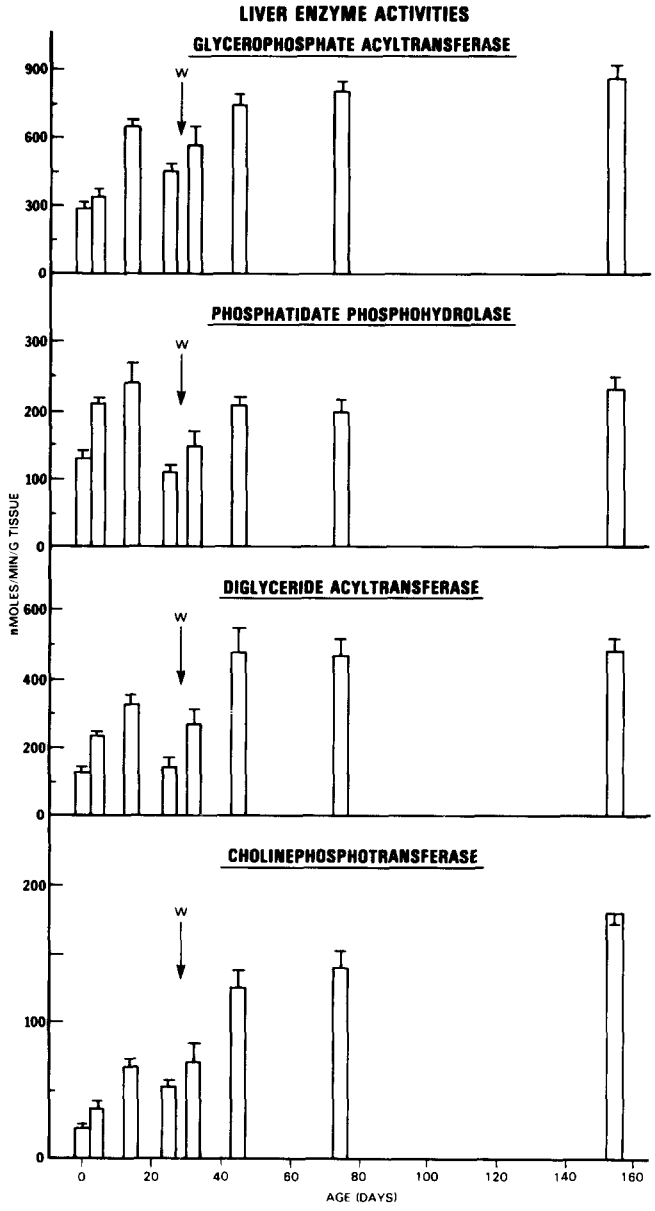

Fig. 2. Swine liver complex lipid synthetic enzyme activity. Data are indicated as nmol/min/g tissue with the standard error indicated by the vertical bars. The age of weaning is indicated (W).

synthesis were low (1–3) with a large portion of the fatty acid precursors for esterification into adipose triglycerides presumably supplied from circulating triglycerides by lipoprotein lipase (28, 29). The capacity for fatty acid biosynthesis was markedly increased in swine adipose tissue after weaning (1–3, 5, 27) along with an elevated lipoprotein lipase activity (28, 29). These postweaning sources of fatty acid potentially provide substrate for incorporation into adipose tissue glycerolipids. During the postweaning period of increased fat deposition, the GPAT, PPH, and especially the DGAT activity, expressed on a cell basis, were increased.

TABLE 2. Liver microsomal enzyme activity

	Age (Days)								
	0	3	14	25	32	45	75	155	SE[g]
N[a]	2	5	5	5	5	5	5	5	
Protein[b]	12.0	15.2	14.8	11.6	13.1	18.0	19.6	22.4	1.3, 0.9
GPAT[c,d]									
SAw	282	319	637	447	562	680	794	849	84, 53
SAp	24	22	43	38	43	38	40	39	6, 4
NL/PL[e]	29	20	14	20	13	9	12	9	3, 2
PPH[c,f]									
SAw	132	206	241	110	146	210	202	228	30, 19
SAp	11	14	16	9	11	12	10	10	1, 1
DGAT[c,f]									
SAw	131	229	322	141	271	481	469	482	57, 36
SAp	11	15	22	12	21	27	24	22	3, 2
CPT[c,f]									
SAw	21	36	67	52	70	126	138	180	15, 10
SAp	1.8	2.4	4.5	4.5	5.3	7.0	7.0	8.1	0.7, 0.5

[a] N, number of animals. Weaning was at ca. day 28.

[b] mg recovered microsomal protein per g tissue.

[c] GPAT, glycerophosphate acyltransferase, PPH, phosphatidate phosphohydrolase, DGAT, diglyceride acyltransferase, CPT, choline phosphotransferase.

[d] Incorporation of [^{14}C]glycerolphosphate into phosphatidate + diglyceride + triglyceride as determined by thin-layer chromatography. The activity is expressed as nmol per min per g tissue (SAw) or per mg recovered microsomal protein (SAp).

[e] Radioactivity recovered in (1,2- + 1,3-diglyceride) + triglyceride/(lysophosphatidate + phosphatidate) × 100. The diglyceride fractions represent about 24–30% of the neutral lipid fractions at all ages except day 14 (17%).

[f] nmol substrate incorporated or product formed per min per g tissue (SAw) or per mg recovered microsomal protein (SAp).

[g] Standard errors for age 0 and other age means, respectively.

TABLE 3. Glycerophosphate acyltransferase activity in microsomal–cytosolic preparations[a]

Age (Days)	Protein[b]	SAw[c]	SAp[c]	Products (%)[d]			NL/PL[e]
				PL	DG	TG	
1) Liver							
0	94	588	6.5	64	6	31	58
3	98	996	10.4	58	5	37	72
10	120	781	6.6	54	12	35	87
25	97	1050	10.9	68	11	22	49
45	106	923	9.1	62	15	24	63
70	105	825	7.9	68	12	21	49
SE[f]	9	64	1.2	3	2	3	
2) Adipose tissue							
0	27	32	1.3	44	35	22	130
3	38	56	1.5	27	46	26	267
10	21	70	3.4	48	29	23	108
25	11	18	1.6	76	15	10	33
45	17	48	2.7	69	18	14	46
70	15	40	2.6	69	19	12	45
SE[f]	3	9	0.4	4	4	2	

[a] Enzyme activity measured with a 10,000 *g* supernatant fraction. There were four animals at each age with weaning at about day 28.

[b] mg recovered 10,000 *g* supernatant protein per g tissue.

[c] Incorporation of [^{14}C]glycerolphosphate into phosphatidate + diglyceride + triglyceride as determined by thin-layer chromatography. The activity is expressed as nmol per min per g tissue (SAw) or per mg recovered 10,000 *g* supernatant protein (SAp).

[d] Percent radioactivity recovered in lysophosphatidate + phosphatidate (PL) or in 1,2- + 1,3-diglyceride (DG) or in triglyceride (TG).

[e] Percent DG + TG/PL × 100.

[f] Standard error.

Comparison of the SAw and SAc (Table 1) values suggests that the dramatic fall in adipose enzyme activities near weaning may be a specific adaptation since all cell parameters (size, number, triglyceride content) were relatively constant between days 14 and 45. Such an adaptation may result from a specific dietary nutrient and/or the energy intake coupled with the stress of weaning itself or the interplay of other hormonal and metabolic factors (9). Whatever the cause, there was a similar pattern in liver, and both tissues rebounded to elevated enzyme activity levels by day 45. The gradual decline in the SAw values during postweaning growth (Table 1) for the most part reflects the decreased cellularity per unit tissue weight caused by increased cell size (2, 21, 28).

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