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Relationship between glycerol-3-phosphate dehydrogenase, fatty acid synthase and fatty acid binding proteins in developing human placenta

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Abstract. The activities of the enzymes glycerol-3-phosphate dehydrogenase and fatty acid synthase are inhibited by palmitoyl-coenzyme A and oleate. The two isoforms of fatty acid binding proteins (PI 6·9 and PI 5·4) enhance the activities of glycerol-3-phosphate dehydrogenase and fatty acid synthase in the absence of palmitoyl-coenzyme A or oleate and also protect them against palmitoyl-coenzyme A or oleate inhibition. Levels of fatty acid binding proteins, the activities of the enzymes fatty acid synthase and glycerol-3-phosphate dehydrogenase increase with gestation showing a peak at term. However, the activity of fatty acid synthase showed the same trend up to the 30th week of gestation and then declined slightly at term. With the advancement of pregnancy when more lipids are required for the developing placenta, fatty acid binding proteins supply more fatty acids and glycerol-3-phosphate for the synthesis of lipids. Thus a correlation exists between glycerol-3-phosphate dehydrogenase, fatty acid synthase and fatty acid binding proteins in developing human placenta.

Keywords. Glycerol-3-phosphate dehydrogenase; fatty acid nthase; ontogeny; placenta.

1. Introduction

The feto-placental unit requires a large amount of energy for its development in the mother's womb. Carbohydrates and lipids serve as the major energy source during its intrauterine development. There is a positive correlation between lipid synthesis and the activities of synthesizing enzymes in animal tissues, whereas an inverse con-elation exists between the concentration of long chain fatty acyl-coenzyme A (CoA) compounds and *de novo* fatty acid synthesis. It suggests that fatty acyl-CoA acts as a feed back inhibitor of fatty acid synthesis (Tubbs and Garland 1964; Goodridge 1968, 1973). The rate of glucose catabolism in the liver also affects the fatty acyl-CoA concentration *via* the ability of α-glycerophosphate to esterify fatty acyl-CoA to triglyceride (Szabo *et al* 1963). It is known that palmitoyl-CoA (Pal-CoA) inhibits, and in some cases inactivates numerous and functionally diverse enzymes (Kawaguchi and Bloch 1974; Glatz and Veerkamp 1985) which are involved directly or indirectly in lipid metabolism. According to the literature (Srere 1965; Pandey and Mead 1968; Dorsey and Porter 1968) inhibition of the enzyme activity by Pal-CoA is a non-specific detergent effect.

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Abbreviations used: Pal-CoA, palmitoyl-coenzyme A; FABPs, fatty acid binding proteins; Gly3PDH, glycerol-3-phosphate dehydrogenase; FAS, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase.

During embryogenesis, rapid cell differentiation and organelle formation take place both of which require lipids. Fatty acids requires for the production of lipids are synthesized *de novo* or taken up from the circulation (Beaconsfield and Ginsburg 1979). Whatever the route through which fatty acids are supplied for the synthesis of lipids, they must be activated to their CoA form. It has been found out that a low molecular weight (14–15 kDa) cytosolic fatty acid binding protein (FABP) is a good candidate for the acylation of long chain fatty acids to their CoA thioesters (McCormack and Brecher 1987; Cistola *et al* 1988). FABPs bind appreciable amounts fatty acids and their CoA esters and thus create a readily available intracellular pool of these substances (Spener and Mukherjea 1990; Kaikaus *et al* 1990). FABPs also regulate the concentrations of fatty acids and acyl-CoA as substrate, modulator or inhibitor of certain enzymes (Sweetser *et al* 1987; Spener *et al* 1989; Veerkamp *et al* 1991) and their transport system (Barbour and Chan 1979). Thus these proteins have important functions in modulating total cellular metabolism.

It is known that triacyl glycerols are actively synthesized from glycerol-3-phosphate and fatty acyl-CoAs. Glycerol-3-phosphate is derived from dihydroxy acetone phosphate (the product of the aldolase reaction of glycolysis), by the NAD-Iinked glyeerol-3-phosphate dehydrogenase (Gly3PDH) of the cytosol. The enzyme which synthesizes fatty acids *de novo* i.e., fatty acid synthase (FAS) as well as the enzymes which furnishes NADPH and acetyl-CoA for fatty acid biosynthes is glucose-6-phosphate dehydrogenase (G6PD) and ATP citrate lyase (respectively), are inhibited by long chain fatty acids and their CoA thioesters (Kawaguchi and Bloch 1974; Tubbs and Garland 1964). Our laboratory has already reported that FABPs protect G6PD and 6-phosphogluconate dehydrogenase from the detrimental effects of fatty acids and fatty acyl-CoA esters in human placenta (Das *et al* 1988; Bandyopadhyay and Mukherjea 1993) and fetal lung (Sa *et al* 1989).

In this paper we have reported the FABP patterns, the changes in activities of Gly3PDH and FAS and their interrelationship during human embryogenesis.

2. Materials and methods

2.1 *Chemicals*

Acetyl-CoA, malonyl-CoA, NADH, bovine serum albumin (BSA), DEAE-cellulose, oleic acid, Pal-CoA, ATP, sephacryl S-200 and dihydroxy acetone phosphate were purchased from Sigma Chemical Co., St. Louis, Mo, USA. All the other chemicals used were of analytical grade and were purchased from local dealers.

2.2 *Clinical materials*

Human placentas of gestational ages between 5–30 weeks were collected from patients undergoing legal abortion either by suction or *via* hysterotomy from the Department of Obstetrics and Gynecology, National Medical College and Hospital, Calcutta. Placentas above 30 weeks were obtained from different Nursing Homes and medical termination of pregnancy clinics in and around Calcutta. Tissues were collected within 15 min of operation/delivery and kept in ice. Gestational ages were calculated from the period of amenorrhea and by crown-rump length of the fetus (Choudhuri *et al* 1982).

2.3 *Preparation of FABPs*

FABPs from human placental cytosol was purified according to the method of Das *et al* (1988) using sephacryl S-200 gel filtration and DEAE-cellulose chromatography. Two isoforms of human placental FABPs (PI 6·9 and PI 5·4) were obtained. The proteins were routinely characterized by UV spectroscopy and by SDS-PAGE to ensure single band of about 14,000 molecular weight and were stored at 0–4°C as lyophilized powder.

2.4 *Preparation and assay of FAS and Gly3PDH*

Human placenta of different gestational ages were dissected, cut into small pieces and homogenized in all glass Potter-Eivehjem homogenizer in 0.25 M sucrose to get 20% homogenate for assay of both the enzymes. The homogenates were spun at 105,000 *g* for 1 h. Fatty acid synthase and GIy3PDH activities were assayed from the soluble supernatant spectrophotometrically in a Hitachi Spectrophotometer Model U3210 following the decrease in absorbance at 340 *nm* according to the method of Smith and Abraham (1975) and White (1975) respectively.

2.5 *Effect of Pal-CoA, oleate and FABPs on FAS and Gly3PDH*

Inhibition of the enzymes were studied in the reaction mixtures in presence of different concentrations of Pal-CoA or oleate. The isoforms of FABPs (PI 6·9 and PI 5·4) were added separately in the reaction mixture in presence or absence of the inhibitors.

2.6 *Estimation of protein*

Protein was estimated according to the method of Lowry *et al* (1951) using BSA as standard.

2.7 *Statistical analysis*

Data were tested statistically using the student's *'t'* test. The variability of the data was presented as mean ± SEM. In order to study the extent of correlation between any two of the relevant variables involved, correlation coefficient were computed. Differences at $P < 0{\cdot}05$ were considered to be significant.

3. Results

3.1 *Development of FAS, Gly3PDH and FABPs in human placenta*

The presence of FAS activity was found in developing human placenta throughout the gestation of 5–40 weeks. The enzyme activity increased with the advancement of pregnancy up to 25–30 weeks and then decreased slightly at term (table 1).

Table 1 also indicates that Gly3PDH activity is discernible in human placenta throughout the gestation. A significant increase occurs at 25–30 weeks of gestation and then the rate of increase slows down up to term.

Table 1. Ontogenic profile of Gly3PDH and FAS in developing human placenta.

Group	Gestational ages (weeks)	Gly3PDH (nmol of NADH oxidized/ min/mg protein)	FAS (nmol of Pal-CoA produced/ min/mg protein)
I	5–10	3·95 ± 0·85	1·01 ± 0·18
II	10–15	5·06 ± 0·81	1·72 ± 0·22
III	15–20	8·18 ± 0·76	2·20 ± 0·38
IV	20–25	11·69 ± 1·01	3·19 ± 0·47
V	25–30	16·58 ± 1·98	5·18 ± 0·50
VI	30–35	17·02 ± 0·78	4·46 ± 0·28
VII	35–40	17·81 ± 1·08	3·78 ± 0·31
Values are mean ± SEM of 3 sets of experiments in each case			
Group II vs I		-	$P < 0·05$
Group II vs II		$P < 0·05$	-
Group IV vs III		$P < 0·05$	-
Group V vs IV		$P < 0·01$	$P < 0·05$
Group VI vs V		-	-
Group VI vs VI		-	-

It has been reported earlier (Das *et al* 1988) that human placental FABPs resolve into two forms PI 6·9 and PI 5·4 having the same molecular weight (14,000) but different isoelectric points and ligand binding affinities. Out of the two forms concentration of PI 6·9 remains maximum throughout the gestation while PI 5·4 was minimum (figure 1). Concentrations of these two proteins increased gradually from 5 weeks up to term.

3.2 *Correlation coefficient between the ontogenic profiles of FABPs, Gly3PDH and FAS*

A positive correlation was observed between the developmental patterns of FABPs, Gly3PDH and FAS in developing human placental tissue.

P values $< 0·001$ (Gly3PDH vs FAS, Gly3PDH vs FABPs)
$< 0·01$ (FABPs vs FAS).

3.3 *Role of Pal-CoA, oleate and FABPs on Gly3PDH activity*

Figure 2 indicates that the enzyme Gly3PDH is inhibited by Pal-CoA (figure 2A curve 'a') and oleate (figure 2B curve 'a'). Nearly 150 μM Pal-CoA or 200 μM oleate is sufficient to inhibit the enzyme completely, but 35 μM Pal-CoA or 45 μM oleate is required for 50% inhibition of the enzyme. FABPs PI 6·9 and PI 5·4 fractions protect the enzyme Gly3PDH against such inhibition (figure 2A curves 'b' and 'c' figure 2B curves 'b' and 'c' respectively). PI 6·9 protein is more effective than PI 5·4 in both the cases to reverse the inhibition created by the inhibitors.

Results of figure 3 indicate that with the increment of concentration of FABPs (PI 6·9 and PI 5·4) there is a parallel increase in the activity of the enzyme in presence (curves 'b' and 'a') or absence (curve 'c') of the inhibitor Pal-CoA.

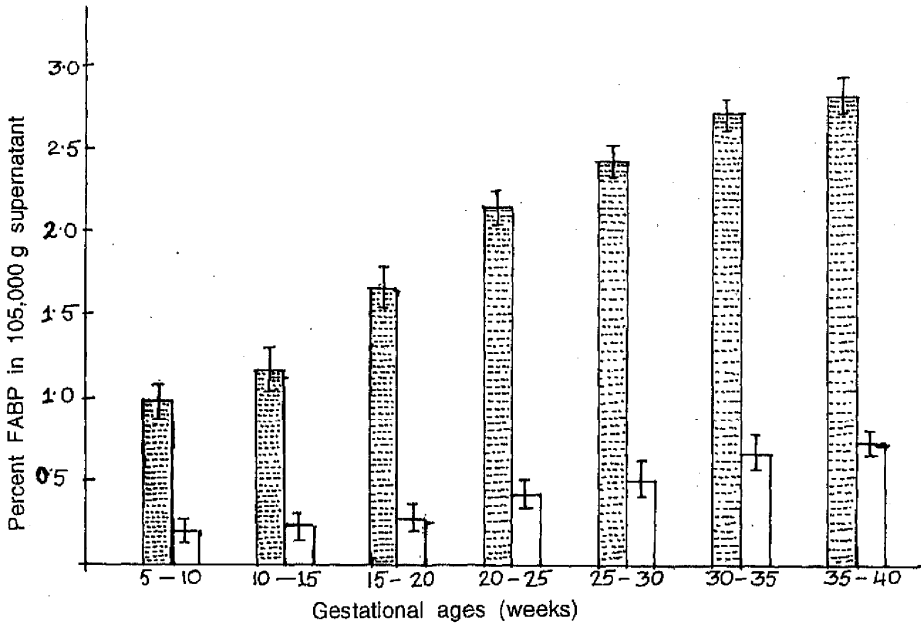

Figure 1. Ontogenic profiles of FABP PI 6·9 (▩) and PI 5·4 (□) of developing human placenta.

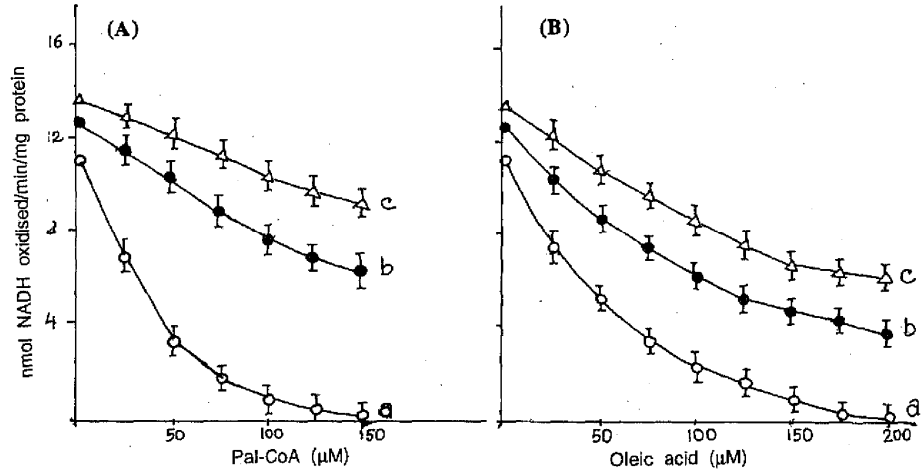

Figure 2. Role of FABPs in regulating inhibition of human placental Gly3PDH by Pal-CoA (**A**)/oleate **(B).** The enzyme activity was measured in the standard assay system in presence of increasing concentrations of Pal-CoA/oleate with fixed concentration (25 μg/ml) of PI 6·9 (Δ) and PI 5·4 (●) fractions of FABP or without FABP (O). Each point is the mean ± SEM of triplicate experiments.

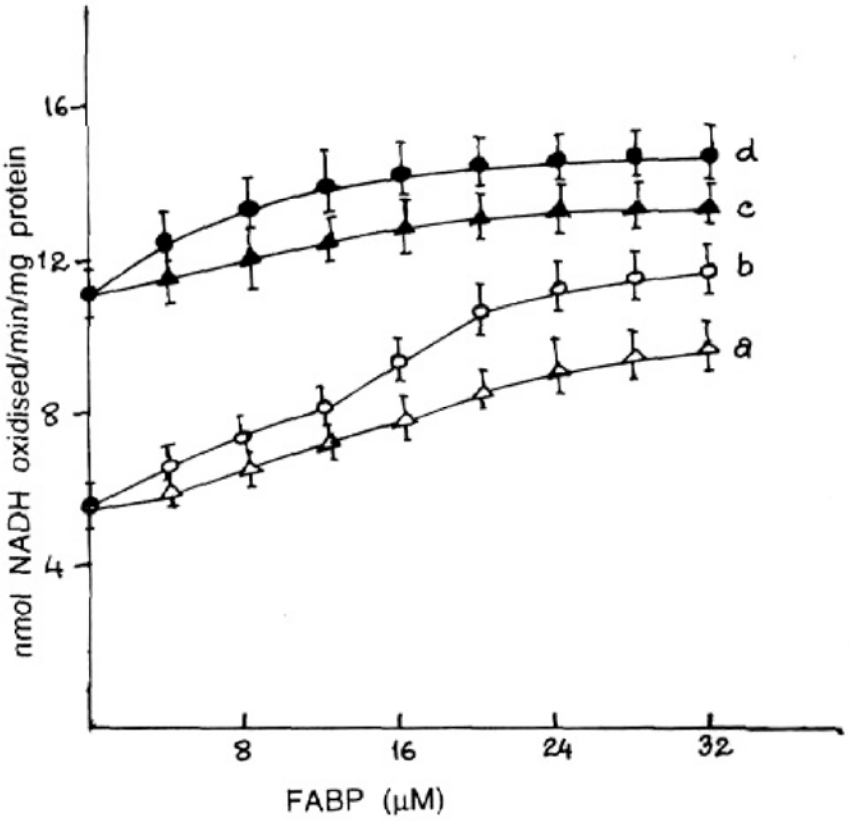

Figure 3. Effect of varying concentration of FAHPs on human placental G1y3PDH activity. The enzyme was assayed in the standard assay system in presence of increasing concentrations of FABP PI 6·9 (O) or PI 5·4 (Δ) with 30 μM Pal-CoA and FABP PI 6·9 (●) or PI 5·4 (▲) without Pal-CoA. Each point is mean ± SEM of triplicate experiments.

3.4 *Effect of FABPs on the inhibited FAS activity*

Figure 4 shows that Pal-CoA and oleate inhibit FAS activity. Only 33% enzyme activity was found by 25 μM of Pal-CoA or 48% by oleate. Progressive increase in the concentrations of PI 6·9 or PI 5·4 in these inhibited system caused parallel increase in the enzyme activity. PI 6·9 protein has been found to be more effective activator than PI 5·4 and Pal-CoA appears to be more potent inhibitor than oleate

4. Discussion

In the placenta, as in other tissues, triglyceride synthesis depends on continued provision of glycerophosphate which in turn depends on glycolysis and on fatty acids supplied either from the maternal circulation (Szabo *et al* 1963; Roux and Myers 1974) or produced as the result of *de novo* placental synthesis (Kleine 1967). The present study shows that the glycerophosphate synthesizing enzyme Gly3PDH activity was found to be closely related to FAS and FABPs. There is an increase

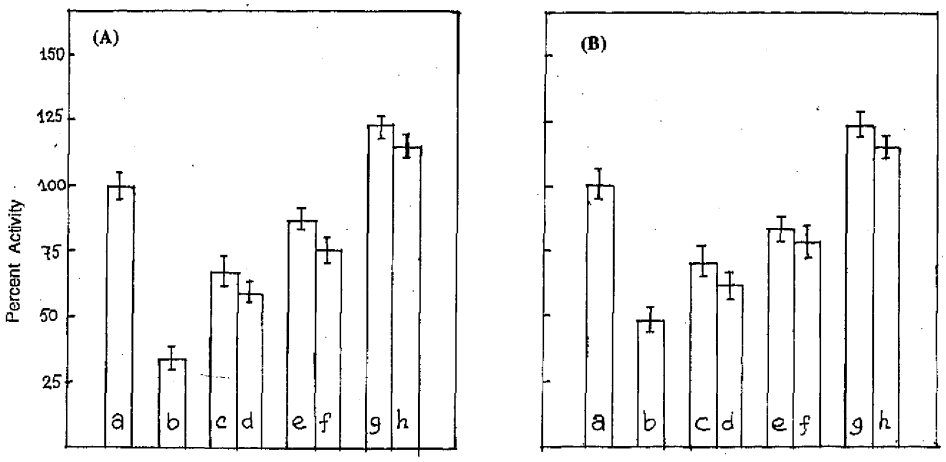

Figure 4. Inhibition of human placental FAS by Pal-CoA (**A**)/oleate (**B**), The enzyme was assayed in the standard assay system in presence of fixed concentration (25 μM) of the inhibitor. Effect of varying concentration of FABPs on the enzyme FAS has been shown. (a), 0 μM Pal-CoA/oleate + 0 μg/ml FABP; (b), 25 μM Pal-CoA/oleate + 0 μg/ml FABP; (c), 25 μM Pal-CoA/oleate + 15 μg/ml FABP PI 6·9; (d), 25 μM Pal- CoA/oleate + 15 μg/ml-FABP PI 5·4; (e), 25 μM Pal-CoA/oleate + 25 μg/ml FABP PI 6·9; (f), 25 μM Pal-CoA/oleate + 25 μg/ml FABP PI 5·4; (g), 0 μM Pal-CoA/oleate + 25 μg/ml FABP PI 6·9; (h), 0 μM Pal-CoA/oleate + 25 μg/ml FABP PI 5·4.

in activities of the enzymes Gly3PDH and FAS with parallel increase in FABP fractions with advancement of pregnancy. PI 6·9 and PI 5·4 fractions of FABP transport long chain fatty acids (Takahashi *et al* 1983) and increase the synthesis of fatty acid and glycerophosphate in meeting the increasing demand for these substances to synthesize lipids by the developing placenta. It is known that the overall rate of placental fat synthesis increases at term (Roux and Green 1968) in spite of lower placental pentose shunt activity (Beaconsfield *et al* 1965) and lower NADPH levels (Villee and Hagerman 1961) at this stage.

The present study indicates that the activities of the enzymes Gly3PDH and FAS are inhibited by long chain fatty acids and their CoA esters. Such inhibitions have been proposed as a possible regulatory mechanism for lipogenesis and ketogenesis (Tubbs and Garland 1964). Since Pal-CoA is an end product of FAS (Flick and Bloch 1975), its action might be classified as negative feed back inhibition. Pal-CoA has the novel feature of causing inhibition by disrupting the quaternary structure of FAS rather than by changing an active to inactive oligomeric conformation (Vance *et al* 1973). The action of Pal-CoA on the native enzyme structure of Gly3PDH is not clear yet. A proposed theory (Kawaguchi and Bloch 1974) is that the change of active to inactive oligomeric conformation of the enzyme occurs by binding of Pal-CoA and oleate. When FABP is added to the enzyme inhibited system, the activity of the enzyme is regenerated indicating the binding of these inhibitors by FABP.

It is supported by the fact that displacement of Pal-CoA from the inactive enzyme protein by a reagent like BSA generates the active oligomeric structure (Knoche *et al* 1973). According to Lunger *et al* (1977) FABPs are the major cytosolic

binder of the inhibitors and the effect may be specific in intact cells. FABPs alone activate the activities of FAS and Gly3PDH suggesting that previous submaximal activities were due to the effect of endogenous long chain acyl CoA esters present in the enzyme preparation. FABP PI 6·9 protein is a more potent activator than PI 5·4, which may be due to different affinities of these proteins towards inhibitors

Thus it seems likely that cytosolic FABP may have diverse roles in cellular function and metabolism. It can also be concluded that there is a correlation between the activities of Gly3PDH, FAS and FABPs in human placenta.

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