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FATTY ACID SYNTHESIS IN FETAL LUNG

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Summary - De novo fatty acid synthesis in lung is significant during fetal growth and development. Specific activity and relative rate of synthesis of fatty acid synthetase increase with the days of gestational age and drop significantly after birth. Fetal lungs contain thyroid hormone receptors and binding capacities of this hormone to the fetal lungs also increase with the days of gestational age. Our results suggest that de novo fatty acid synthesis in fetal lungs may make a significant contribution towards surfactant synthesis.

De novo fatty acid synthesis has been shown to be an important source of fatty acids for the production of pulmonary surfactant (10,12, 15-16). Lung surfactant is a complex mixture of lipids and proteins in which dipalmitoyl phosphatidyl choline (DPL) comprises over 50% (w/w) of the total surfactant lipids (5). The newborns suffering from respiratory distress syndrome appear to suffer from deficiency of DPL (7). Contribution of de novo fatty acid synthesis for the production of surfactant lipids in fetal lungs would, therefore, be of considerable interest.

Glucocorticoids have been found to accelerate fetal lung maturation as it pertains to the synthesis and secretion of pulmonary surfactant in animals (6,8,9). Recently several workers reported similar stimulatory effect of thyroid hormone on fetal rabbit lung development (3,11). Thyroid hormone is also known to activate synthesis of fatty acids in lungs of diabetic and hypophysectomized rats (12-14). Triiodothyronine (T_3) has been found to be effective in stimulating fatty acid synthesizing enzymes in normal rat lungs (17). Recently, Morishige and Guernsey demonstrated the presence of T_3 receptor in rat lungs (4). The stimulatory effect of

*Abbreviations: T_3, Triiodothyronine; DPL, Dipalmitoyl phosphatidyl choline; MBC, maximum binding capacity; KRB, Krebs Ringer bicarbonate buffer.

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thyroid hormone on the fatty acid synthesis may be initiated through the binding of T_3 to specific nuclear receptors.

In the present study we attempted to quantify the rate of synthesis of fatty acid synthesizing enzymes in fetal lungs and livers of various gestational ages. Specific activities of fatty acid synthetase were also evaluated. We were able to demonstrate the binding of T_3 by nuclei from lungs and livers of rabbit fetuses of various gestational ages. A definite correlation was found between relative rate of synthesis of fatty acid synthesizing enzymes and binding capacities of (^{125}I) T_3 with the gestational ages. These results might help elucidate the mechanism of thyroid influence on surfactant production and morphological development of fetal lungs. A preliminary report of these findings was presented in the form of an abstract (26).

MATERIALS AND METHODS

Animals: Adult female and time-dated pregnant New Zealand white rabbits were purchased from Marland Breeding Farms, New Jersey. Rabbits were killed by i.v. injection of about 5 ml sodium pentobarbital (60 mg/ml) after fetuses were obtained by uterotomy. Fetuses were killed immediately by decapitation and fetal lungs and livers were quickly removed and kept in ice-cold Krebs Ringer bicarbonate buffer, pH 7.4.

Chemicals: Acetyl CoA, malonyl CoA, NADPH, fat-free bovine serum albumin, dithiothreitol and L-triiodothyronine were purchased from Sigma Chemical Company, St. Louis, Missouri. L-(^{125}I) T_3 and (4,5,^{3}H) leucine were purchased from New England Nuclear Co., Boston, Massachusetts. All other chemicals were reagent grade and were obtained from commercial suppliers.

Methods: Preparation and fractionation of liver and lung homogenates. Lungs were dissected free of trachea and major bronchi and then homogenized in 3 volumes (w/v) of Krebs Ringer bicarbonate buffer using a hand held homogenizer. Livers were similarly homogenized. High speed (105,000xg) supernatants of lungs and livers were prepared as described elsewhere (18). The supernatants were dialyzed for 2 hours at 4C against 100 volumes of 0.1M phosphate buffer, pH 7.0 containing 1 mM EDTA and 0.5 mM dithiothreitol.

Preparation of nuclei: Nuclei from lungs and livers were isolated according to Samuels et al (19). Krebs Ringer bicarbonate homogenates were filtered through a double layer cheese cloth and then centrifuged at 800xg for 15 minutes. The pellet was resuspended in 1 ml STM buffer and layered over 5 ml of 2.4 M sucrose and centrifuged at 40,000xg for 1 hour. The 40,000xg pellet was washed twice with STM containing 0.5% Triton X-100, washed two more times with STM buffer and finally suspended in STM buffer containing 1 mM EDTA and 5 mM dithiothreitol. This procedure yielded whole nuclei free of cytoplasmic contamination as judged by examining trypan blue stained preparations by light microscopy.

Assay for fatty acid synthetase activity: Fatty acid synthetase was assayed as described before (12). Assay mixture contained: 100 uM malonyl CoA, 30 uM acetyl CoA 100 uM NADPH, 0.2 M potassium phosphate, pH 7.0 and 1 mM EDTA, pH 7.0. Reaction was carried out at 30 C in a Beckman Spectrophotometer using 340 mM wave length. Reaction was initiated by the addition of supernatant protein (100 ug/ml) for liver and 400 ug/ml for lung). The specific activity was expressed in units (nmoles of NADPH oxidized/min/mg protein).

Relative rate of fatty acid synthesis: The differences in rates of synthesis of lung and liver fatty acid synthetase enzymes were determined by using pulse-labeling technique using (4,5,^{3}H) leucine as described by Kumar et al (12). Fatty acid synthetase from lung and liver was purified according to Burton et al (21) and anti-fatty acid synthetase γ-globulin was prepared and purified as described by Volpe and Vagelos (20). The radioactivity per mg of the purified enzyme was estimated in antigen-antibody complex as described before (12).

Nuclear binding: Nuclear binding capacity was measured according to Silva et al (22). Nonspecific binding was estimated by the use of 500-fold excess unlabeled L-T_3. Incubations were carried out at 30 C for 3 hours and then incubates were centrifuged at 1500 x g for 10 minutes at 4 C. The pellets were washed twice with Triton-STM and finally with STM before measuring the radioactivity by a spectrometer. Binding parameters were assessed by Scatchard analysis of dose-response experiments using duplicate determinations (23).

Protein and DNA estimation: Protein was determined by the method of Lowry et al using fat-free bovine serum albumin as standard (24). DNA was estimated according to Wannemacher et al (25).

Statistical analysis: Statistical comparisons used regression analysis, correlation coefficient and Student's t-test.

RESULTS

Specific activities of fatty acid synthetase: Fatty acid synthetase enzyme activities were determined in 105,000 x g supernatant fractions of fetal lungs and livers. Synthetase activities were compared between lungs and livers of fetuses of different gestational ages. Fatty acid synthetase activity was detected in fetal lung of 20-day gestational age. Enzyme activities were found to be increasing with the increasing days of gestational ages (Table I). Synthetase activity dropped significantly after birth and dropped further after one month. Further change of activities did not occur during one month to three months of neonatal ages. Hepatic fatty acid synthetase activity, on the other hand, remained constand during different gestational ages and did not change appreciably

TABLE 1

SPECIFIC ACTIVITIES OF PULMONARY AND HEPATIC FATTY ACID SYNTHETASE ENZYMES OF FETAL AND ADULT RABBITS

GESTATIONAL AGE (DAYS)	SPECIFIC ACTIVITY OF FATTY ACID SYNTHETASE (UNITS) LUNG	LIVER
20	5.2 ± 0.5	15.0 ± 2.5
22	6.0 ± 0.7	14.6 ± 1.9
24	6.8 ± 0.2	14.7 ± 2.7
26	6.6 ± 0.4	15.3 ± 1.8
28	7.4 ± 0.8	16.1 ± 4.0
30	7.1 ± 1.0	15.8 ± 1.3
NEW BORN	5.2 ± 0.7	17.0 ± 2.2
ONE MONTH	4.9 ± 0.6	16.8 ± 3.1
THREE MONTHS	4.8 ± 0.5	19.5 ± 2.6

after birth (Table I). Although specific activities of fatty acid synthetase enzymes are much lower in fetal lungs (about 30%) compared to those in livers, variation of synthetase activities in fetal lungs suggests the significance of fatty acid synthesis in this organ during growth and development.

Relative rates of fatty acid synthetase synthesis: It was of interest to know if increase in specific activities of fatty acid synthetase was due to increased rate of synthesis of this enzyme. Relative rate of synthesis of fatty acid synthetase was, therefore, determined in lungs and livers of fetal and neonatal rabbits. Once again, liver showed no variation in rate of synthesis during the fetal and neonatal growth. To the contrary, relative rates of fatty acid synthetase synthesis in lungs increased with the increasing days of gestational ages and dropped significantly after birth. The rate of synthesis dropped further after one

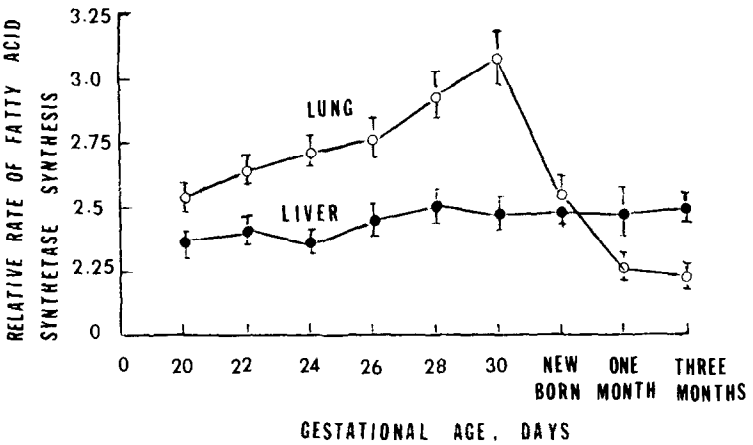

FIG. 1. Comparison of relative rates of fatty acid synthetase synthesis in lungs (o - o) and livers (● - ●) of rabbit fetus. Pregnant rabbits were anesthetized with lidocaine. The uterus was opened by midline abdominal incision under semisterile operating conditions. Each fetus in each litter was given 20 uCi of (4,5,^{3}H)- leucine. Continuous suture was used to close abdominal incisions and rabbits were allowed to recover. After 2 hours, the abdomens were reopened under anesthesia and fetuses were delivered by hysterotomy, and sacrificed immediately by decapitation. 105,000 x g supernatants were prepared and counts per minute per mg. of protein determined as described under methods. Total counts per minute in fatty acid synthetase were determined from antibody precipitate per mg of purified protein multiplied by the total protein. Values are means ± SEM.

month but remained constant during one to three months period (Figure 1). Thus the increase in specific activity parallels increase in relative rate of synthesis of fatty acid synthetase during fetal and neonatal ages indicating that increase in specific activities during fetal growth might be due to accelerated rate of synthesis of this enzyme.

Nuclear uptake of (^{125}I) T_3: Since thyroid hormones are known to influence fatty acid synthesis and metabolism involving stimulation of both RNA and protein synthesis, binding of this hormone to specific nuclear receptors seems to be a logical step in this mechanism. Figure 2 shows the variation of nuclear uptake of (^{125}I) T_3 in fetal lungs and livers with gestational ages. Uptake of T_3 remained constant during early gestational ages, but increased after 26 day of gestation (Figure 2). Pulmonary uptake of T_3 in liver remained fairly constant during fetal and neonatal ages.

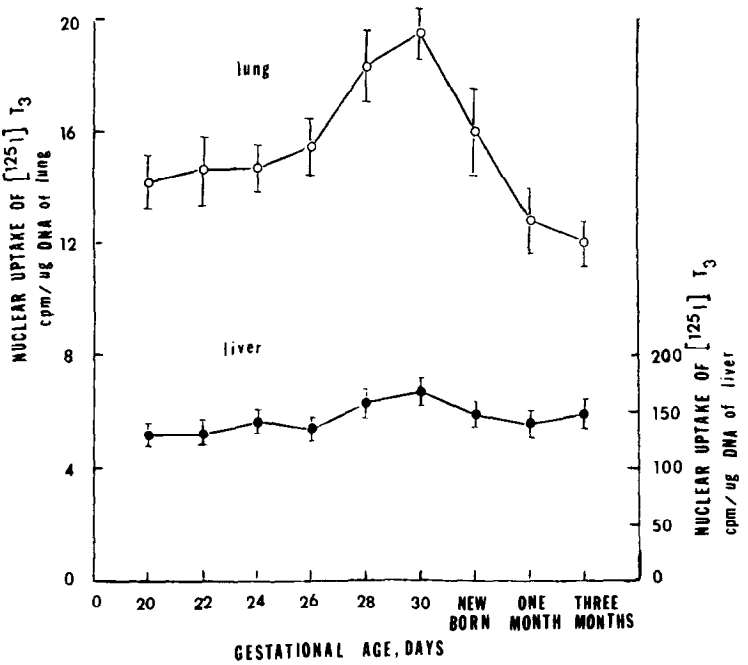

FIG. 2. Comparison of nuclear uptake of (^{125}I) T_3 by lungs (o - o) and livers (● - ●) of rabbit fetus. Nuclei from fetal lungs and livers were prepared, purified and then incubated with radiolabeled T_3 at 30 C for 3 hours as described in methods. Values are means ± SEM.

Binding of (^{125}I) T_3 with the nuclear portions of lungs and livers were assessed by Scatchard analysis as described before and results were compared for various gestational and neonatal ages. Maximum binding capacities for fetal lungs increased with the increasing days of gestational ages and dropped after birth (Figure 3). Once again fetal livers did not exhibit any significant change in maximum binding capacities with (^{125}I) T_3.

Statistical analysis: Statistical analysis was performed to determine the relation between relative rate of lung fatty acid synthetase synthesis (or specific activities of pulmonary fatty acid synthetase) and maximum binding capacity of (^{125}I) T_3 for lung during growth and development. An excellent correlation coefficient was found ($p<0.01$) demonstrating a possible relationship between fatty acid synthesis and thyroid hormone action.

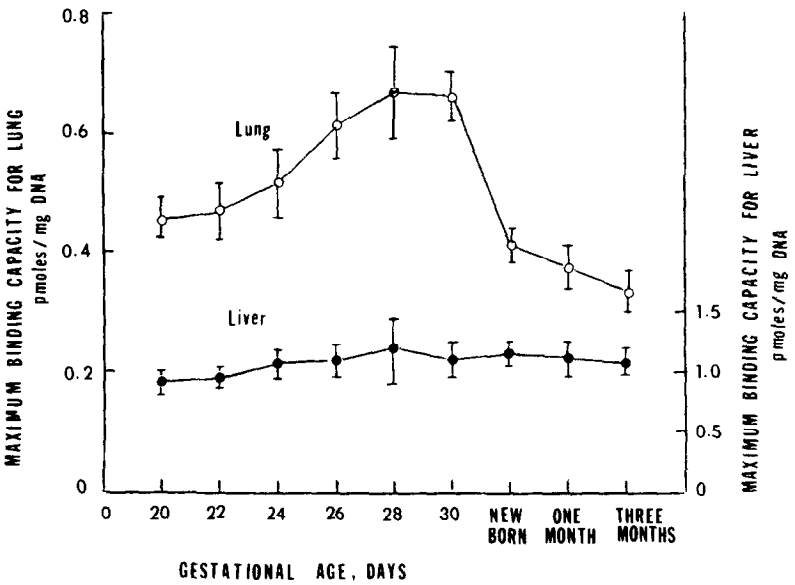

FIG. 3. Comparison of maximum binding capacity of (^{125}I) T_3 for lungs (o - o) and livers (● - ●) of rabbit fetus. Nuclei from lungs and livers of rabbit fetus were prepared, purified and incubated with radiolabeled T_3 as described in methods. Binding parameters were assessed by Scatchard analysis of doseresponse experiments. Values are means ± SEM.

DISCUSSION

The principal objective of this study was to demonstrate the contribution of de novo fatty acid synthesis towards phospholipid production during growth and development of fetal lungs. Results of our experiments clearly indicated that specific activities of fatty acid synthesizing enzymes increased with the increasing day of gestational ages. Moreover, relative rate of synthesis of pulmonary fatty acid synthetase also increases with the advancement of gestational age. The fatty acids synthesized de novo in the fetal lungs, thus, might make a significant contribution towards the surfactant production.

A critical question still remains unanswered as to what stimulus is responsible for de novo synthesis of fatty acids in fetal lung. Numerous investigators have suggested different stimuli of fetal origin for phospho lipid biosynthesis. Fetal cortisol is known to be the most important of

these stimuli (1,6). Prolactin is also known to accelerate phosphatidylcholine biosynthesis in fetal lungs (2). More recently thyroid hormones have been found to play an important role in surfactant synthesis (3). Moreover, specific receptors of triiodothyronine have been found in the lungs (4). Triiodothyronine has also been reported to stimulate fatty acid synthesis in the lungs of diabetic and hypophysectomized animals (12-14). Based on these analogies, we attempted to investigate the possible role of fetal thyroid hormone for the stimulation of fatty acid synthesis in fetal lungs. If this were true, one would expect at least a temporal relationship between the binding capacities of T_3 receptors in fetal lungs and the relative rate of fatty acid synthesis. Indeed we were able to find such a relationship between T_3 receptors and de novo fatty acid synthesis in fetal lungs. Nuclear uptake of (^{125}I) T_3 and maximum binding capacities of (^{125}I) T_3 for lung were found to be increased with the increasing days of gestational ages. Specific activities of fatty acid synthetase and more importantly, relative rate of synthesis of fatty acid synthetase followed a similar pattern. An excellent correlation has been found from statistical analysis suggesting that de novo fatty acid synthesis in fetal lungs might be stimulated through some molecular events involving fetal thyroid hormones. In this study we were unable to find such a relationship in fetal livers.

The present results also suggest the importance of de novo fatty acid synthesis during fetal growth and development. Relative rate of synthesis of fatty acid synthetase dropped significantly after birth and remained fairly constant up to a period of three months of neonatal age. The relatively higher activity of pulmonary fatty acid synthetase synthesis during the later part of fetal life probably suggests the contribution of de novo fatty acid synthesis towards surfactant production, because increased quantity of surfactant is usually found to be present during that time. Frequency of occurrance of respiratory distress syndrome is higher among the babies born from diabetic or hypophy-

sectomized mothers whose fatty acid synthesizing systems are significantly impaired. Although there is no evidence that depression of fatty acid synthesis would mean deprivation of surfactant production, it is conceivable that surfactant formation might be lower if there is a defect in fatty acid synthesizing systems.

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