Indian J Physiol Pharmacol 1993; 37(3) : 176-182

ALTERED MATERNAL THYROID FUNCTION : FETAL AND NEONATAL HEART CHOLESTEROL AND PHOSPHOLIPIDS

RATAN KUMAR* AND B. N. CHAUDHURI

Defence Institute of Physiology and Allied Science, Delhi Cantt - 110 010

(Received on October 1, 1992)

Abstract : The influence of maternal thyroid function on the fetal and neonatal myocardial cholesterol and phospholipid content was studied in rats. Fetuses born to hyperthyroid mothers had decreased total cholesterol and increased esterified cholesterol while offsprings born to hypothyroid mothers had increased total, free and esterified cholesterol during late gestation and/or at term. Phospholipid fractions phosphatidyl choline and phosphatidyl ethanolamine in offsprings born to hyperthyroid mothers were not significantly changed. offsprings born to hypothyroid mothers had decreased total phospholipids, phosphatidyl choline and phosphatidyl ethanolamine at fetal and neonatal stages. ^{3}H-acetate incorporation in phosphatidyl choline and phosphatidyl ethanolamine was also decreased. Maternal thyroid seems to have important role in the regulation of cholesterol and phospholipid metabolism in fetal and neonatal hearts.

Key words : maternal thyroid fetal neonatal heart cholesterol phospholipids

INTRODUCTION

During early developmental period, particularly during fetal development, there is a marked need for sterols. As body mass increases exponentially, cholesterol is required for membrane synthesis, for its maintenance and for synthesis of hormones and bile acids (1). In cardiac muscle phospholipids are needed to participate in complex biological processes (2). In our previous studies (3-4) we observed that maternal thyroid status played important role in the fetal and neonatal growth and in myocardial metabolism. However, no report is available on fetal and neonatal myocardial cholesterol and phospholipids content in relation to the maternal thyroid status. This study examines fetal and neonatal heart cholesterol and phospholipid contents in relatioin to altered maternal thyroid status.

METHODS

Colony bred Sprague Dawley female rats weighing 150±10 g were used. Animals had free access to drinking water and food in the pellet form (Hindustan Lever Ltd., Bombay). Animals were kept at 24°C±1°C. They were divided into three groups. Group I rats served as controls (without any treatment). Group II rats were made hypothyroid by intraperitoneal (ip) injection of 1 mci I^{131} obtained from Bhabha Atomic Research Center (BARC), Bombay. They were kept separate for 6-8 weeks before their use for mating. Group III rats were made hyperthyroid by ip injection of 70 µgm of L-thyroxine, obtained from M/s Sigma Chemical Co. Ltd., USA, per rat per day. Thyroxine injections were started two weeks prior to the use of rats for the purpose of mating and continued during pregnancy and lactation.

The untreated, hypothyroid and hyperthyroid groups of rats were mated with normal male rats of same strain and age after periods stated above. Estrus cycle of rats was daily monitored by vaginal smear. The sperm positive day of the Vaginal smear was taken as day 01 of pregnancy. Age groups studied were day 19 and 21 in fetal rats and the day of birth (0 day), 7 days, 14 days, 21 days and 28 days after birth in neonates.

*Corresponding Author

Experimental

I. Plasma thyroxine: Plasma thyroxine (T_4) concentration was estimated using radio-immunoassay kits obtained from BARC, Bombay.

II. Incorporation of sodium ^3H acetate into heart cholesterol and phospholipids: For fetal studies mothers were injected ip with a sterile solution of sodium- ^3H-acetate (BARC, Bombay) at a dose of 25 µci/100 g body weight on the 19 and 21 day of pregnancy. One hr after injection of the radiotracer, mothers were subjected to nembutal anesthesia (40 mg/kg body weight, ip) and laporotomy carried out. Fetuses were removed and viable hearts were dissected out. For neonatal studies, newborns and neonates were ip injected with sodium-^3H-acetate (25 µci/100 g body weight) 1 hr before sacrifice. Neonates were also anesthetized with nembutal for the removal of hearts.

Fetal and neonatal hearts were washed with saline, dried with filter paper, weighed and processed for lipid extraction (5). Free and esterified cholesterol were separated by thin layer chromatography (TLC) by the method of Misra (6). Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) were separated by TLC using the method of Abramson and Blecher (7) cholesterol and phospholipid spots were identified with iodine vapours and cholesterol and phospholipids from individual spots were eluted with hexane: ether (1:1 v/v) and chloroform: methanol (2:1 v/v) respectively and evaporated to dryness. The residue was dissolved in known volumes of chloroform. Gel blanks were similarly treated. This solution was divided in two parts. One part was used for chemical analysis of cholesterol (8) and phospholipid phosphorus (9). Suitable aliquots from second part were transferred to scintillation counting vials containing 15 ml toluene based scintillation fluid (4 g PPO and 200 mg POPOP/ liter of toluene). The radioactivity was measured in Beckman LS-7000. Liquid scintillation counter and data were statistically analysed using student 't' test.

RESULTS

Maternal, fetal and neonatal plasma thyroxine: Plasma thyroxine levels in mothers, fetuses and neonates are shown in Table I. Plasma thyroxine levels were significantly high in hyperthyroid mothers at all age groups while the reverse has been observed in hypothyroid mothers (Table I). Plasma thyroxine levels in fetuses and neonates born of hyperthyroid mothers were significantly higher at all age groups studied excepting at 28 days. Plasma thyroxine levels were significantly low in offsprings born of hypothyroid mothers at 21 day fetal age, newborns and 7 days neonatal age groups. Because no offspring born to hypothyroid mother could survive beyond eight days so in this group values beyond 7 days neonatal age are not available.

TABLE I : Plasma thyroxine concentration of mothers and their offsprings during gestational and lactational period.

Group		*Gestation/Fetal age (days)*		*Day of birth*	*Lactational/neonatal age (days)*			
		19	21	0	7	14	21	28
Control	Mother	3.5±0.1	3.3±0.1	3.4±0.2	5.4±0.2	3.9±0.3	5.2±0.2	5.4±0.3
	Offsprings	0.4±0.1	0.5±0.1	0.4±0.1	1.5±0.2	2.3±0.2	2.9±0.3	3.6±0.2
Hyperthyroid	Mother	7.4±0.2 P<0.001	6.3±0.5 P<0.001	5.2±0.4 P<0.01	8.2±0.1 P<0.001	7.9±0.1 P<0.001	8.4±0.2 P<0.001	9.2±0.2 P<0.001
	Offsprings	0.9±0.1 P<0.01	1.0±0.1 P<0.02	1.0±0.1 P<0.001	1.8±0.1 P<0.05	3.2±0.2 P<0.01	4.0±0.1 P<0.01	3.8±0.2
Hypothyroid	Mothers	0.8±0.1 P<0.001	0.7±0.1 P<0.001	0.9±0.1 P<0.001	0.7±0.1 P<0.001	—	—	—
	Offsprings	ND	0.2±0.05 P<0.05	0.2±0.04 P<0.02	0.5±0.1 P<0.001	—	—	—

Values have been expressed as plasma thyroxine µg/dl and all the values are mean of 5 samples ± SEM in each group.
ND = Not detected

Cholesterol :

(i) *Total :* Results in Table II show that in fetuses of hyperthyroid mothers there was a decrease in total cholesterol but in neonates there was increase in total cholesterol at 7 days and 28 days of age. In the fetuses of hypothyroid mothers, there was an increase in total cholesterol during late gestational period and at birth.

(ii) *Free and esterified cholesterol :* Results in Table III and table IV show fetal and neonatal

TABLE II : Total phospholipid and cholesterol content of fetal and neonatal rat heart.

Group		*Fetal age (days)*		*Day of birth*	*Neonatal age (days)*			
		19	21	0	7	14	21	28
Control	Total Phospholipid	50±4	40±4	37±3	43±4	128±7	85±9	80±3
	Total Cholesterol	229±6	133±3	123±5	191±15	292±23	229±23	167±10
Hyperthyroid	Total Phospholipid	38±4 P<0.05	31±2 P<0.05	39±3	42±2	138±5	81±7	75±4
	Total Cholesterol	188±17 P<0.05	105±7 P<0.01	118±5	273±17 P<0.01	285±25	249±12	271±11 P<0.001
Hypothyroid	Total Phospholipid	27±2 P<0.001	29±2 P<0.05	28±5	23±2 P<0.001	—	—	—
	Total Cholesterol	216±20	182±11 P<0.05	184±13 P<0.01	174±10	—	—	—

Values have been expressed as µgm phospholipid phosphorus/100 mg wet weight of tissue for total phospholipid and as µgm cholesterol (total)/100 mg wet weight of tissue.

Values are mean of 5 samples ± SEM in each group.

TABLE III : Free cholesterol (FC) content and incorporation of ^{3}H-acetate in free cholesterol of fetal and neonatal rat heart.

Group		*Fetal age (days)*		*Day of birth*	*Neonatal age (days)*			
		19	21	0	7	14	21	28
Control	FC	64±7	29±2	28±4	50±2	60±5	50±2	45±4
	^{3}H-acetate incorporation in FC	119±12	89±10	87±8	105±10	78±9	65±7	42±5
Hyperthyroid	FC	56±2	32±3	38±2	51±6	53±8	54±4	60±4 P<0.05
	^{3}H-acetate incorporation in FC	135±6	67±8	96±15	48±4 P<0.001	78±7	63±5	56±5
Hypothyroid	FC	80±6 P<0.05	49±9 P<0.05	74±6 P<0.001	71±5 P<0.01	—	—	—
	^{3}H-acetate incorporation in FC	91±10	110±13	100±8	40±5 P<0.001	—	—	—

Values have been expressed as µgm free cholesterol/100 mg wet weight of tissue and as DPM/100 mg wet weight of tissue equivalent free cholesterol for ^{3}H-acetate incorporation. All the values are mean of 5 samples ± SEM in each group.

TABLE IV : Esterified cholesterol (EC) content and incorporation of ^{3}H-acetate in esterified cholesterol of fetal and neonatal rat heart.

Group		*Fetal age (days)*		*Day of birth*	*Neonatal age (days)*			
		19	21	0	7	14	21	28
Control	EC	88±6	47±6	76±6	96±15	162±11	124±6	94±5
	^{3}H-acetate incorporation in EC	174±19	135±13	145±16	178±12	161±14	148±7	156±25
Hyperthyroid	EC	106±5 P<0.05	67±7 P<0.05	68±7	122±8	93±5 P<0.001	138±4	129±7 P<0.01
	^{3}H-acetate incorporation in EC	158± 12	112±10	184±13	187±8	176±14	186±19	192±13
Hypothyroid	EC	97±6	61±5	109±8 P<0.02	101±6	—	—	—
	^{3}H-acetate incorporation in EC	176±20	175±18	158±13	78±7 P<0.001	—	—	—

Values have been expressed as µgm esterified cholesterol/100 mg wet weight of tissue and as DPM/100 mg wet weight of tissue equivalent esterified cholesterol for ^{3}H-acetate incorporation. All the values are mean of 5 samples ± SEM.

myocardial free and esterified cholesterol content and incorporation of ^{3}H-acetate into these fractions. In the offsprings born to hyperthyroid mothers at 7 days neonatal age incorporation of ^{3}H-acetate into free cholesterol was found decreased while in neonates of 28 days age heart free cholesterol increased alongwith unchanged ^{3}H- acetate incorporation into free cholesterol fraction (Table III). Myocardial esterified cholesterol content was found increased during fetal age and 28 days neonatal age but it decreased at 14 days neonatal age. However, there was no change in ^{3}H-acetate incorporation into myocardial esterified cholesterol of offsprings born to hyperthyroid mothers at all age groups studied (Table IV).

Offsprings born to hypothyroid mothers had increased heart free cholesterol content at all the fetal and neonatal age groups studied while ^{3}H-acetate incorporation in myocardial free cholesterol decreased at 7 day neonatal age (Table III). At day of birth offsprings of hypothyroid mothers showed increased esterified cholesterol content while ^{3}H-acetate incorporation in myocardial esterified cholesterol dereased at 7 days neonatal age (Table IV)

Phospholipids :

(i) Total : Results in Table II show that in fetuses born to hyperthyroid mothers total phospholipid of heart decreased. The offsprings born to hypothyroid mothers showed decrease in heart total phospholipid during gestational and at 7 days neonatal age (Table II).

(ii) Phosphatidyl choline and phosphatidyl ethanolamine : Resuits in Table V and Table VI show fetal and neonatal myocardial phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) content and incorporation of ^{3}H-acetate into these phospholipid fractions. Offsprings born to hyperthyroid mothers showed decrease in PC content alongwith increased ^{3}H-acetate incorporation in PC at 14 days neonatal age (Table V). Offsprings born to hypthyroid mothers showed reduced PC content and reduced ^{3}H-acetate incorporation in myocardial PC during fetal and neonatal stages studied (Table V) alongwith reduced PE content and ^{3}H-acetate incorporation in PE at 19 days fetal and 7 days neonatal age (Table VI).

TABLE V: Phosphatidyl choline phosphorus (PC-P) content and incorporation of ^{3}H-acetate in phosphatidyl choline (PC) of fetal and neonatal rat heart.

Group		*Fetal age (days)*		*Day of birth*	*Neonatal age (days)*			
		19	21	0	7	14	21	28
Control	PC-P	16±1	15±1	14±1	19±1	49±5	29±3	33±3
	^{3}H-acetate incorporation in PC	276±19	267±11	257±21	395±26	468±47	295±40	243±18
Hyperthyroid	PC-P	18±1	15±1	13±1	17±2	36±2 P<0.05	34±5	31±2
	^{3}H-acetate incorporation in PC	294±28	226±22	264±16	305±13	688±35 P<0.01	316±18	256±18
Hypothyroid	PC-P	9±1 P<0.001	13±1	11±1 P<0.05	8±1 P<0.001	—	—	—
	^{3}H-acetate incorporation in PC	168±4 P<0.001	290±18	154±21 P<0.01	164±8 P<0.01	—	—	—

Values have been expressed as µgm phosphatidyl choline phosphorus (PC-P)/100 mg wet weight of tissue and as DPM/100mg wet weight of tissue equivalent PC-P for ^{3}H-acetate incorporation. All the values are mean of 5 samples±SEM in each group.

TABLE VI: Phosphatidyl ethanolamine phosphorus (PE-P) content and incorporation of ^{3}H-acetate in phosphatidyl ethanolamine (PE) of fetal and neonatal rat heart.

Group		*Fetal age (days)*		*Day of birth*	*Neonatal age (days)*			
		19	21	0	7	14	21	28
Control	PE-P	10±1	11±1	13±2	15±2	35±1	33±3	25±3
	^{3}H-acetate incorporation in PE	138±7	134±6	142±11	208±13	297±30	266±30	146±19
Hyperthyroid	PE-P	12±1	11±1	12±1	13±2	32±2	30±3	26±2
	^{3}H-acetate incorporation in PE	150±20	121±9	134±12	189±15	364±13	273±12	148±16
Hypothyroid	PE-P	7±1 P<0.01	10±1	11±2	7±1 P<0.01	—	—	—
	^{3}H-acetate incorporation in PE	73±7 P<0.001	139±10	128±20	56±4 P<0.001	—	—	—

Values have been expressed as µgm phosphatidyl ethanolamine phosphorus (PE-P)/100 mg wet weight of tissue and as DPM/100 mg wet weight of tissue equivalent PE-P for ^{3}H-acetate incorporation. All the values are mean of 5 samples ± SEM in each group.

DISCUSSION

The observed increase in plasma T_4 levels in offspring born to hyperthyroid mothers reveal their hyperthyroid state while those born to hypothyroid mothers reveal their hypothyroid state with low plasma T_4 levels. These finding also support transfer of maternal T_4 to fetal side as observed earlier (10).

No report is available on cholesterol contents of developing heart in relation to maternal thyroid status. It appears that increased serum cholesterol concentration of the hypothyroid mothers (11) may be a factor for increased total cholesterol in hypothyroid pups. We also observed increase in free cholesterol (FC) during fetal and neonatal periods studied as well as increased esterified cholesterol (EC) content at day of birth in offsprings born to hypothyroid mothers. The findings of increased total, free and esterified cholesterol despite normal or low ^{3}H-acetate incorporation in FC and EC indicated a decreased utilization of cholesterol in heart of offsprings born to hypothyroid mothers. In hyperthyroid neonates at 7 and 28 days age increase in total cholesterol may be due to more availability of cholesterol from hyperthyroid mothers milk during lactation, as the milk is also a source of cholesterol (12). In offsprings born to hyperthyroid mothers myocardial total cholesterol was low and EC was marginally high during gestation period, which should make free cholesterol low but we observed no change in FC during this period. However, ^{3}H-acetate incorporation was unaltered in both FC and EC fractions. Similarly in offsprings born to hyperthyroid mothers EC and FC contents were observed increased at 28 days neonatal age with unchanged ^{3}H-acetate incorporation. The results suggested that during these periods utilization of EC and FC was reduced.

Total phospholipid contents were observed decreased in fetuses born to hyperthyroid and hypothyroid mothers (Table II). Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) are the two major phospholipid fractions of myocardial phosopholipids (13) and beside these other phospholipid fractions of the heart are phosphatidyl serine (PS), Lysophosphatidyl Choline (LPC) and phosphatidic acid (PA) (14). Steven et al (15) have reported that milk serves as a source of choline and PC. As the hypothyroid mothers are hypolactating (16), it is quite likely that neonates from hypothyroid mothers did not receive adequate choline and other substrates via milk resulting in a decreased PC as observed in hypothyroid neonates of this series. Decrease in total phospholipids during gestation period may be due to observed low contents and synthesis of PC and PE during these periods in hypothyroid pups. We also observed decreased total phospholipids in fetuses born to hyperthyroid mothers. While there was no change in contents of PC and PE and incorporation of ^{3}H-acetate into these phospholipid fractions. Beside PC and PE, there are some other myocardial phospholipid fractions also such as PS, LPC and PA (14) which were not estimated in this study. Decrease in myocardial total phospholipids of hyperthyroid fetuses may be due to changes in these phospholipid fractions.

REFERENCES

1. Presti FT, Pace JR, Chan SI. Cholesterol-Phospholipid interaction in membranes. 2. Stoichiometry and molecular packing of cholesterol rich domains. *Biochemistry* 1982; 21 : 3831-3835.
2. Hanahan DJ, Nelson DR. Phospholipids as dynamic participants in biological processess. *J Lipid Res* 1984; 25 : 1528-1534.
3. Kumar R, Chaudhuri BN. Altered maternal thyroid function: Fetal and neonatal development of rat. *Indian J Physiol Pharmacol* 1989; 33 : 233-238.
4. Kumar R, Chaudhuri BN. Altered maternal thyroid function: Fetal and neonatal myocardial metabolism. *Biol Neonate* 1990; 57 : 300-312.
5. Folch J, Lees M, Slonae-stanley GH. A simple method for the isolation and purificatioin of total lipids from animal tissue. *J Biol Chem* 1957; 226 : 497-509.
6. Misra UK. Thin layer chromatographic estimation on rat plasma and tissue lipids. *Biochim Biol Speri* 1968; 7 : 57-60 .

7. Abramson D, Blecher M. Quantitative two dimensional thin layer chromatography of naturally occuring phospholipids. *J Lipid Res* 1964; 5 : 628-631.

8. Zlatkis A, Zak B, Boyle AJ. A new mthod for the direct determination of serum cholesterol. *J Lab Clin Med* 1953; 41 : 486-492.

9. Raheja RK, Charanjit K, Ajit S, Bhatia IS. New colorimetric method for the quantitative estimation of phospholipids without acid digestion. *J Lipid Res* 1973; 14 : 695-701.

10. Obregon MJ, Malloe J, Pastor R, Morreale De Escobar G, Escobar Del, Rey F. L. Thyroxine and 3,5,3 Triiodothyronine in rat embryos before onset of fetal thyroid function. *Endocrinology* 1984; 114: 305-307.

11. Schs ML. Abnormalities of cholesterol metabolism in hypothyroidism and the effects of treatment with thyroid hormones and thyroxine analogues; in Crispel KR (ed): Current Concepts in hypothyroidism. Pergman Press, New York, 1963, pp 83-107.

12. Connor WE, Lin DS. Origin of milk cholesterol in the rabbit and guinea pig. *J Lipid Res* 1967; 8 : 558-564.

13. Gudmundsdottir A, Gudbjaranson S. Neonatal changes in fatty acid profile of phospholipids in rat heart-muscle. *Biochim Biophys Acta* 1983; 752 : 284-290.

14. Vasdev SC, Kako KJ. Incorporation of fatty acids into rat heart lipids. *J Mol Cell Cardiol* 1977; 9 : 617-631.

15. Steven HZ, Douglas C, Nancy FS. Choline phosphatidyl choline and sphingomyelin in human and bovine milk and infant formulas. *J Nutr* 1986; 116 : 50-58.

16. Babicky A, Novakova V. Influence of thyroxine and propyl thiouracil administration on the intake of maternal milk in suckling of the laboratory rat. *Physiol bohemoslovaca* 1985; 34 : 193-200.