ALTERED MATERNAL THYROID FUNCTION: EFFECT OF L-CARNITINE SUPPLEMENTATION ON FETAL AND NEONATAL MYOCARDIAL FREE FATTY ACID OXIDATION, *IN VITRO*.

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ABSTRACT

Effect of L-carnitine supplementation on myocardial free fatty acid oxidation, *in vitro*, in offsprings born of hypothyroid and hyperthyroid mothers was studied in rats. L-carnitine supplementation stimulated myocardial fatty acid oxidation during gestational period in offspring born of control and hyperthyroid mothers. In contrast L-carnitine supplementation induced stimulation in myocardial fatty acid oxidation was very less in fetuses born of hypothyroid mothers. However, in neonates born of hypothyroid mothers L-carnitine stimulated myocardial fatty acid oxidation to a great extent. The results suggested that during maternal hypothyroidism low availability of thyroid hormones to fetuses through maternal circulation and availability of less carnitine to neonates due to hypolactation might be responsible for decreased myocardial free fatty acid utilization. In neonates born of hypothyroid mothers and with cardiac energy insufficiency parenteral carnitine supplementation might be of great help to prevent mortality and morbidity of such offsprings.

KEYWORDS: Maternal, Thyroid, Fetal, Heart, FFA

INTRODUCTION

The developing heart undergoes remarkable metabolic transformations. During gestation, glycolysis and lactate oxidation constitute the major sources of adenosine triphosphate (ATP) for the fetal heart. After birth, however, there is a rapid shift from carbohydrate to fatty acid utilization (1). Thyroid hormones exert influence on various physiological functions, metabolism and fetal development (2,3). During development in cardiac muscle biomolecules essential for its membrane synthesis and maintenance viz. cholesterol and phospholipids were also observed to be regulated by maternal thyroid status (4). The offsprings born of hypothyroid mothers showed subnormal cardiac lipid metabolism (5) resulting in reduced free fatty acid oxidation at birth and afterwards. Carnitine is an important factor in the oxidation of

fatty acids (6). In adult heart it has been shown that changes in fatty acid metabolism may underlie the decline in cardiac function in hypothyroid animals. Rates of mitochondrial fatty acid oxidation as well as carnitine-palmitoylcarnitine exchange reactions were depressed in heart mitochondria isolated from hypothyroid rats (7). In heart mitochondria from hyperthyroid rats carnitine-palmitoylcarnitine exchange reactions were stimulated (8). In failing mitochondria and cardiomyopathy, importance of carnitine supplementation has been studied. It was observed that extra carnitine caused a considerable increase in β -oxidation rate of isolated muscle mitochondria (9). No study is available on the influence of maternal thyroid status on myocardial fatty acid oxidation with respect to L-carnitine. The present study was undertaken to find out the effect of maternal thyroid status on myocardial fatty acid oxidation *in vitro*, with respect to L-carnitine supplementation, in offsprings of hypothyroid and hyperthyroid female rats mated with normal male rats.

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MATERIALS AND 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 $24^\circ \text{C} \pm 1^\circ \text{C}$. They were divided into three groups. Group I rats served as controls (without any treatment). Group II rats were made hypothyroid by intra-peritoneal injection (i.p.) of 1 mCi ^{131}I obtained from Bhabha Atomic Research Center (BARC), Mumbai. They were kept separate for 6-8 weeks before their use for mating. Group III rats were made hyperthyroid by i.p. injection of $70 \mu\text{g}$ of L-thyroxine, obtained from Sigma Chemical Co. Ltd. U.S.A., 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 (control), hypothyroid and hyperthyroid groups of female rats were mated with normal male rats of same strain and age. Estrus cycle of rats was daily monitored by vaginal smear. The sperm positive day of the vaginal smear was taken as day 1 of pregnancy. Age groups studied were day 19 and 21 in fetal rats and day of birth (0 day), 7 days, 14 days, 21 days and 28 days after birth in neonates.

Plasma thyroxine

To determine exact thyroid status of mothers, fetuses and neonates, their plasma thyroxine (T_4) concentration was estimated using radio-immunoassay kits obtained from BARC, Mumbai.

Fatty acid oxidation *in vitro*

On days 19 and 21 of gestation, the pregnant mothers were anesthetized with nembutal (40 mg/kg body weight), i.p. and the litters were removed surgically. Fetal hearts were dissected out and were pooled together. Neonatal rats of different age groups were anesthetized by injecting nembutal (40 mg/kg body weight i.p.) and hearts removed. The heart tissues were homogenized in cold 0.25 M sucrose, containing 1mM EDTA and the homogenate was centrifuged at 5°C in cold centrifuge at 700 g. The supernatant obtained was again centrifuged at 12,000 g to obtain the mitochondrial fraction. The mitochondrial pellet

was suspended in 0.25 M sucrose. Protein was estimated by method of Lowry et. al. (10).

Aliquots of mitochondrial suspension from fetal and neonatal heart tissues were used for *in vitro* studies on fatty acid oxidation. Oxidation of ^{14}C -palmitic acid into $^{14}\text{CO}_2$ by fetal and neonatal heart was determined by incubating heart mitochondrial fraction for 1 hr at 37°C in 25 ml flasks containing a total volume of 2.5 ml incubation medium (11). Components of incubation medium were 1 mM ATP, 1mM magnesium chloride, 7×10^{-5} M palmitate- $1\text{-}^{14}\text{C}$ with total activity of 1 μCi per vessel, 0.15 M potassium chloride, 0.015 M potassium phosphate buffer, pH 7.4, 0.05 mM CoA, heart mitochondrial fraction containing 200-250 μg protein and water to make up a total volume of 2.5 ml in the main compartment. L-carnitine, 0.5 mM was added in experiments in which fatty acid oxidation was estimated in presence of L-carnitine. Glass cups containing 0.2 ml 1N KOH (CO_2 free) and a thin strip of Whatman No. 1 filter paper were fixed in the center of each flask to absorb metabolic $^{14}\text{CO}_2$. Glass cups containing absorbed $^{14}\text{CO}_2$ were transferred to vials containing 15 ml dioxane based scintillation cocktail (12). Radioactivity was determined in a Beckman LS 7000 Liquid scintillation counter.

Results were analysed using Student's 't' test.

RESULTS

Maternal, fetal and neonatal plasma thyroxine

Plasma thyroxine levels in mothers, fetuses and neonates are shown in Table 1. Plasma thyroxine levels were significantly high in hyperthyroid mothers at all age groups studied while the reverse has been observed in hypothyroid mothers. Plasma thyroxine levels in fetuses and neonates born of hyperthyroid mothers were also significantly higher at all age groups studied. Plasma thyroxine levels were significantly low in offsprings born of hypothyroid mothers at 21 days fetal age, newborns and 7 days neonatal age groups. Because no offspring born of hypothyroid mothers could survive beyond eight days, in this group values beyond 7 days neonatal age are not available for plasma thyroxine and myocardial FFA oxidation.

Table 1. Plasma thyroxine concentration of mothers and their offsprings during gestational and lactational period.

Group	Gestational/fetal age (Days)		Day of birth	Lactational/neonatal age (Days)			
	9	21	0	7	14	21	28
CONTROL							
Mother	3.8 ±0.2	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	0.5 ±0.1	0.5 ±0.1	0.4 ±0.1	1.6 ±0.2	2.3 ±0.2	2.9 ±0.3	3.6 ±0.2
HYPERTHYROID							
Mother	7.6* ±0.2	6.4* ±0.5	5.3* ±0.4	8.2* ±0.1	7.9* ±0.1	8.4* ±0.2	9.2* ±0.2
Offsprings	1.0* ±0.1	1.1* ±0.1	1.0* ±0.1	1.8* ±0.1	3.2* ±0.2	4.0* ±0.1	3.8 ±0.2
HYPERTHYROID							
Mother	0.8* ±0.1	0.7* ±0.1	1.0* ±0.1	0.7* ±0.1	—	—	—
Offsprings	ND	0.2* ±0.05	0.2* ±0.04	0.5* ±0.01	—	—	—

Values have been expressed as plasma thyroxine µg/dl and are mean of six samples ±S.E in each group.

ND = Not detected

* = Values significantly different at P<0.05 level in comparison to control values.

Table 2. Altered maternal thyroid: Effect of carnitine on ¹⁴C-palmitate oxidation *in vitro* by fetal and neonatal heart.

Group	Gestational/fetal age (Days)		Day of birth	Lactational/neonatal age (Days)			
	19	21		7	14	21	28
CONTROL							
(-) L-Carnitine	272 ±13	228 ±8	394 ±34	409 ±29	940 ±34	1371 ±67	858 ±26
(+)L-Carnitine	575 ±71	687 ±35	650 ±45	712 ±21	1662 ±66	1262 ±44	833 ±39
% Change with carnitine	+153%	+209%	+65%	+74%	+77%	-8%	-3%
HYPERTHYROID							
(-)L-Carnitine	228 ±30	271 ±20	457 ±15	474 ±10	928 ±11	1229 ±48	933 ±33
(+)L-Carnitine	1084* ±32	1073* ±96	831* ±50	792 ±69	1481 ±15	1312 ±62	1004* ±21
% change with carnitine	+375%	+296%	+82%	+67%	+60%	+7%	+8%
HYPOTHYROID							
(-)L-Carnitine	235 ±10	239 ±11	224* ±18	310* ±32	—	—	—
(+)L-Carnitine	393* ±40	416* ±10	797* ±18	979* ±32	—	—	—
% change with carnitine	+67%	+74%	+255%	+216%	—	—	—

Values have been expressed as ¹⁴CO₂ counts per minute/hr/mg protein at 37°C and are mean of six samples ±S.E. each group.

* = Values significantly different at P<0.05 level in comparison to control values.

Myocardial FFA oxidation *in vitro*

In presence of L-carnitine the myocardial fatty acid oxidation was very much stimulated in control and offsprings of hyperthyroid mothers during gestational period but in offsprings of hypothyroid mothers stimulation was less at this time (Table 2). After birth and in neonatal stages the stimulation of fatty acid oxidation by carnitine supplementation in offsprings born of control and hyperthyroid mothers was not as high as in fetal stages (Table 2). The stimulation of fatty acid oxidation by carnitine in neonatal stages was almost comparable in offsprings from control and hyperthyroid mothers. But stimulation of fatty acid oxidation by carnitine in newborns and 7 days neonates born of hypothyroid mothers was very high.

DISCUSSION

The observed increase in plasma T_4 levels in offsprings born of hyperthyroid mothers reveal their hyperthyroid state while those born of hypothyroid mothers reveal their hypothyroid state with low plasma T_4 levels.

The myocardial oxidation of ^{14}C -palmitic acid in to $^{14}CO_2$ by fetal hearts was very low in all the groups in comparison to neonatal stages irrespective of maternal thyroid status (Table 2). The observed low FFA oxidation by fetal hearts is in agreement with the previous findings (13,16). However, in offsprings born of hypothyroid mothers FFA utilization by heart remained low even at day of birth and 7 days neonatal age in comparison to control rats. The observed decrease in FFA oxidation by hypothyroid pups after birth might be due to non-availability of sufficient thyroxine, fatty acids and carnitine via milk due to inability of

hypolactating hypothyroid mothers to feed sufficient milk to pups (14). The primary source of carnitine in neonatal tissues at early stages has been shown to be the maternal milk in which carnitine contents are very high at this time (15).

Carnitine is an important component for FFA oxidation. In presence of L-carnitine the fatty acid oxidation was stimulated in the hearts of fetuses born of control and hyperthyroid mothers. However, increase in % FFA oxidation was more and significant in hyperthyroid mothers fetuses (Table 2). The carnitine supplementation induced stimulation in myocardial FFA oxidation was less in hypothyroid mothers fetuses which might be due to very low level of thyroid hormones available to them at this time. But on the day of birth and 7 days neonatal stage carnitine induced increase in myocardial FFA utilization by the hypothyroid pups was significantly very high in comparison to pups of same age and born of control and hyperthyroid mothers. It suggested that at this time hypothyroid pups had low level of carnitine but at the same time pups born of control and hyperthyroid mothers had better carnitine levels resulting in to a smaller rise of FFA oxidation by their hearts, when carnitine was supplemented in assay system.

The observed findings indicate that both carnitine and thyroid hormones are important for FFA oxidation by fetal and neonatal hearts. As both are not available sufficiently to the hypothyroid pups due to hypothyroid and hypolactating state of the mothers, their myocardial capacity to oxidize FFA was very low. This may be one of the causes for heavy mortality rate of the hypothyroid pups just after birth and in early neonatal stages of one week (3). It is concluded that in neonates born of hypothyroid mothers and with cardiac insufficiency parenteral carnitine supplementation might be of great help to prevent mortality and morbidity of such offsprings.

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