

Effect of Hypothyroidism and Thyroxin replacement on growth Of long bones in prenatally treated Albino Rats

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Abstract

Objective:To study the effect of induced hypothyroidism and thyroxin replacement on bone growth.

Design:An animal study carried out on experimental Albino rats.

Place and duration of study: The study was carried out in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from June 1999 to May 2002.

Method:Pregnant female Albino rats obtained from the animal house of Basic Medical Sciences Institute, JPMC, Karachi were treated with carbimazole and carbimazole plus thyroxin from 10th day of gestation till parturition. Another group of pregnant rats did not receive any treatment and acted as controls. Pups born to the treated as well as control animals were sacrificed on 10th postnatal day and fixed in formal saline. They were then processed through 95% ethanol and acetone, bulk stained with alizarin red and cleared in 4% KOH to reveal their bony and cartilaginous elements. The ulna and tibia of both sides were disarticulated from the treated and control animals and measured for intact bone length and diameter. The measurements of the three groups were then compared statistically.

Results:The retardation in length observed at the end of experimental period in ulna was by 13.67% and in tibia by 27.84% in carbimazole treated group while in carbimazole plus thyroxin treated group the reduction in length of ulna was 5.08% and of tibia 3.91% when compared with their age matched controls.

Conclusion:Prenatal hypothyroidism has an adverse effect on bone growth and results in reduction of long bone length (JPMA 53:18;2003).

Introduction

Thyroid hormones exert an important effect on growth and differentiation of tissues in many mammalian species. They are particularly essential, optimal and critical for normal growth and maturation of skeletal, nervous and reproductive tissues.¹ For development and growth of these tissues in fetus the hormone comes from the fetal thyroid gland and not the maternal hormone crossing placenta. Congenital deficiency of thyroid hormones is associated with short stature and mental retardation. Delay and retardation in skeletal growth have been described in human cretins at birth.² Thyroid deficiency therefore has an adverse effect on skeletal growth.³ Carbimazol is a thionamid compound and a good agent to produce hypothyroidism.⁴

The purpose of this paper is to evaluate the effect of prenatally induced hypothyroidism on growth of long bones in albino rats and the effect of simultaneous thyroxin replacement.

Materials and Method

Nine adult virgin female albino rats, weighing 175-200 gms, were mated with adult male albino rats of same age and strain in a ratio of three females with one male rat in one cage.⁵ Next morning each female rat was examined for any sign of mating in form of blood stained vagina or a vaginal plug.^{6,7} Absence of any apparent sign of mating led to vaginal wash which was obtained on glass slide and observed under microscope for presence of spermatozoa. Their presence was again considered as day zero of pregnancy. The pregnant albino rats were then separated from male rats and divided into three groups, P1, P2 and P3 each comprising three rats. Animals of group P1 were given carbimazole (neomercazole) at a dose of 6µg/gm body weight subcutaneously from 10th day of pregnancy to parturition.⁸ Group P2 animals were given carbimazole subcutaneously at a dose of 6µg/gm body weight as well as thyroxin 5µg intraperitoneally⁹ daily from 10th day of gestation until parturition. Group P3 animals received 0.5 c.c unossified bone remained unstained.

Appendicular skeleton was then separated from the axial skeleton and long bones of fore and hind limbs were disarticulated. Gross length and diameter of the ulna and tibia of two sides were then measured with the help of an electronic digital caliper with a measuring range of 0-300mm (up to 12 inches) and an error of ± 0.03 mm of indicating value.

Results

Intact bone length

Ulna: The mean bone length of ulna in pups born to control mothers was 12.58 ± 0.01 mm while in pups of carbimazole treated mothers it was 10.86 ± 0.04 mm and in pups born to carbimazole plus thyroxin treated mothers it was 11.94 ± 0.05 mm. The decrease in the length of ulna was 13.67% in carbimazole treated group as compared to controls ($P < .001$). The decrease was 5.08% in carbimazole plus thyroxin treated group when compared with age matched controls ($P < .001$).

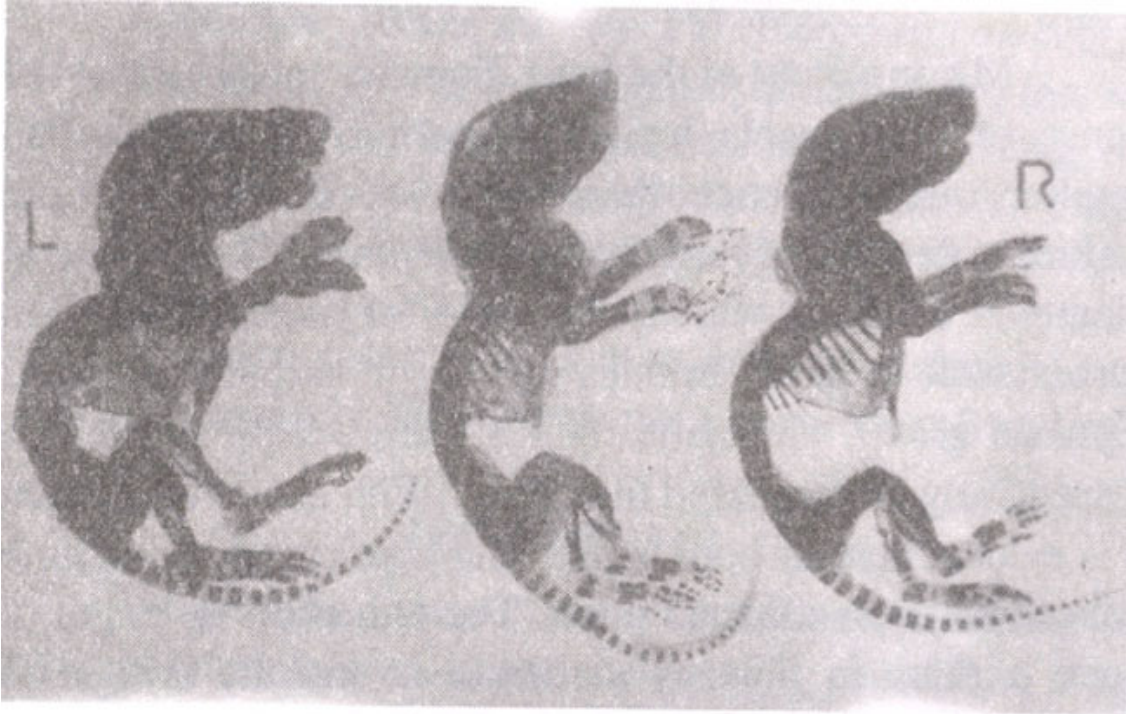


Figure 1. Photograph showing cleared and Alizarin red stained prenatally treated albino rats. In the centre is the rat born to Carbimazole treated mother showing shorter length of long bones, of fore and hind limbs as compared to the rats born to control and Carbimazole plus Thyroxine treated mothers present on left (L) and right (R) respectively.

Tibia: The mean bone length of tibia in pups born to control mothers was 13.54 ± 0.04 mm while in pups of carbimazole treated mothers it was 9.77 ± 0.03 mm and in pups born to carbimazole plus thyroxine treated mothers it was 13.01 ± 0.05 mm. The decrease in the length of tibia was found to be 27.84% in carbimazole treated group ($P < .001$) and 3.91% in the carbimazole plus thyroxine treated group ($P < .001$).

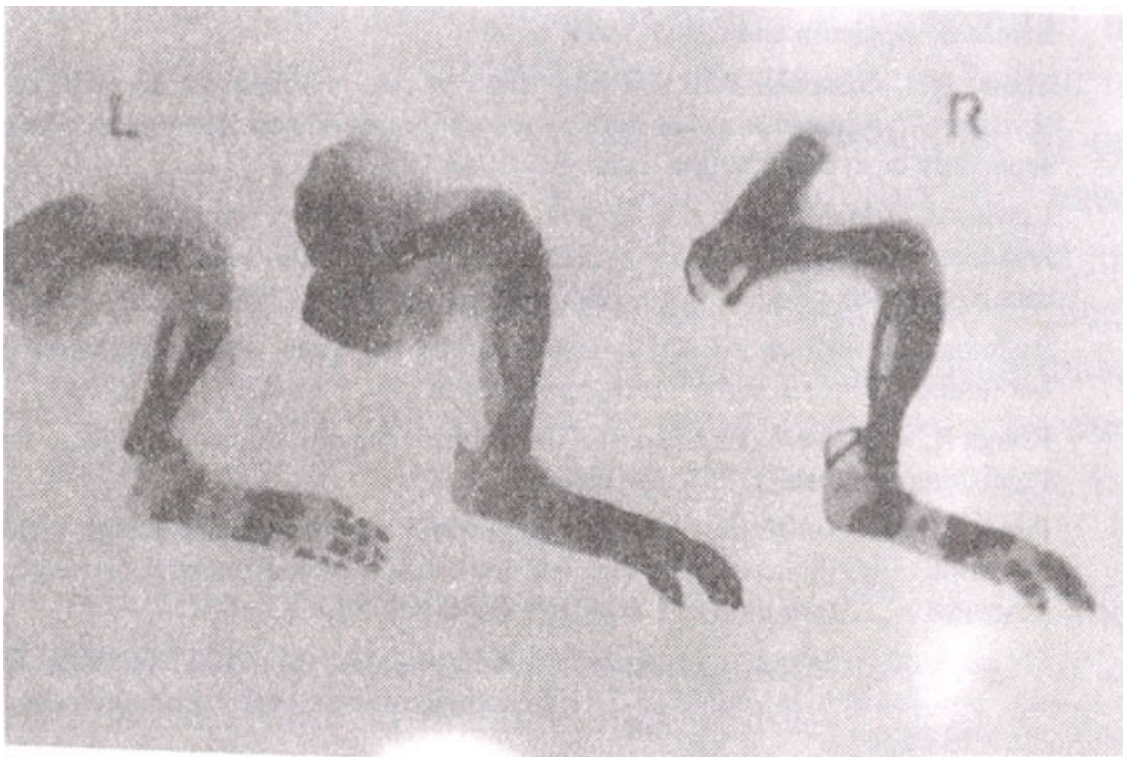


Figure 2. Photograph showing the left hind limb bones of cleared and Alizarin red stained prenatally treated albino rats. On the left (L) is the hind limb of the rat born to Carbimazole treated mother showing a shorter tibia and fibula as compared to the bone of the rats born to control mother present in center and rats born to Carbimazole plus Thyroxin treated mothers present on right (R).

Diameter of the bone

Ulna: The mean diameter of ulna in the pups born to control mothers was 1.10 ± 0.02 mm while in pups born to carbimazole treated mothers was 0.90 ± 0.02 mm and in pups born to carbimazole plus thyroxin treated mothers was 1.03 ± 0.02 mm. The reduction in diameter as compared to age matched control was 18.18% in carbimazole treated group ($P < 0.001$) and approx: 6.36% in carbimazole plus thyroxin treated group ($P < 0.02$).

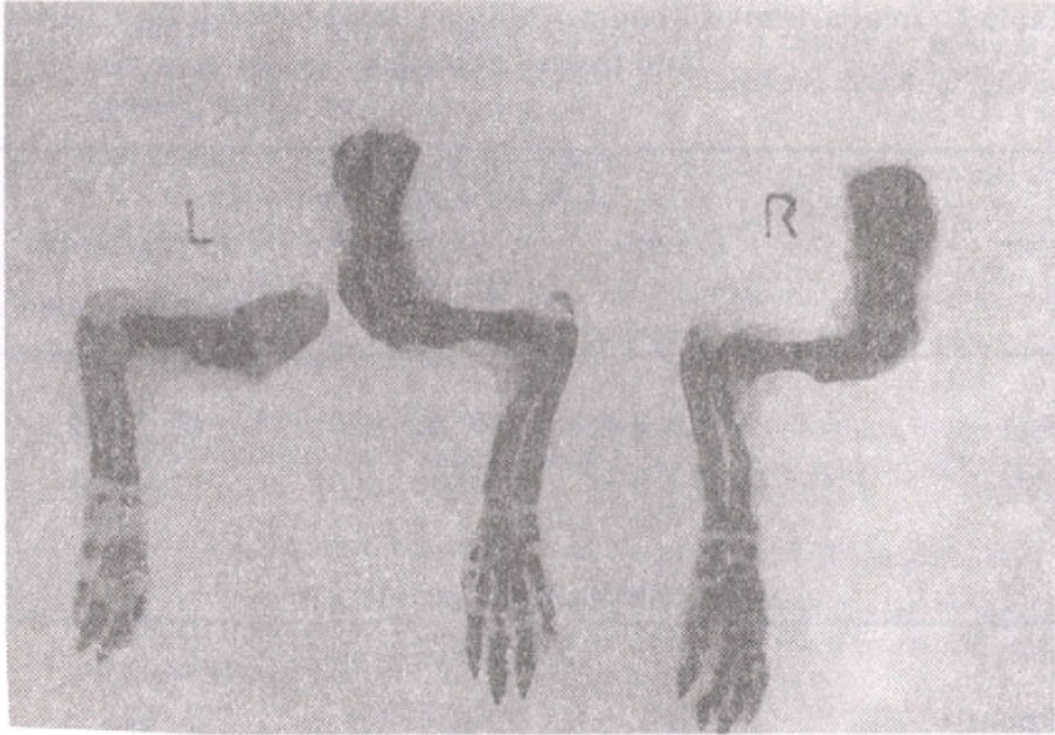


Figure 3. Photograph showing the fore limb bones of the cleared and Alizarin red stained treated albino rats. On the left (L) is the fore limb of the rat born to Carbimazole treated mother showing a shorter ulna and radius as compared to the bones of the rat born to control mother present in center and the rat born to Carbimazole plus Thyroxin treated mothers present on right (R).

Tibia: The mean diameter in tibia in pups born to control mothers was 1.26 ± 0.01 mm while in pups born to carbimazole treated mothers it was 0.95 ± 0.01 mm and in pups born to carbimazole plus thyroxin receiving mothers it was 1.22 ± 0.01 mm. The reduction in diameter as compared to age matched control was 24.60% in carbimazole treated group ($P < 0.001$) and approximately 3.17% in carbimazole plus thyroxin treated group ($P < 0.02$).

Table 1. Comparison of intact Bone Length (mm) between the Control and Treated animals

Bone	Prenatally Treated		Carbimazole plus thyroxin treated
	Control	Carbimazole	
Ulna	12.58 $\pm$ 0.12	10.86 $\pm$ 0.04	11.94 $\pm$ 0.05
Tibia	13.54 $\pm$ 0.04	9.77 $\pm$ 0.03	13.01 $\pm$ 0.05

Statistical Comparison			
Groups	Prenatally treated		S / N.S.
	P Value		
	Ulna	Tibia	
C vs Ca	<0.001	<0.001	H.S.
C vs Ca + T4	< 0.001	< 0.001	H.S.
Ca vs Ca + T4	< 0.001	< 0.001	H.S.

Key: C= Control; Ca= Carbimazole; T4 = Thyroxine; S= Significant; N.S. = Non-significant; H.S. = Highly Significant.

Table 2. Comparison of Diameter of Bone (mm) between the Control and treated animals.

Bone	Prenatally Treated		
	Control	Carbimazole	Carbimazole plus Thyroxin treated
Ulna	1.10 $\pm$ 0.02	0.90 $\pm$ 0.01	1.03 $\pm$ 0.02
Tibia	1.26 $\pm$ 0.01	0.95 $\pm$ 0.01	1.22 $\pm$ 0.01

Statistical Comparison			
Groups	Prenatally Treated		
	P Ulna	Value Tibia	S / N.S.
C vs Ca	<0.001	<0.001	H.S.
C vs Ca + T4	< 0.02	< 0.001	S.
Ca vs Ca + T4	< 0.01	< 0.01	H.S.

Key: C= Control; Ca= Carbimazole; T4 = Thyroxin; S = Significant; N.S. = Non- significant; H.S. = Highly Significant

Discussion

The carbimazole administered in pregnant rats crosses placenta.^{12,13} and concentrates in the thyroid of developing pups. It thus blocks the synthesis of fetal thyroid hormones which are ultimately required for growth and differentiation in developing pups since maternal thyroxin crosses the placenta in a very limited amount.^{14,15} Placenta acts as a very effective barrier, preventing maternal iodothyronins from reaching the fetus. This would be a consequence of both poor permeability and active deiodinating mechanism of fetal members.¹⁶ However some maternal T4 and T3 do get transferred to the fetus in both rats and humans. But administered carbimazole in pregnant rats also results in marked reduction in maternal levels of circulating T3 and T4¹⁷ thus reducing the chances of maternal hormones for crossing the placenta to nil. The effectiveness of carbimazole as a chemical method of inducing hypothyroidism in rats is shown by studies conducted by Redmond et al⁴ and Berthier et al.¹⁸

We have observed in our study that prenatally induced hypothyroidism reduced the growth of long bones in the albino rat. The reduction was however more in tibia

(27.84%) than in ulna (13.67%). This may have been due to the fact that there is a postnatal dominance in growth of hind limb bones.¹⁹ Patton et al²⁰ have also reported a hind limb dominance in growth postnatally. Although we administered the antithyroid drug during gestation but the data was collected on 10th postnatal day. It is likely that the effect of carbimazole may have persisted up to 10th postnatal day and had affected the growth of hind limb bone (tibia) more than that of fore limb (ulna).

Simultaneous treatment with thyroxine mitigated the effect of carbimazole but the full restoration of bone length was not achieved. There was still a decrease in length of the tibia by 3.9% and that of ulna by 5.08% in pups born to carbimazole plus thyroxine treated mothers as compared to those of control mothers.

Measurement of the bone diameter revealed that the bones of carbimazole treated (hypothyroid) animals had smaller circumference than those of the control and carbimazole plus thyroxine treated animals. Reduction in diameter of tibia was 24.6% and of ulna 18.18% in carbimazole treated animals while in carbimazole plus thyroxine group. The same figures were 3.17% and 6.36% respectively as compared to control. With above discussion it is evident that for both length and diameter the hind limb bone (tibia) was affected more. The reduction in length of bone is more in tibia by carbimazole and the mitigation effect of thyroxine is also more in this bone.

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