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ORIGINAL ARTICLE

Effect of carbimazole induced Hypothyroidism and thyroxine replacement on the growth of the long bones in Albino rats of different age groups

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Abstract

To evaluate the effect of carbimazole induced Hypothyroidism and thyroxine replacement, on the growth of long bones of albino rats of different age groups. Experimental albino rats were developed with carbimazole and carbimazole plus thyroxine for a period of six weeks. At the end of the experiment the animals were sacrificed, fixed and processed to demonstrate the bony and cartilaginous parts. The ulna and tibia of both sides were measured for intact bone length & diameter and the data compared. The reduction in length and circumference observed, at the end of experiment, in ulna was 10.89%, & 11.94% and in tibia it was 12.52%, 14.81% in carbimazole treated group respectively, while in carbimazole plus thyroxin treated group the reduction in length & circumference of ulna was 1.37% & 1.88% and in tibia it was 1.86% & 3.08% respectively. They were compared to their age matched controls. The reduction in length and circumference in ulna was 5.58% & 6.25% and 6.42% and 5.88% in tibia respectively among the carbimazole treated animals while in the carbimazole plus thyroxine treated animals the reduction was only 0.63% and 3.12% in ulna and 0.91% and 1.06% in tibia respectively.
The results show that hypothyroidism and its replacement therapy affects the endochondral as well as periosteal bone growth and results in reduction in length as well as circumference of long bones.

INTRODUCTION

Thyroid hormone is essential for the recruitment and maturation of bone cells and other soft viscera of the body during development (Escobar *et al.* 1993; Ganong, 1999; Adin *et al.* 2005; Freitas *et al.* 2005; Sarwar & Parveen, 2005). A deficit of thyroid hormone during pregnancy and in the newborn leads to retardation 2n the formation of skeleton and other tissues. (Arey, 1974; Liu *et al.* 1986; Inuwa *et al.* 1996). Hypothyroidism during later life causes a relevant decrease of bone turnover (Moore *et al.* 1998; Ahmed & Janjua, 2003). Excessive amounts of thyroid hormone induce increased activity of osteoblasts and osteoclasts leading to high bone turnover and a loss of BMD (Bone Mass Density), as the activity of osteoclasts predominates over the activity of osteoblasts (Scow *et al.* 2000; Cull-

ing, 1974). Men seem to have a limited, but significant bone loss in subclinical hypothyroidism (Ganong, 1999), however, the postmenopausal women face a significant BMD loss. (Gartner, 2006). Therapeutic consequences focus on the normalization of thyroid function, which can lead to restoration of BMD and normalization of bone turnover. (Gam *et al.* 1991; Brtalena *et al.* 1996; Pantazi & Peter, 2000; Mikosch, 2005). Thyroid hormone preparations, especially thyroxin, are widely used either as replacement doses to correct hypothyroidism or as suppressive doses to abolish thyrotropin (thyroid-stimulating hormone) secretion in patients with differentiated thyroid carcinoma. The major clinical consequences by this therapy include cardiovascular changes (shortening of systolic time intervals, increased frequency of atrial premature beats and, possibly, left ventricular hypertrophy) and bone changes (reduced bone density and bone mass). The risk of these adverse effects can be minimized by carefully monitoring serum free thyroxin and free triiodothyronine measurements and adjusting the dosage accordingly. (Langdahl, 1996; Hadji, 2000).

Apart from thyroxia thionamides [thiamazole (methimazole), carbimazole and propylthiouracil are the most widely used antithyroid drugs. (Brtalena *et al.* 1996). In most cases, adverse effects are minor and transient (e.g. skin rash, itching, mild leucopenia). However agranulocytosis and decreased BMD, can also occur in 0.1 to 0.5% of patients. These life-threatening conditions can now be effectively treated by granulocyte colony-stimulating factor administration and proper mineral therapies respectively. (Pantazi & Peter, 2000).

The patients suffering from hypothyroidism can be considered as more prone to osteoporosis (Langdahl, 1996; Hadji, 2000), a major public health problem. In histomorphometric studies, reconstruction of the remodeling sequence in patients with hypothyroidism discloses a marked shortening of both the resorptive and formative phases of the remodeling cycle, with a negative balance of 9–10 μm/remodeling cycle (Gam *et al.* 1991). Osteoclastic and osteoblastic activities are enhanced, with a predominance of bone resorption resulting in increased levels of bone turnover markers (Lewinson, 1989; Bancroft, 1990) and in decreased bone mass, as determined by single photon absorptiometry, dual photon absorptiometry, and dual x-ray absorptiometry, which is the most rapid, accurate, and reproducible methods to evaluate bone mineral density (Lewinson, 1989; Inuwa, 1996)

The mechanisms of thyroid action on bone are in accordance with a direct effect of thyroid hormones on bone in thyrotoxicosis. Therapy to thyroid disorders could be instrumental for the changes in bone turnover, in hyper- and hypothyroidism. (Bijlsma *et al.* 1983; Bolander, 1999).

Long-term, non-suppressive L-Thyroxin treatment in women with goiter or hypothyroidism was associated with a slight reduction in quantitative ultrasonometry (QUS) values, which was more pronounced in postmenopausal women. These women could be at higher risk for osteoporotic fractures. (Hadji, 2001). The non-suppressive levothyroxine treatment for a long time on quantitative ultrasonometry of bone in women revealed that these were more at risk having early fractures due to decreased bone mass density. (Bijlsma *et al.* 1983; Bolander, 1999).

Serum alkaline phosphatase and type I collagen C-terminal telopeptide (ICTP) have been introduced recently for evaluation of formation and resorption of bone turnover markers, respectively (Fottner & Weber, 2006). These precise bone markers could clarify, the study, existence of a bone mass recovery period after attainment of euthyroidism that has been suggested in patients treated with radioiodine. Moreover, recent reports suggests the reversibility of thyrotoxic bone disease after more than or even 1 yr of euthyroidism (Hadji, 2000). Some Studies suggest that a significant proportion of patients, particularly women after menopause and at old age, with a history of thyrotoxicosis continue to be at risk of reduced bone mass density (BMD) and always at risk for fractures. (Gartner, 2006; Majima *et al.* 2006). In our study the relationship between hypothyroidism and its effects on the long bones, was investigated with the facts that hypothyroidism and the treatment of hypothyroidism may lead to decrease in bone mass density of bones generally and long bones particularly.

MATERIAL AND METHODS

Thirty-six albino rats of different age groups were included. These animals were obtained from the Animal House of Basic Medical Sciences Institute, JPMC, Karachi, This *strain* of albino rats was originally obtained from Charles River Laboratory, Brooklyn, Massachusetts, USA, and was cross bred at the said animal house.

The animals were kept in experimental room for one week prior to the commencement of study, for accumutalization to the experimental conditions with 12 hours light and dark cycles. The animals were fed laboratory chow and water *ad libitum*.

Out of 36 rats, 18 were two weeks old and the another 18 were six weeks old at the commencement of study. The 36 animals underwent experimentation for a period of six weeks.

Groups

A – Two weeks old

B – Six weeks old

Groups A and B were further divided into three sub groups: A1, A2, & A3, Bl,B2, & B3, each containing six animals.

Sub group A1&B1 received antithyroid agent carbimazole dissolved in 0.9% NaCI subcutaneously at a dose of 6 μg/ body weight daily for six weeks. Sub groups A2&B2 received carbimazole subcutaneously at a dose of 6μg/ body weight and Thyroxine 5 μg/animal in 0.9%saline and 0.01 M NaOH solution intraperitoneally daily for six weeks.

Sub group A3&B3, received 0.5 cc (volume equal to the volume of Thyroxine) of normal saline (0.9% NaCI) intraperitoneally and acted as control group.

The animals were weighed weekly and dosage reviewed accordingly. At the end of study period the animals were sacrificed by deep ether anaesthesia, eviscerated and fixed in formal saline[11,12]

The animals were processed through 95% ethanol, acetone and then placed in 4% KOH for a period of 4–6 weeks for clearing. Upon clearing they were bulk stained using 0.001% Alizarine red in 1% KOH [13] .This is technique clearly demonstrated the ossified parts while the unossifid parts remained unstained.

Appendicular skeleton was disarticulated and the length and the diameter of the ulna and tibia of the two sides was measured with the help of electronic digital caliper with measuring range of 0–300 mm and error of ±0.03mm of indicating value.

Statistical Analyses

The data was subjected to student's t-test. Difference between the means of the parameter between different groups was evaluated and regarded as significant if the "p-value" was less than 0.05 and non significant if the "p-value" was greater than 0.05

RESULTS

The results were obtained in intervals of two weeks and six weeks of age of the albino rats. They were assessed on the basis of bone length and bone diameter.

NTAORT BONE LENGTH

Two weeks age group

Ulna

The mean length of bone in the control animals was 21.11±0.06 mm, in Carbimazole treated group l8.81±0.06 mm, and in Carbimazole plus Thyroxin treated group it was 20.82±0.04 mm. (Table 1**).**

The decrease in the length of ulna in the Carbimazole treated group was 10.89% and observed to be significant ($p<0.05$), whereas in Carbimazole plus Thyroxin treated group was 1.37% which was significant ($p<0.05$) statistically, when compared with their age matched controls.

Tibia

The mean bone length in the control group was 26.83±0.06 mm, in Carbimazole treated group 23.47±0.04 mm, and in Carbiriazole plus Thyroxine treated group 26.33+0.14 mm. (Table 1**).**

The decrease in the length of tibia in the Carbimazole treated group was approximately 12.52% and found to be significant ($p<0.05$) statistically, whereas in the Carbimazole plus Thyroxin treated group was 1.86% which was statistically significant ($p<0.05$), when compared with the age matched controls.

Six weeks age group

Ulna

The mean bone length in control group was 23.65±0.06 mm, in Carbimazole group was 22.33±0.20 mm, and in Carbimazole plus Thyroxine treated group was 23.50±0.07 mm. (Table 2**).** Decrease in the length of ulna in the Carbimazole treated group was approximately 5.88% and observed to be significant ($p<0.05$), while in Carbimazole plus Thyroxin treated group was 0.63% which was statistically non significant ($p>0.05$), when compared to their age matched controls.

Tibia

The mean bone length in the control group was 28.35±0.07 mm, in Carbimazole treated group was 26.52±0.08 mm, and in Carbimazole plus Thyroxine treated group was 28.09±0.04 mm. (Table 2**).**

Decrease in the length of the tibia in the Carbimazole treated animals was 6.45% and was significant ($p<0.05$) statistically, while in Carbimazole plus Thyroxin treated animals it was 0.91% and significant ($p<0.05$) statistically, when compared with their age matched control.

DIAMETER OF THE BONES

Two weeks age group

Ulna

The mean diameter of the ulna in the control group was 1.59±0.02 mm; in Carbimazole treated was 1.40±0.02 mm, and in Carbimazole plus Thyroxin treated group was 1.56±0.02 mm. (Table 3). Reduction in diameter in the Carbimazole treated groups was 11.94% and observed to be significant ($p<0.05$) statistically, whereas in the Carbimazole plus Thyroxin treated group the reduction in the diameter of ulna was 1.88% and found to be non significant ($p>0.05$), when compared with their age matched controls.

Tibia

The mean diameter of tibia in the control group was 1.62±0.02 mm, in Carbimazole treated group was 1.38±0.01 mm, and in Carbimazole plus Thyroxin treated group it was 1.57±0.01 mm. (Table 3**).**

Reduction in the diameter of the tibia in the Carbimazole treated group was14.81% and was significant ($p<0.05$) statistically, whereas in the Carbimazole plus Thyroxin treated group reduction in the diameter was 3.08% which was non significant ($p<0.05$) statistically, when compared as their age matched controls.

Six weeks age group

Ulna

The mean diameter in the control group was 1.92±0.02 mm, in Carbimazole group it was 1.80±0.02 mm, and in Carbimazole plus Thyroxine treated group the diameter was 1.86±0.01 mm. (Table 4**).**

Table 1. Comparison of intact bone length (mm) between the control & treated animals at two weeks age group.

Bone	Control	Carbimazole treated	Carbimazole & Thyroxin treated
Ulna	21.11±0.06	18.81±0.06**	20.82±0.04**
Tibia	26.83±0.06	23.47±0.04**	26.33±0.14

** p<0.05

Table 2. Comparison of intact bone length (mm) between the control & treated animals at six weeks age group.

Bone	Control	Carbimazole treated	Carbimazole & Thyroxin treated
Ulna	23.65±0.06	22.33±0.20**	23.50±0.07
Tibia	28.35±0.06	26.52±0.08**	28.09±0.04

** p<0.05

Table 3. Comparison of diameter of bone (mm) between the control & treated animals at two weeks age group.

Bone	Control	Carbimazole treated	Carbimazole & Thyroxin treated
Ulna	1.59±0.02	1.40±0.02**	1.56±0.02
Tibia	1.62±0.02	1.38±0.01**	1.57±0.01

** p<0.05

Table 4. Comparison of diameter of bone (mm) between the control & treated animals at six weeks age group.

Bone	Control	Carbimazole treated	Carbimazole & Thyroxin treated
Ulna	1.92±0.02	1.80±0.02**	1.86±0.01
Tibia	1.87±0.02	1.76±0.01**	1.85±0.01

** p<0.05

Reduction in the diameter of the ulna in the Carbimazoie group was 6.25% and was significant (p<0.05) statistically, while in the Carbimazole plus Thyroxine treated group was 3.12% and statistically significant (p<0.05), when compared to their age matched controls.

Tibia

The mean diameter in the control group was 1.87±0.02 mm, in Carbimazole group was 1.76±0.02 mm, and in Carbimazole plus Thyroxin treated group the diameter was 1.85±0.01 mm. (Table 4).

Reduction in the diameter of the tibia in the Carbimazole group was 5.88% and was significant (p<0.05) statistically, while in the Carbimazole plus Thyroxin treated group the reduction was 1.06% which' was non significant (p>0.5), when compared with age matched controls.

DISCUSSION

We demonstrate, that, a significant reduction in axial BMD in hypothyroid rats. This reduction was similar in magnitude to that observed previously and was partially restored after attainment of the euthyroid state. Some studies with smaller number (Patton & Kaufman, 1995; Ahmed & Janjua, 2003) and using different methodologies (Ronning & Kantoma, 1988) have suggested a potential hypothyroid bone disease. to this was attributed to abnormal glucose metabolism and increased bone turn over were hallmarks of the untreated Hyperthyroidism. (Al-shoumer *et al.* 2006). The results of the present study suggest that the length of hind limb bone (Tibia) was affected more than the fore limb (Ulna). This is in agreement with the previous studies (Patton and Kaufman, 1995, Ahmed & Janjua, 2003) who have reported hind limb dominance in growth postnatally. Recently that postnatal dominance in growth of hind limb bones has been considered as a rule of growth and development. (Sofi *et al.* 2006). Comparing the effect on bone length it was observed that retardation in the length of tibia (hind limb) was higher than the forelimb (ulna) in animals of two weeks age group where as in animals of six weeks age group the difference in retardation was not significant. Furthermore, the difference in growth of the two limbs has reduced to almost half in six weeks age group (10.89% vs. *5.58%* and 12.50% vs 6.42%) respectively.

Measurement of bone diameter has shown a decrease in the diameter of the bones in carbimazole treated rats as compared to their age-matched controls and carbimazole plus thyroxin treated rats. The decrease in the diameter is marked in tibia and it is maximum in two weeks age group while in the present study in the six weeks age group surprisingly ulna was slightly more reduced in diameter than tibia. These results agree with the study of Ahmed & Janjua in 2003.

The process of ossification has been found to progress in generally a proximal to distal manner with fore limb being advance of hind limb, as was previously observed (Escobar, 1993). However, after birth the order is reversed so that the distal hind limb long bones have the highest growth rate. This observation is in agreement with another study (Fretias *et al.* 2005). The findings of the present study are consistent with this since hind limb bone (Tibia) has shown more retardation in growth. Our study shows that this retardation was at its maximum next to younger age group (two weeks).

In six-week age group either two limbs were affected equally or with slight difference. This increase in the bone length is the function of endochondral ossification while increase in diameter is the function of periosteal ossification. (Ahmed *et al.* 2003).

Urabe *et al.* (1999) in their study developed a rat fracture model and demonstrated that Hypothyroidism inhibits endochondral ossification, resulting in an impaired fracture repair process. This may be due to osteopenia resulting from the reduced bone growth, inhibi-

tion of bone apposition and return of bone resorption as was reported (Lewinson *et al.* 1989. Patton & Kaufinan, 1995. Bartalena *et al.* 1996 and Ribeiro, *et al.* 2004).

From the findings of the present study, it can be concluded that carbimazole produced hypothyroidism which retards the endochondral, periosteal bone growth as well as causes the shrinkage of adrenal cortex in 10 days (Sarwar *et al.* 2005) and thyroxin replacement though have mitigated the effect of hypothyroidism but a full recovery was not achieved. As is shown in present study it must be remembered that long-term, non-suppressive thyroxin treatment in goiter or hypothyroidism was associated with a slight reduction in QUS values, which was more pronounced in postmenopausal women. This group could be at higher risk for osteoporotic fractures. (Hadji, 2000). Our results do agree with others observations (Scow *et al.* 1949. Lewinson *et al.* 1989, Patton & Kaufinan, 1995, and Bartalena *et al.* 1996). It can be concluded that carbimazole induced hypothyroidism impede the endochondral as well as periosteal bone growth and bone mass density, with significant retardation in younger age group animals for hind limb.

Besides, further studies should be designed to evaluate the same cases on histological basis to see any change in the cartilages. The findings of the study are suggestive that the treatment of hyperthyroidism with carbimazole requires extra care and patient of the same must be evaluated on regular basis.

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