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Original Article

Role of Thyroxine (T_4) in Calcium, Phosphorus Metabolism in Tissues of Amphibia (Bufo melanostictus)

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Abstract

Current studies, find the role of thyroid hormone, thyroxine (T_4) in regulating the levels of calcium and phosphorous metabolism in bone, blood and muscle of Indian toad, *Bufo melanostictus*. Following seven days of thyroxine treatment, the body weight of toad increased significantly at both dose levels as compared to the control values. Thyroxine at lower doses showed a more pronounced effect with respect to the calcium and phosphorous parameters, corroborating the earlier view that the hormone is anabolic at lower doses, at least in poikilotherms. Both calcium and phosphorous in three tissues (blood, bone, muscle) showed higher levels following thyroxine treatment at higher doses. Such an observation possibly points to speculations that the hormone influences their levels by regulating their rate of absorption in the digestive tract or rate of excretion in kidney tubules. Alternatively one might suggest inhibitor of thyrocalcitonin by exogenous thyroxine directly through feed-back control or indirectly via the actions of parathyroid hormone.

Keywords: Thyroxine (T_4); Calcium; Phosphorus and Bufo Melanostictus.

Introduction

Ionic calcium is an integral requirement for many biological metabolic processes such as muscular contraction, nerve stimulation, change in permeability of cell membrane, the mediation of hormonal and cell receptor activities, fertilisation of the ovum, separation of chromosome in dividing cells, the beating of flagella, and the enzymatic cascade concerned with blood coagulation and so on. Its determination in biological system is extremely complex. Ionic calcium acts as a bridge between stimulus on one hand and biological and chemical responses on the other. It is useful for formation of bone and teeth. Deficiency develops rickets and muscle spasms.

Phosphorus or phosphate is important for formation of bone, teeth and bio-membranes. It keeps muscle and nerve activity normal, synthesis of nucleic acid (DNA, RNA) and ATP. Deficiency of phosphate causes loss of bone minerals, many metabolic disorders including cardiac muscle nerve disorder. It helps in energy transfer, cell division, and phosphorylation reactions etc.

Small quantity of calcium and inorganic phosphates present in the extracellular fluid are in equilibrium with the immense reservoir of calcium and phosphate in bones. The ions of calcium and phosphate are widely distributed in the extracellular fluid is under control by the parathyroid hormone (PTH). PTH influences both intestinal absorption of calcium, renal re-absorption of calcium and phosphates which are mediated through cholecalciferol (Vit-D_3). It mobilises calcium from the skeleton and calcitonin inhibits the release of calcium from the bones. Together they may be responsible for the remodelling and growth of bones. Cholecalciferol not only a vitamin to prevent rickets but also modulates the effects of PTH on osteoclasts in bone. Calcitonin is secreted when calcium level is high in the blood, then it lowers the calcium level by suppressing release of calcium ions from the bones. It influences tubular re-absorption of many ions like Ca, P, Na, K, Mg. Role of calcitonin in man is not clear. Thus calcitonin has an action opposite to that of PTH hormone on calcium metabolism.

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Bone formation involves the formation of matrix and deposition of calcium salts. These processes are influenced by a number of endocrine factors. The role of parathormone and of thyrocalcitonin in the control of calcium and phosphorous metabolism in tissues of vertebrates are not withstanding. There are few reports on the involvement of thyroid hormones in the metabolism of two parameters like estimation of calcium and phosphorous particularly in bone tissues. Adequate thyroid hormone is necessary for normal bone development. In hypothyroidism, skeletal growth and maturation are in paired while in hyperthyrodism children, linear growth is accelerated (Mundy and Raisz, 1979; Schlesinger et al., 1973). In adult hyperthyrodism, decreased bone mass is associated with increased osteoclastic activity and a high rate of bone turnover (Melsen and Mosekilde; 1977).In cartilage, thyroid hormone can enhance growth by increasing somatomedin production or sensitivity (Phillips and Vassilopoulou-Sellin, 1980 a; b; Thorngren and Hansson, 1977). The molecular mechanism by which thyroid hormone affects skeletal development is unclear (Raisz and Kream, 1981). In spite of increased osteoclastic bone re-absorption by thyroid hormones invitro, it could be responsible for enhanced bone turnover and remodelling (Mundy et al., 1976).In the view of above findings, in the present study, the calcium and phosphorus turnover in some tissues like bone, muscle and blood of Indian toad, *Bufo melanostictus* by thyroxine (T_4) treatment is accessed.

Materials and Methods

The common Indian toad, *Bufo melanostictus* of mixed sexes were collected from nature during evening time and were transferred to the laboratory within 12 hours. They were maintained in lab condition in wire netted wooden cages (75 x 40 x 35 cm in size) containing a moist sand bed for about 5 days. They were forced feed with about 1 gm of goat liver (Composition mg/gm wet-weight: 110 $\pm$41 protein, 84 $\pm$ 16 lipid, 2.3 $\pm$1.1 glycogen) each on every day and water was provided *ad libitum*.

Treatment

After laboratory acclimation, animals were divided into control and experimental groups. The experimental groups of toad were injected intramuscularly with thyroxine ($T_{„}$) Na-salts (fluke AG) at a dose of 0.5 µg / gm (Treated – I) and 2.0 µg / gm (Treated – II) in separate batches, dissolved in 0.65 % of NaCl solution pH = 8.3. The control animals received an equal volume of 0.65 % of NaCl solution pH = 8.3. This injection schedule was continued daily for 7 days so that each animal receives 7 doses. On the eighth day of treatment, the animals were sacrificed for estimation of biochemical parameter after taking their final body weight.

Collection of Tissues extract

At the end of the treatment, the animals were sacrificed by pitching on the head; blood, muscles & bone were quickly dissected out. Blood was collected from heart of the animal with the help of a hypodermic syringe containing 2ml of 2% sodium citrate solution. The muscles from hind limb were transferred to cold Amphibian Ringer solution and adherent connective tissues, blood vessels, nerve fibres were removed. Then blotted off with whatman filter paper No – 1. Long bones (Humerus) were taken out and cleaned off adherent materials in distilled water. All these 3 tissue extracts were collected by centrifuging at 2000 rpm for 10 minutes; those were used for estimation of biochemical parameters.

Estimation of Calcium

The calcium contents of 3 extracts of Bone, Muscle &Blood were estimated independently by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925).

Estimation of Phosphorus

The phosphorus contents of 3 extracts of Bone, Muscles & Blood were estimated by the method of Fiske and Subbarow (1925).

These data were statistically analyzed by the student ***t – test*** (Abramoff and Thomson, 1966; Bishop, 1966.

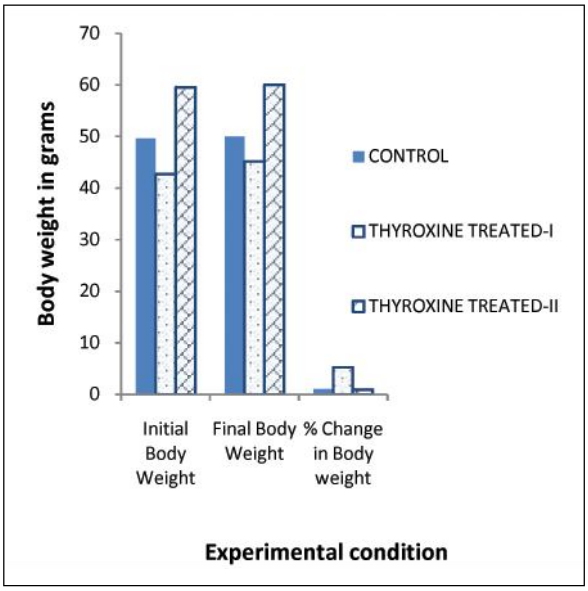
Results

Body weight: During the course of experiment the body weight of all animals have been found to increase. But the rate of increase (% change) in body weight based on individual values were found to be significantly high in T_4 treated animals as compared to that of the control animals (table-1, Fig-1).

Table-1: *In vivo* affects of thyroxine (2 doses) on body weights of Indian toad, *Bufo melanostictus. Values for initial and final body weights in grams (mean values). The % changes in body (mean value) are based on the individual values of initial and final body weights. Numbers in parentheses indicate the number of animals used.*

Experimental Condition	Initial Body Weight	Final Body Weight	% Change in Body weight
Control	49.7 (24)	50 (24)	1.058
Thyroxine treated-i	42.77 (22)	45.18 (22)	5.29
Thyroxine treated-ii	59.55 (20)	60.1 (20)	0.91

Fig. 1: Effects of thyroxine (0.5µg/gm; 2 µg/gm) on body weight and % change in body weight after 7 days of treatment, of *Bufo melanostictus*. Values for body weight are in gram. Columns represent the mean values



Calcium content

Thyroxine treatment for 7 days did not change the calcium content in any of the tissues studied at lower doses (0.5 mg / g) when compared to the control values. Thyroxine treatment at higher doses (2.0mg/g) increased significantly (P< 0.001) the levels of calcium in blood, muscle & bone after 7 days of treatment.

A comparison of data from two treated doses showed significantly high levels of calcium in all the 3 tissues of higher dose treated animals. (Figs. 2-4, table 2-4).

Phosphorus content

Thyroxine treatment for 7 days did not change the phosphorous content in bone & muscle tissues studied at lower doses (0.5mg/g) when compared to the control values, but in blood the content decreased significantly when compared to the control values.

Thyroxine treatment at higher doses (2.0mg/g) increased significantly (P<0.001) the levels of phosphorus in blood, bone, and muscle after 7 days of treatment.

A comparison of data from two treated doses showed significantly high level of phosphorus in bone, muscle (P0.001) and blood (P<0.01) of higher dose treated toads (Fig. 2-4; table 2-4).

Table 2: *In vivo* effects of thyroxine (2 doses) on calcium and phosphorous levels in blood of Indian toad, *Bufo melanostictus. Values are mg/100 ml of blood (Mean±SEM). Numbers in parentheses indicate the number of animals used, NS, not significant at 0.05 confidence level.*

Experimental Condition	Calcium	Phosphorous
Control	40.79 ± 2.18 (12)	3.1 ± 0.204 (9)
P	NS	< 0.05
Thyroxine treated-i	43.67 ± 1.02 (6)	2.47 ± 0.241 (8)
P	< 0.001	< 0.01
Thyroxine treated-ii	80.74 ± 4.26 (9)	4.24 ± 0.36 (8)
P	< 0.001	< 0.001

Fig. 2: Effects of thyroxine (0.5µg/gm;2µg/gm)on the calcium and phosphorous content of blood after 7 days of treatment.Values for calcium and phosphorous are mg/100 ml of blood; columns represent the mean values and vertical bars SEM.

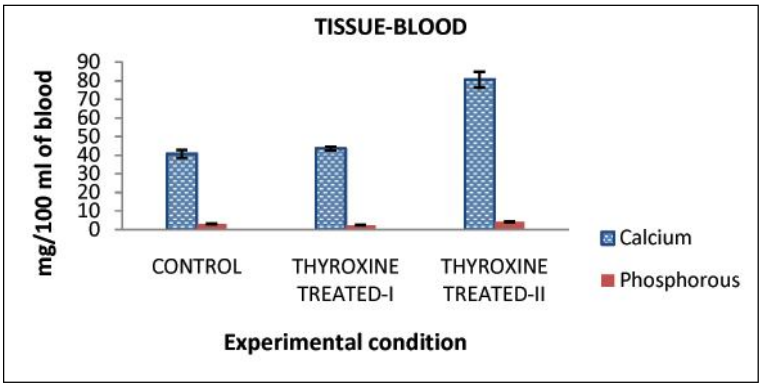


Table 3: *In vivo* effects of thyroxine (2 doses) on calcium and phosphorous levels in muscle of Indian toad, *Bufo melanostictus. Values are mg/gm t*issue wet-weight *(Mean±SEM). Numbers in parentheses indicate the number of animals used, NS, not significant at 0.05 confidence level*

Experimental Condition	Calcium	Phosphorous
Control	0.409 ± 0.018	0.177 ± 0.0089
P	(10)	(14)
	NS	NS
Thyroxine treated-i	0.442 ± 0.026	0.179 ± 0.015
P	(9)	(8)
	< 0.001	< 0.001
Thyroxine treated-ii	0.94 ±0.030	0.38 ± 0.014
P (between control and treated-"ii")	(9)	(10)
	< 0.001	< 0.001

Fig. 3: Effects of thyroxine (0.5µg/gm;2µg/gm)on the calcium and phosphorous content of muscle after 7 days of treatment. Values for calcium and phosphorous are mg/gm tissues wet weight; columns represent the mean values and vertical bars SEM.

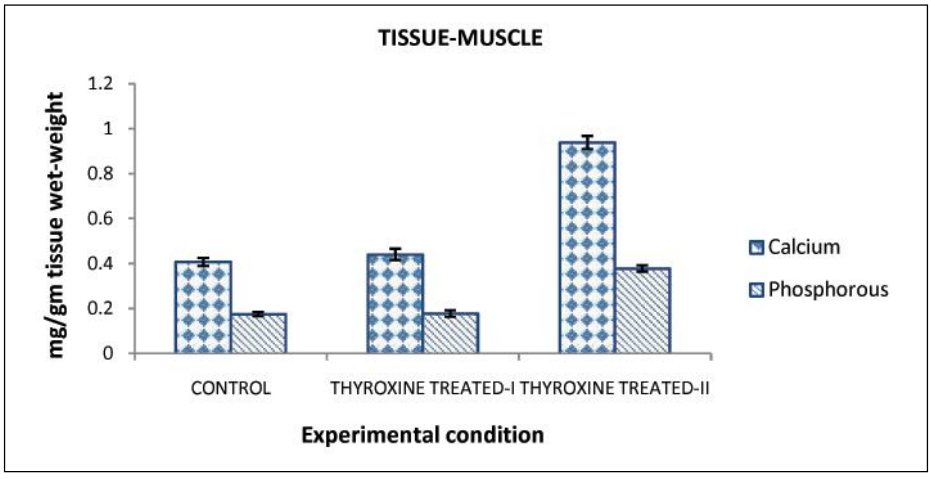
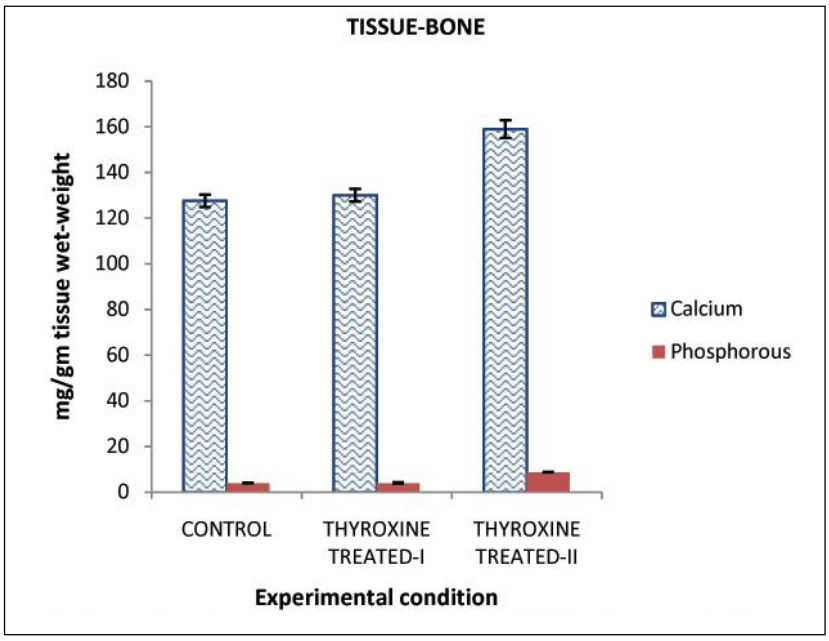


Table 4: *In vivo* effects of thyroxine (2 doses) on calcium and phosphorous levels in bone of Indian toad, *Bufo melanostictus. Values are mg/gm t*issue wet-weight *(Mean±SEM). Numbers in parentheses indicate the number of animals used, NS, not significant at 0.05 confidence level.*

Experimental Condition	Calcium	Phosphorous
	127.63 ± 2.72	3.99 ± 0.275
Control	(14)	(15)
P	NS	NS
	130.12 ± 2.74	3.95 ± 0.37
Thyroxine treated-i	(6)	(9)
P	< 0.001	< 0.001
	159.06 ± 3.95	8.73 ± 0.16
Thyroxine treated-ii	(10)	(10)
P	< 0.001	< 0.001

Fig. 4: Effects of thyroxine (0.5µg/gm;2µg/gm)on the calcium and phosphorous content of bone after 7 days of treatment. Values for calcium and phosphorous are mg/gm tissues wet weight; columns represent the mean values and vertical bars SEM.



Discussion

The thyroid gland synthesises and releaseT_3 and T_4. The biological active hormones T_3 (K.Boelaert, JA Franklyn, 2005) and T_4 play a significant role in growth, development and function of all major tissues. The hormone thyroxine appearing during the course of evolution in vertebrates only, has attracted the attention of biologists as a substances involved in the control of cell multiplication (Rudland and Jumnez, 1976; Gibson et al., 1978). The thyroid hormones increase cellular respiration and thereby increase the basal metabolic rate (BMR) (Jack DeRuiter, 2001).This hormone appears to have regulatory influence on almost all the metabolic events, although the mechanism of action at subcellular level has become a subject of intensive investigative effort and furious debate in recent years. Like steroid hormones, the thyroid hormones are relatively hydrophobic and lipophilic, solubilised and transported in the plasma with aid of carrier proteins and exert their action intra-cellularly after penetrating the plasma membrane (Kochupillai and Ramalingaswami, 1990).

Thyroid hormone synthesis and secretion is regulated by the negative feedback system that involves the hypothalamus, pituitary and the thyroid gland (Shupnik et al, 1989). Thyroid hormones are particularly important as regulators of differentiation during development. A closely related function is that of a stimulator of oxidative reactions and general regulators of metabolic rates in the body. Increased thyroid hormone is associated with increased oxygen consumption, body temperature, pulse systolic blood pressure, mental and physical vigour, irritability, the lipolysis and decreased cholesterol level in blood. Although a number of effects of thyroid hormones on specific metabolic reactions have been demonstrated, a unifying concept of mechanism is not yet apparent. This is, in part, due to the different effects noted when the hormone is studied at physiologic levels or at unphysiologic high doses.

Thyroid hormone involvement in ionic regulation is general and metabolic pathway like calcium & phosphorus in particular. Small amounts of thyroxine given to young growing animals enhance the retention of calcium. This is probably secondary effect resulting from the protein anabolic action of the hormone which facilitates the deposition of new bone matrix. In hyperthyroid stages, there is increased mobilisation of calcium from the skeleton and increased loss through urine and faeces without affecting the Ca concentration of blood (Turner, 1966). Thyroid hormone not only controlling the Ca & P metabolism, but also influences the metamorphosis of tadpole larvae in general and ossification process in particular (Duellman and Trueb, 1986) promoted us to carry out this study. The dose and route of administration of thyroxine to these animals was determined following the reports of several workers (Medda & Ray; Ghosh, 1982, Begum *et al*, 1984, Achary, 1986). These authors have clearly shown that thyroid hormones are anabolic at lower

doses and catabolic at higher doses. However species specific differences in the responses to doses of thyroxine have been observed. A dose considered anabolic for a species may have catabolic effects on the other.

Administration of thyroxine to toads, every day for 7 days caused an overall increase in the body weight at both dose levels. However the percentage increase in the body weights in animal treated with lower (0.5 µg / g) dose of thyroxine was higher than those treated with higher (2µg / g) dose. However these results are consistent with the observations in certain groups fishes (Barrington *et al.* , 1961 ; Higgs *et al.* , 1961; Higgs *et al.,1976*) , reptiles (Achary, 1986) and birds (Thapliyal *et al*.,1983). Administration of thyroid hormones to premetabolic tadpoles induced myofibrillar protein synthesis and muscle fibre formation in undifferentiated tadpole hind limb tissues (Dhanarajan, 1980).

One might, therefore speculate similar anabolic changes with respect to nitrogen metabolism in adult amphibians of the present study. Adequate thyroid hormone is necessary for normal bone development. Data on Ca & P content of bone, blood & muscle of adult toads showed increased levels following thyroxine treatment at higher doses.

These observation clearly points to increased retention of these substances in the tissues of toads by thyroxine, making them sufficiently available for incorporation into bones and / or to be utilised by other tissues for different metabolic processes. Such retentions of Ca & P could possibly be mediated through an increase in somatomedin production or sensitivity (Phillips and Vassilopoulou-Sellin, 1980 a, b; Thorngren and Hansson, 1977). Another possibility is that thyroxine might be causing Ca & P retention by way of influencing the rates of their absorption in the digestive tract or the rates of their excretion by the kidney tubules.

Exogenous thyroxine as is administered in the present study, might be inhibiting thyroid activity in toads, thus reducing the release of such a factor, consequently leading to increase in the concentrations of both calcium & phosphorus. PTH involved in Ca & P metabolism, however there has been lot of debates as to which of these two (parathormone or thyrocalcitonin) is the actual agent for the control of Ca & P (Turner, 1966).

It has been suggested that parathyroid itself is not the source of hypocalcemic principle but that the parathyroid produce a humoral substance (releasing factor) which stimulate the thyroid to release thyrocalcitonin. In view implies that parathyroid and thyroid tissues are needed for the regulation of Ca & P in animals (Gittes and Irvin, 1965)

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