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Impact of T_3 on Calcium and Phosphorus Metabolism in Tissues of *Bufo Melanostictus*

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Abstract

The two most important macrominerals calcium and phosphorus are required for bone growth and body function of animals. The present study is designed to investigate the role of thyroid hormone i.e. triiodothyronine (T_3) in regulating the levels of calcium and phosphorus in blood, muscle and bone of Indian toad, *Bufo melanostictus*. The response of poikilotherms to the thyroid hormones is dose dependent. The calcium and phosphorus content of three extracts of blood, muscle, bone tissues were estimated independently by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925) and the method of Fiske and Subbarow (1925) respectively. There is an enhancement of calcium and phosphorus concentrations in blood, muscle and bone tissue at both the dose levels except for bone at higher dose T_3 treatment for 7 days. Treated animals show an increased level of calcium and decreased level of phosphorus of bone. Tissue specific action showing the increase in calcium and phosphorus levels at higher dose is much more as compared to that of lower dose level in blood and muscle tissue. In contrast to young and immature animals, the adults and old animals may show certain differential response because of the interplay of other hormones such as gonadal and corticosteroids & growth hormone which appears in adult stage.

Keywords: Triiodothyronine(T_3); Calcium; Phosphorus; *Bufo Melanostictus.*

Introduction

Thyroid hormone (T_4& T_3) arguably the most important hormone in anuran development and affects through exogenous and endogenous means. It also plays an important role in calcium and phosphorous homeostasis by their action on turnover of bone. The thyroid hormone stimulates the ossification of cartilage, linear growth of bone, maturation of epiphyseal bone centres and closure of epiphyses (Reddy et al., 2012). Thyroid hormone plays a vital role in the metabolic processes of animals.

T_3 does not act directly on bone cell and cartilage but potentiate the effect of growth hormone or other growth factors on bone growth. Therefore, T_3 does not act directly on bone growth process but helps in bone maturation. Thyroid hormone T_3 is required for skeletal development during childhood. It regulates bone turnover and mineralisation in adult. Thyroid hormone (T_3) is essential for normal development of endochondral and intra-membranous bone and plays an important role in the linear growth and maintenance of the bone mass. In childhood, retardation of skeletal development and growth are arrested by deficiency of T3 whereas excess T3 accelerates the growth and bone formation. In thyrotoxicosis, there is increase in bone remodelling with imbalance between bone resorption and formation (Bassett and Williams, 2003). T_3 exerts anabolic action during growth to stimulate peak bone mass accrual but has catabolic effects on the adult skeleton that increases the bone turnover. Recent studies suggest that TSH may have direct action in bone cells (Williams, 2009).

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The two elements like calcium and phosphorus are most important macrominerals required for bone growth and body function of animals. Bone tissue serves as reservoir of calcium to maintain the constant concentration in blood, muscle and intracellular fluids. The balance between bone resorption and formation changes with age. The mineral content of bone increases throughout childhood (Lu et al., 1994), peak in adolescence(Bailey, 2000) and remain relatively constant in early or late adulthood (Teegarden et al., 1995) and declined in old age. Calcium ion functions as a signal for many cellular processes like hormonal control of cell function and influences a wide variety of metabolic processes (Martin et al., 1975) play an important role in muscle contraction, working of heart, metabolism, blood clotting, enzyme activity, neuromuscular function, cell adhesion and intracellular signalling (Veum, 2010). Phosphorus plays a major role in multiple biological processes (Berndt et al., 2005) like energy metabolism, cell signalling, acid-base regulation, buffering, regulation of protein synthesis, skeletal development and bone mineralization or bone integrity (Alizaden- Naderi & Reilly, 2010).

Materials and Methods

The common Indian toad, *Bufo melanostictus* of mixed sexes were collected from wild 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 wetweight: 110 +41 protein, 84 + 16 lipid, 2.3 +1.1 glycogen) each on every day and water was provided adlibitum.

Treatment

After laboratory acclimation, animals were divided into control and experimental groups. The experimental groups of toad were injected intramuscularly with thyroxine (T_3) Na-salts (flukeAG) 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 pithing 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 analysed by correlation (correlation coefficient "r").

Results

From our findings it is clear that thyroid hormone action (T_3) is tissue specific.

In long term (7 days) T_3 treatment to these animals caused enhancement of calcium and phosphate concentrations in blood, muscle and bone tissue at both the dose levels except for in bone at higher dose treatment. There is an increased level of calcium and decreased level of phosphorus of bone. The increase in calcium and phosphorus levels at higher dose is much more as compared to that of lower dose level in blood and muscle tissue.

Seven days lower dose (0.5μg/gm): The calcium content of blood decreased upto 1 Yr then remained constant in contrast to controls where the calcium content decreased significantly, whereas the calcium content in muscle and bone show biphasic (decreased up to 1 yr, then increased after 1 yr up to 2 yrs, and declined thereafter with the age) and triphasic (decreased up to 1 yr then remained constant after 1 yr up to 3 yrs and further declined with the age)

characteristics respectively as compared to control animals (Fig. 1.1, Fig. 1.2, Fig. 1.3).

The Phosphorous content of blood decreased with increasing body weight upto 2 yrs, then remained constant upto 3 yrs and finally declined thereafter. In both muscle and bone tissue the phosphorus content decreased up to 1 yr with increasing body weight. Then increased gradually up to 2+ yrs and subsequently declined with the ageas compared to control animals (Fig. 2.1, Fig. 2.2, Fig. 2.3).

Seven days higher dose (2µg/gm): The calcium of blood tissue of treated animals decreased significantly compared to control animals. T_3 treated ($P<0.001$) animals it exhibited triphasic characteristic. It decreased up to 1 yr, then increased after 1 yr up to 2 yrs with increase in the body weight. Finally it declined after 2 yrs with the age in both muscle and bone (Fig. 1.4, Fig. 1.5, Fig. 1.6).

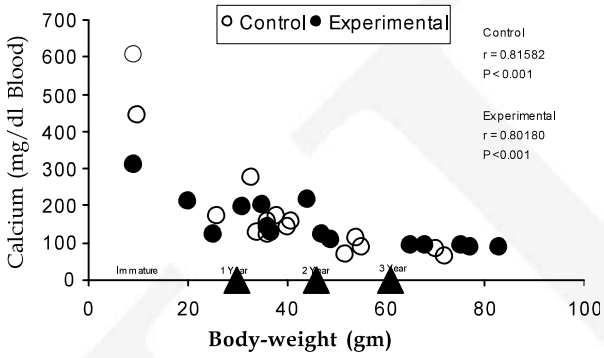

Fig. 1.1: Correlation of calcium content in blood of control (r = 0.81582; P < 0.001) and T3 (r = 0.80180; P < 0.001) treated toads, *Bufo melanostictus* through different ages. Values are mg/dl of blood. Dose - low dose (0.5 µg/g). Duration – 7 days (multiple treatments).

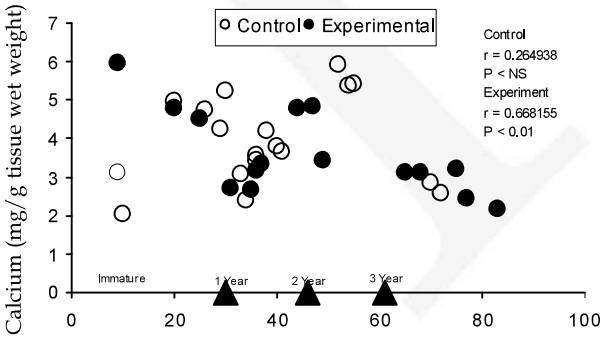

Fig. 1.2: Correlation of calcium content in muscle of control (r = 0.264938; P, NS) and T3 (r = 0.668155; P < 0.01) treated toads, *Bufo melanostictus* through different ages. Values are mg/g tissue wet weight. Dose - low dose (0.5 µg/g). Duration – 7 days (multiple treatments).

The phosphorus content of all the 3 tissues (blood, muscle and bone) exhibited a triphasic characteristic. It primarily decreased up to 1 yr, then increased after 1 yr up to 2+yrs and terminal decrease beyond that with increase in the body weight as compared to control animals. However, enhancement of calcium and phosphate concentrations in blood, muscle and bone tissue at both the dose levels except for in bone at higher dose treatment Fig. 2.4, Fig. 2.5, Fig. 2.6).

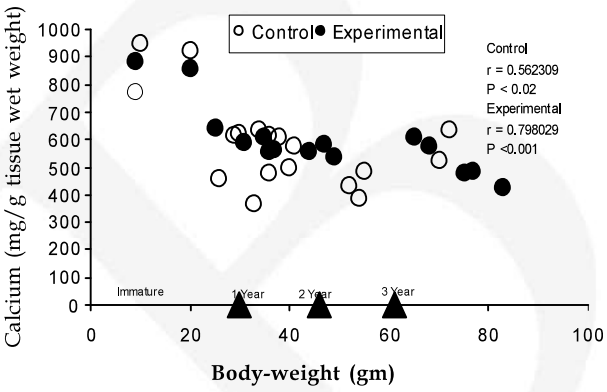

Fig. 1.3: Correlation of calcium content in bone of control (r=0.562309; P< 0.02) and T3 (r = 0.798029; P< 0.001) treated toads, *Bufo melanostictus* through different ages. Values are mg / g tissue wet weight. Dose-low dose (0.5 µg/g). Duration–7 day (multiple treatments)

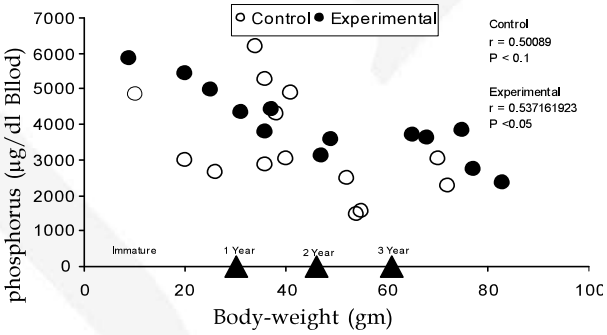

Fig. 2.1: Correlation of phosphorus content in blood of control (r = 0.50089; P<0.1) and T3 (r=0.537161932; P<0.05) treated toads, *Bufo melanostictus* through different ages. Values are µg/dl of blood. Dose-low dose (0.5 µg/g). Duration–7 days (multiple treatments)

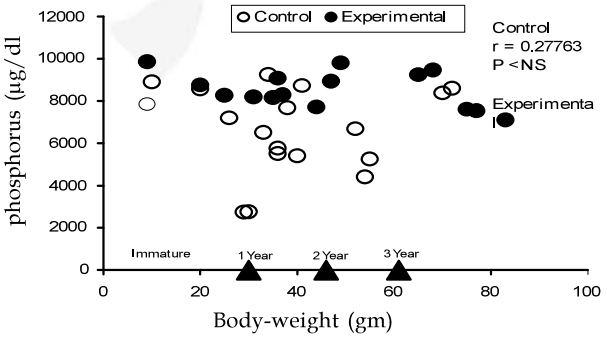

Fig. 2.2: Correlation of phosphorus content in muscle of control (r = 0.27763; P, NS) and T3 (r=0.426358; P< 0.2) treated toads, *Bufo melanostictus* through different ages. Values are µg/g tissue wet weight. Dose-low dose (0.5 µg/g). Duration –7days (multiple treatments)

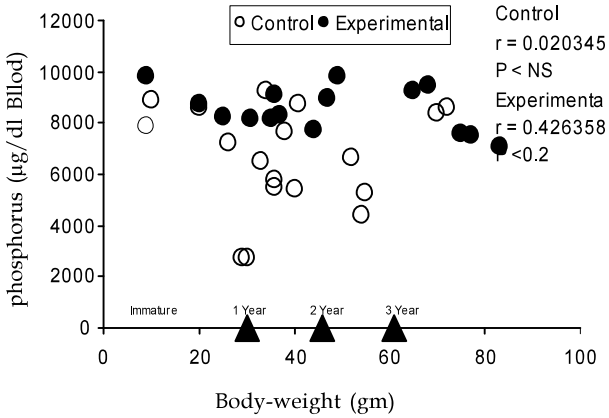

Fig. 2.3: Correlation of phosphorus content in bone of control (r = 0.020345; P, NS) and T3 (r = 0.426358; P < 0.2) treated toads, *Bufo melanostictus* through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/g). Duration – 7 days (multiple treatments)

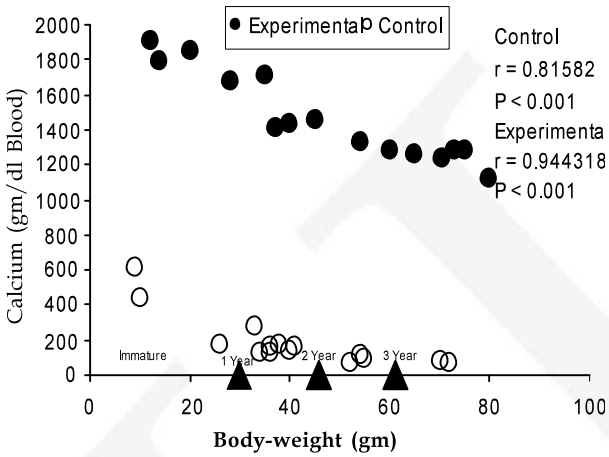

Fig. 1.4: Correlation of calcium content in blood of control (r = 0.81582; P < 0.001) and T3 (r = 0.944318; P < 0.001) treated toads, *Bufo melanostictus* through different ages. Values are mg/dl of blood. Dose - high dose (2 µg/g). Duration – 7 days (multiple treatments)

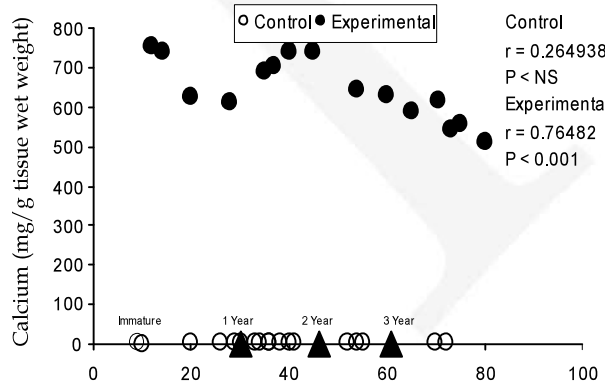

Fig. 1.5: Correlation of calcium content in muscle of control (r = 0.264938; P,NS) and T3 (r = 0.76482; P<0.001) treated toads, *Bufo melanostictus* through different ages. Values are mg/g tissue wet weight. Dose - high dose (2 µg/g). Duration–7 days (multiple treatments).

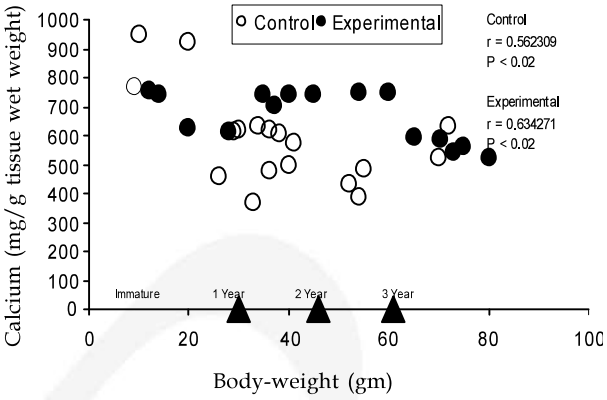

Fig. 1.6: Correlation of calcium content in bone of control (r= 0.562309; P<0.02) and T3 (r=0.634271; P<0.02) treated toads, *Bufo melanostictus* through different ages. Values are mg/g tissue wet weight. Dose-high dose (2 µg/g). Duration–7day (multiple treatments)

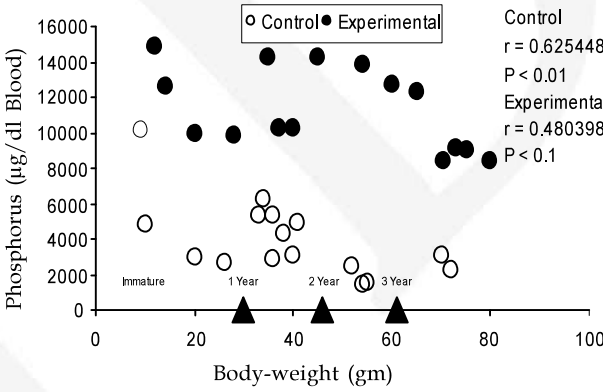

Fig. 2.4: Correlation of phosphorus content in blood of control (r =0.625448; P<0.1) and T3 (r=0.480398; P<0.1) treated toads, *Bufo melanostictus* through different ages. Values are µg/dl of blood. Dose-high dose (2 µg/g). Duration–7 days (multiple treatments)

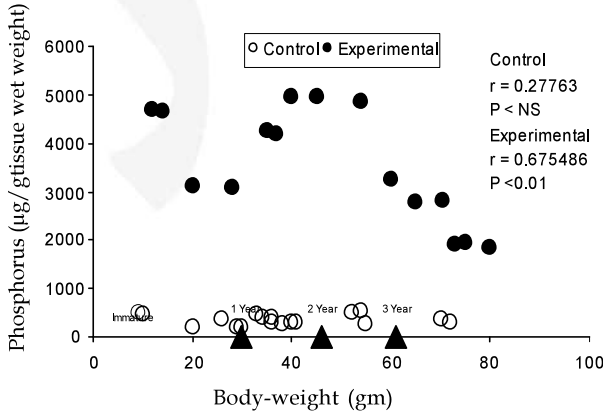

Fig. 2.5: Correlation of phosphorus content in muscle of control (r = 0.27763; P, NS) and T3 (r = 0.675486; P < 0.01) treated toads, *Bufo melanostictus* through different ages. Values are µg/g tissue wet weight. Dose - high dose (2 µg/g). Duration – 7 days (multiple treatments)

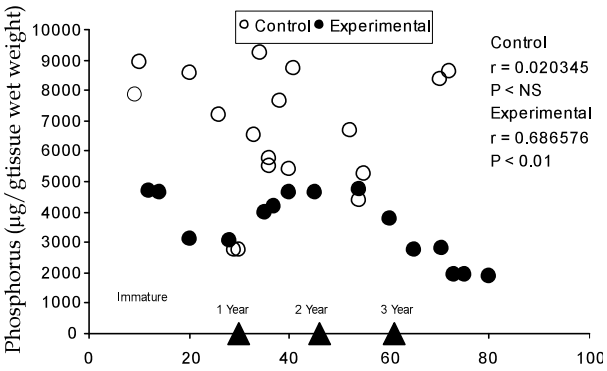

Fig. 2.6: Correlation of phosphorus content in bone of control (r = 0.020345; P, NS) and T3 (r = 0.686576; P < 0.01) treated toads, *Bufo melanostictus* through different ages. Values are µg/g tissue wet weight. Dose - high dose (2 µg/g). Duration – 7 days (multiple treatments)

Discussion

In 7 days T_3 treatment to these animals caused enhancement of calcium and phosphate concentrations in blood, muscle and bone tissue at both the dose levels except in bone at higher dose treatment. There is an increased level of calcium and decreased level of phosphorus of bone. The increase in calcium and phosphorus levels at higher dose is much more as compared to that of lower dose level in blood and muscle tissue. It clearly indicates that the thyroid hormone action is both catabolic and anabolic at lower and higher doses respectively. In bone it showed a reciprocal relationship in between calcium and phosphate levels. The increasing trend or the rate of increase at high dose is much high as compared to the lower dose in muscle and blood in subsequent age groups. These observations clearly point to increased retention of these metabolites in tissues of toads by the thyroid hormone making them sufficiently available for incorporation into bones and/or to be utilized by other tissues for different metabolic purposes. Such retentions of both calcium and phosphorus could possibly be mediated through an increase in the somatomedin production or sensitivity (Phillips and Vassilopoulou-Sellin, 1980a, b). Another possibility is that this hormone might be causing calcium and phosphorus retention by way of influencing the rates of their absorption in the digestive tract or the rates of their excretion by the kidney tubules.

Such an observation possibly points to speculation that the hormone influences their levels by regulating their rate of absorption in the digestive tract or rate of excretion at kidney tubules. Calcium and Phosphate homeostasis implies the balance between the calcium & phosphorus minerals among serum and bone while muscle is greatly responsible for utilization of both calcium and phosphorus. In 7 days T_3 treatment, calcium and phosphorus concentration in the blood, muscle and bone at both the dose level decreased showing biphasic and triphasic characteristics. It is clearly indicated that the catabolic effect of thyroid hormone in lower dose and anabolic effect in higher. In our investigation, an age related variations in the response to T_3 is found out. Moreover, in sharp contrast to young and immature animals, the adults and old animals may show certain differential response because of the interplay of other hormones such as gonadal and corticosteroids which appears in adult stage. The action of thyroid hormone is also dependent on the state of thyroidal activity, peripheral deiodination and the level of circulating thyroid stimulating hormone.

The dose of the hormone used, the route of administration, the duration of treatment, environmental temperature, photoperiod, nutritional status, age and seasonal activation appears to be the important factors involved in thyroid hormone action. Moreover, one should not ignore the fact that, a very significant role is played by the thyroid hormones during amphibian metamorphosis when these organisms transform the cartilaginous larval skeleton to calcified and bony skeleton of terrestrial young ones. Therefore, as far as the influence of thyroid hormones on the calcium and phosphorus metabolism is concerned, an extensive study is warranted taking metamorphosing tadpole larvae and the freshly migrated terrestrial young toads as the experimental models before drawing any significant conclusion.

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