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Thyroxine Induced Changes in Collagen Characterstics in the Tissue of Bone in Common Toad

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Abstract

The tensile strength of bone derived from collagen fibres, extend primarily along the lines of tensile force. For normal development and function of human skeleton, thyroid hormones are vital, which evidently visible in hyper- and hypothyroid conditions. Earlier studies suggest that, Thyroid effect on collagen is dose dependent, tissue specific and sometimes controversial. Hypothyroidism causes impaired bone formation and growth retardation whereas hyperthyroidism may retard collagen synthesis and decelerate the bone mass. Hydroxyproline and hydroxyllysine are essential for the collagen cross linking and strength of the bone. Hydroxyproline estimation is considered as an index of collagen metabolism. The aim of the present study is to find out the changes in collagen characteristics in bone tissue of thyroxine treated common Indian toad. As common toad (*Duttaphyrnus melanostictus*) is studied extensively to have an elementary knowledge on the anatomy of vertebrates, the above species is used as the experimental organism in the present study. The method of Neuman and Logan (1950) modified by Leach (1960) was used to find out the hydroxyproline content from which the collagen contents were found out at two different doses of thyroxine (2.0 µg/gm & 0.5 µg/gm).

Keywords: Bone; Collagen, Thyroxine, Hydroxyproline.

Introduction

Thyroid hormones secreted from thyroid gland are important regulators of connective tissues both in vivo and in vitro. Bone as a connective tissue is largely influenced by thyroid hormone concentration, which is evidently visible in hyperthyroidism and hypothyroidism. It is a unique tissue made up of minerals, various proteoglycans, collagens, glycoproteins, gamma-carboxyglutamic acid-containing proteins and many others. The organic matrix of bone is 90 to 95 percent collagen fibres which gives powerful tensile strength to it. In the other hand thyroid hormones have drastic effect on collagen synthesis and degradation. Any direct and indirect effect can tend to alter the structure and strength of collagen fibres are likely to have significance repercussions on the physical structure of organism as a whole. Hydroxyproline and hydroxyllysine are essential for the collagen cross linking and strength of the fibre. Most, if not all, of the hydroxyproline in collagen appears to be derived from proline. It has been proposed by several investigators on indirect evidence, that it is a bound form of prolinewhich is hydroxylated and incorporated into collagen (Ernest et al., 1960).

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Examination of hydroxyproline status can be considered as an index of collagen metabolism.

Aim and Objective

The present study aims to find out the changes in bone collagen characteristics in *Duttaphrynus melanostictus* through T_4 administration at two different doses of 2.0 µg/gm (High dose) and 0.5 µg/gm (Low dose).

Choice of Parameters

The various biochemical parameters were found outfollowing Thyroxine (T_4) treatment at doses of 2.0 µg/gm and 0.5 µg/gm (Low dose) in bone tissue of toad. The parameters are as follows.

1. Salt soluble collagen,
2. Acid soluble collagen,
3. Insoluble collagen,
4. Total collagen content
5. Salt soluble/salt insoluble collagen ratio
6. Acid soluble/acid insoluble collagen ratio
7. % of salt solubility
8. % of acid solubility

Materials and Method

Common toad (*Duttaphrynus melanostictus)* were collected from the nature of both sex types and transferred into the wire-netted wooden cage (75 × 14 × 35 cm in size) in the laboratory containing sand bed. They were maintained in laboratory condition for about 5 days. The animals were forced fed with goat liver, (composition mg/g wet weight: 110 ± 41 protein, 84 ± 16 lipid, 2.3 ± 1.1 glycogen) and water was provided *ad libitum.*

After laboratory acclimation animals of mixed sexes were divided into two groups, i.e. control (CONT) and experimental (EXPT). Experimental groups were again divided into two groups & were treated intramuscularly with Thyroxine (T_4) Na salts (fluka AG) of doses 2.0 µg/gm and 0.5 µg/gm body wet weight. The control animals were received an equal volume of 0.65% of NaCl solution of pH 8.3. The treatment was carried out for 7 days and on the 8th day, body weights were taken and were sacrificed in separate batches for estimation of biochemical parameters.

Tissue Prosseing and Collagen Estimation

Bone tissue was taken for extraction of different collagen fractions by using Neuman and Logan (1950) method modified by Leach (1960). To evaluatethe statistical significance of the data student's *t* test was applied.

Results

Salt Solubility

The salt soluble collagen decreased significantly (p < 0.002) at T_4 high dose and increased insignificantly p, NS) at T_4 low dose as compared to control animals. Wherever, the % of salt solubility and salt soluble/ salt insoluble collagen ration increased insignificantly in T_4 high dose, but bothdecreased in T_4 low dose, as compared to control animals (Table. 1, 2 & Figs. 1,3,4).

Acid Solubility

There was an insignificant decrease in acid soluble collagenat T_4 high dose and insignificant increase in in T_4 low dose as compared to control animals. The % of acid solubility and acid soluble/insoluble

Table 1: Effect of thyroxine (T_4) (2.0 µg/gm body wt.) on collagen characteristics of bone tissue in Common Indian Toad. Values for soluble, insoluble and total collagen are mg/gm tissue wet-weight (Mean ± SEM), Numbers in parentheses indicate sample size, NS, Not significant, at 0.05 confidence level.

	T4 High Dose							
	Salt soluble	**Acid soluble**	**Insoluble**	**Total**	**% of salt solubility**	**% of acid solubility**	**Salt soluble/ Salt Insoluble**	**Acid soluble/ Acid insoluble**
Control	10.83 ± 0.89 (8)	8.48 ± 0.64 (8)	20.03 ± 2.14 (8)	39.35 ± 3.20 (8)	27.75 ± 1.37 (8)	21.87 ± 1.35 (8)	0.387 ± 0.026 (8)	0.445 ± 0.04 (8)
p	p < 0.002	p, NS	p < 0.001	p < 0.001	p, NS	p, NS	p, NS	P,NS
Experimental	6.98 ± 0.476 (8)	7.02 ± 0.72 (8)	6.13 ± 0.526 (8)	20.12 ± 1.105 (8)	34.72 ± 1.413 (8)	34.30 ± 2.44 (8)	0.53 ± 0.034 (8)	1.23 ± 0.21 (8)

Table 2: Effect of thyroxine (T_4) (0.5 µg/gm body wt.) on collagen characteristics of bone tissue in Common Indian Toad. Values for soluble, insoluble and total collagen are mg/gm tissue wet-weight (Mean ± SEM), Numbers in parentheses indicate sample size, NS, Not significant, at 0.05 confidence level.

T4 Low Dose								
Experimental condition	**Salt soluble**	**Acid soluble**	**Insoluble**	**Total**	**% of salt solubility**	**% of acid solubility**	**Salt soluble/Salt insoluble**	**Acid soluble/ Acid insoluble**
Control	5.67	6.53	20.84	33.07	17.61	19.95	0.219	0.338
±	±	±	±	±	±	±	±	
0.60	0.809	2.233	1.94	2.52	2.32	0.039	0.057	
(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	
p	*p*, NS	*p*, NS	*p*, NS	*p*, NS	*p*, NS	*p*, NS	*p*, NS	*p*, NS
Experimental	6.445	6.94	26.05	39.43	16.51	17.73	0.198	0.27
±	±	±	±	±	±	±	±	
0.298	0.228	1.74	1.56	1.112	0.847	0.0159	0.017	
(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	

collagen ratio increased insignificantly by the administration of T4 at a dose of 2.0 µg/gm and decrease in dose of 0.5 µg/gm body wt. (Table. 1, 2 & Figs. 1,3,4).

Insoluble Collagen

The average insoluble collagen decreased significantly ($p < 0.001$) at T_4 (2.0 g/gm) and increased insignificantly (*p*, NS) at T_4 (0.5 g/gm) as compared to control animals (Table: 1, 2 & Fig: 1).

Total Collagen Content

The average total collagen from bone tissue of T4 (2.0 µg/gm) treated animals decreased significantly ($p < 0.001$) and increased insignificantly (*p*, NS) at T4 (0.5 µg/gm) in bone tissue of treated animals as compared to control animals statistically (Table. 1, 2 & Fig. 2 a,b).

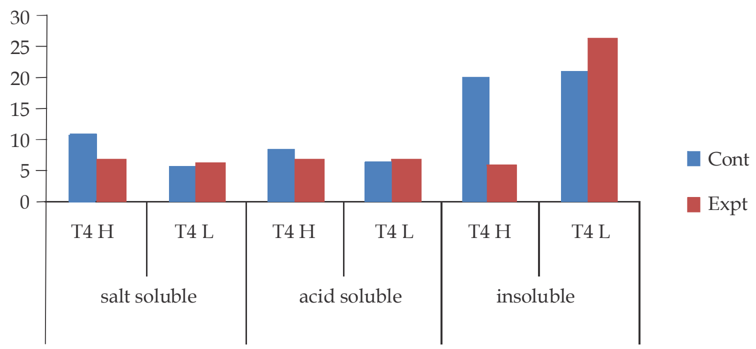

Fig. 1:

Salt soluble, acid soluble, insoluble collagen fraction in bone tissue of *Duttaphrynus melanostictus* by administration of T_4 (2.0 µg/gm and 0.5 µg/gm). Values are mg/gm tissue wet wt., columns represents the mean values.

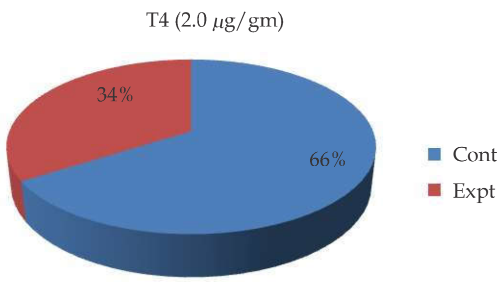

Fig. 2a:

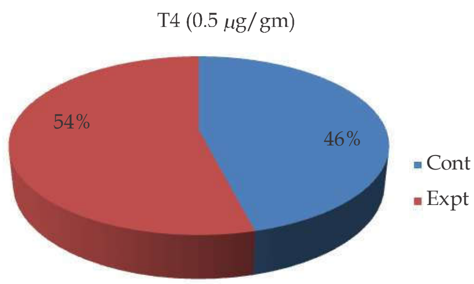

Fig. 2b:

Total collagen fraction in bone tissue of *Duttaphrynus melanostictus* by administration of T_4 (2.0 µg/gm and 0.5 µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values.

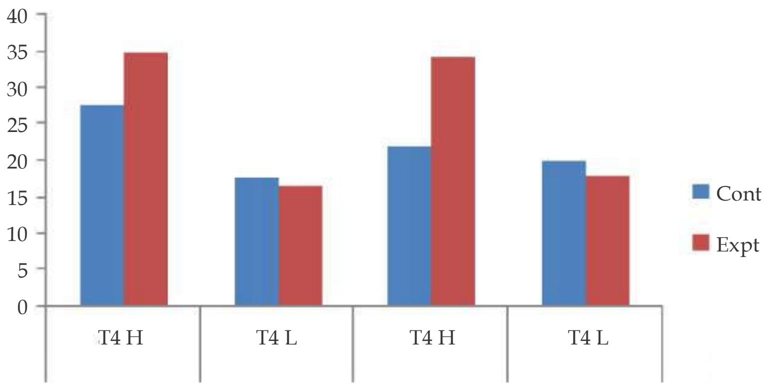

Fig. 3: % **o**f Salt soluble and acid soluble collagen fraction in bone tissue of *Duttaphrynus melanostictus* by administration of T_4 (2.0 μg/gm and 0.5 μg/gm). Values are mg/gm tissue wet wt., columns represent the mean values.

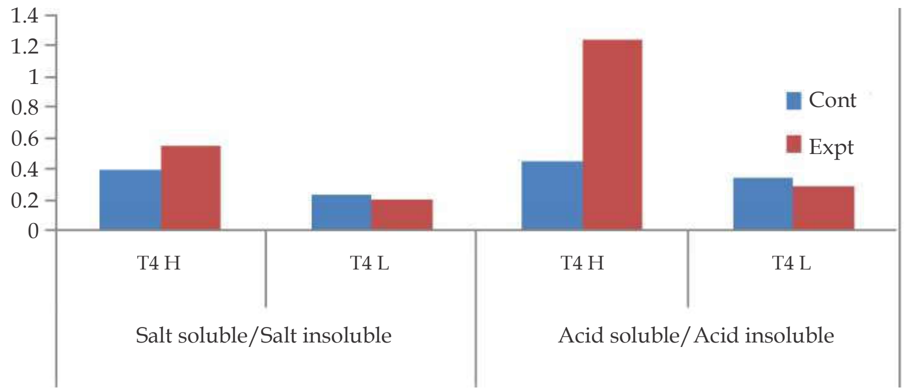

Fig. 4: Soluble/insoluble collagen fraction in bone tissue of *Duttaphrynus melanostictus* by administration of T_4 (2.0 μg/gm and 0.5 μg/gm). Values are mg/gm tissue wet wt., columns represents the mean values.

Discussion

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue. In fact, it is the single most abundant protein in the animal kingdom. The various collagens and the structures they form all serve the same purpose, to help tissues withstand stretching. Collagens were considered as the only major resource of hydroxyproline. The colossal amount of hydroxyproline in collagen has made it a valuable measure of collagen and collagen metabolism (Srivastava, 2016).

The thyroid hormone is seen to stimulate both the synthesis of collagen and non-collagenous proteins. Excess thyroid hormone treatment seems to have accelerated bone degradation (Taimela et al.,1994). Collagen seemed to exhibit the biphasic effect with thyroid hormone. This term refers to the fact that thyroid hormone can exhibit an optimal level and either a deficiency or excess of this level will move a given parameter in a similar direction. Thyroid hormone effect on collagen is dose dependent, tissue specific and controversial (Hell et al., 2011). Thyroxine and triiodothyronine seem to affect both collagen biosynthesis and degradation (Eugene, 1992). The changes in the collagen content in tissues reflect a cumulative effect of thyroid hormone and other connective-tissue-activating factors on collagen turnovers. The rate of collagen synthesis is decreased in hypothyroidism. In addition hypothyroidism is accompanied by decreased rate of catabolism of collagen (Guria et al., 2011). Isotopic studies have revealed that in experimental hypothyroidism the decreased excretion of hydroxyproline due to decreased rate of collagen synthesis and to decreased rates of degradation of both soluble and insoluble collagen.

In hyperthyroidism, the increased excretion of Hyp by increased rates of degradation of both soluble and insoluble collagen. Whereas the rate of collagen synthesis does not seems to be increased, but probably is decreased. Both hyperthyroidism and hypothyroidism are associated with bone degradation but in some tissues hypothyroidism increases collagen accumulation (Drobnik et al., 2009).

From the present research, it was found that salt soluble collagen decreased significantly when treated with T_4 (2.0 µg/gm) and increased insignificantly at T_4(0.5 µg/gm). Salt soluble collagen refers to newly synthesized collagen. Acetic acid extract, a form of collagen cross-linked into fibres by aldimine bond. More concentration of acid soluble collagen give an indication of presence of cross linked collagen by aldimine bond recently after synthesis and they are less stabilized. Acid soluble collagen content increased insignificantly at T_4 (0.5 µg/gm). It was decreased at T_4 (2.0 µg/gm). As collagen synthesis decreased at 2.0 µg/gm of $T_{4,}$ crosslinking formation was also halted but at 0.5 µg/gm it was increasing insignificantly. The insoluble collagen are due to stabilization of the collagen fibres by inter and intramolecular bonding/crosslinking. But in the case of Insoluble collagen it show significant increase in stability at T_4 (0.5 µg/gm) and decreased at other dose. The total collagen content reflects a balance between collagen synthesis and degradation. As per present study, total collagen content decreased significantly on administration of T_4 (2.0 µg/gm) and the other dose showed insignificant output. The changes in solubility and soluble/insoluble collagen ratio are indirect indicators of alterations in the degree of cross linkages of collagen molecules (Everittet al., 1970; Walford et al., 1969: Hall, 1976; Kohn, 1978). In T4 high dose increase in salt and acid soluble collagen ratio and increased solubility in both salt and acid media signifies the prevention of cross link formation. In contrast T4 low dose enhanced the collagen synthesis simultaneously caused its degradation.

Conclusion

Thyroxine hormone have dynamic effect on bone collagen and is mainly dose dependent. The degree of cross-link formation in collagen affects the physiological functions of the concerned tissue (Kohn, 1978). It is also likely that factors influencing the characteristics of collagenmay affect other physiological indices as well. The salt solubility, acid solubility, salt soluble/insoluble and acid soluble/insolubleratio of bone collagen were increased upon T_4 treatment (2.0 µg/gm) but all these decreased at 0.5 µg/gm body wt. It is suggested that T_4 possibly prevents cross-link formation thus retarding the aging of collagen in bone tissues at a higher dose. Conversely it enhanced the collagen synthesis and simultaneously matrix metabolism at the lower dose.

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