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Thyroid Hormone Induced changes in Collagen Metabolism of *Duttaphrynus Melanostictus* in Relation to Age

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Abstract

The present study investigated the correlation between thyroid hormones (i.e. thyroxine (T4) & triiodothyronine (T3)) and total collagen content in tissues of Duttaphrynus melanostictus. Administration of both T4 and T3 (0.5 µg/gm) separately for seven consecutive days led to changes in the total collagen content of dorsal skin, ventral skin and muscle of both young and adult common Indian toads. Thyroxine is important for both collagen synthesis and matrix metabolism (Yen, 2001). Duttaphrynus melanostictus were collected from Berhampur University campus and acclimated at laboratory conditions for 2/3 days. Levels of collagen were estimated following the method of Neuman and Logan (1950), as modified by Leach (1960). T4 treatment appeared to stimulate the deposition of collagen as evident from values of total collagen of dorsal and ventral skin. On the other hand, T4 treatment inhibits the increase in total collagen in the muscle region. On the contrary, T3 treatment decelerated the collagen deposition in dorsal skin and muscle tissue. A tissue-specific action of T3 administration in common Indian toad was shown in ventral skin where the total collagen content increased though differing to some extent in a degree of response. There is an age-related response to collagen metabolism which is tissue specific. The total collagen content declined during maturity whereas showing an acceleration in the post-maturity period.

Keywords: T4; T3; Collagen; Duttaphrynus melanostictus.

Introduction

Collagen may be a fibrous structural macromolecule gift within the animate thing matrix and animal tissue of animals (Ramshaw et al., 2009). It is the sole most plentiful protein in the set of all animals. It is missing in plants and unicellular life forms where polysaccharides and cellulose takes up its job. In the invertebrates, collagen is found in the body walls and cuticles. Particularly in mammals, collagen contains 25-30% of the macromolecule substance of the complete body (Muller & Werner, 2003) and represents well over 70% of the dry weight of human skin (Rycker et al., 1984). It is found in the corneas, bones, blood vessels, cartilage, dentin of teeth, etc. It is found as elongated fibrils in fibrous tissues such as the skin, tendons and ligaments. It comprises 1-2% of muscle tissue wherever it's an important element of the endomysium. Collagen is created largely by the embryonic cell of animal tissue and conjointly by sort of alternative animal tissue cells (Kadler et al., 2007; Silvipriya et al., 2015).

Thyroid hormones are responsible for the early development of vertebrates, especially in amphibian metamorphosis (White and Nicoll, 1982). Thyroxine is arguably the most important hormone in anuran development and affects development through exogenous and endogenous means (Storz, 2003). Amphibian metamorphosis is a highly synchronized mechanism in which essentially all tadpole tissues are transformed (Storz, 2003)

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and understanding the role of the environment in triggering ontogenetic polyphenism is important in understanding the coordinated evolution of the physiological systems involved in metamorphosis (Storz, 2003, Denver 1997).

Thyroxine is important for both collagen synthesis and matrix metabolism (Yen, 2001). Hypothyroidism is the cause for accumulation of glycosaminoglycans (GAGs) in the extracellular matrix, which may, in turn, predispose to tendon calcification (Oliva et al., 2013). Impacts of select Na and Ca- channel blockers on collagen synthesis and deposition were estimated in cultured human dermal fibroblasts and aortic smooth muscle cells by immunoassay. Channel blockers tested demonstrated inhibitory effects on collagen type I deposition to the ECM by fibroblasts, each to a different degree. Ascorbic acid significantly increased collagen I ECM deposition. (Ivanov et al., 2016). Thyroid hormones have been reported to stimulate collagen and GAG production, but reported outcomes, including which specific collagen types are affected, are variable throughout the literature. The ability of thyroxine (T4) to preferentially stimulate collagen production, as compared with GAG, in articular chondrocyte derived scaffold-free engineered cartilage (Whitney et al., 2017). Collagen formation was found to be variable but generally slower than increase in the weight of the thyroid (Harkness, et al., 1953).

Materials and Methods

For the present study, the common Indian toads of both sexes were collected and reared.

Collection and Maintenance: They were acclimated in the laboratory condition at room temperature for 3–4 days in wire-netted plastic cages (75*40*35 cm) size containing a moist sand bed. They were forced-fed with goat liver (composition mg/g wet wt: 110±41 protein, 84±16 lipid, 2.3±1.1 glycogen) every day, and water was provided ad libitum. All collected animals were used within five to seven days of collection. The estimation of various biochemical parameters were completed with all the batches of animals of various sizes irrespective of their sexes.

Treatment: After laboratory acclimation, animals of mixed sexes of different age groups were divided into control and treated groups. The control and treated group contain both young and adult group animals. Each group consists of five animals. There were two treatments of T4 and T3 separately. The treated group of toads were injected intramuscularly with thyroxine (T4) and T3, Na-salts (Fluka A.G.) at a dose of 0.5 µg/gm dissolved in 0.65% NaCl solution, pH 8.3 in separate batches; while the control animals received an equal value of 0.65% NaCl solution, pH 8.3. This injection schedule continued for seven days at a fixed time. The animals were sacrificed on the eighth day for the estimation of biochemical parameters.

Tissue Processing and Statistical analysis: Following the method of Neuman and Logan (1950) as modified by Leach (1960), dorsal skin and ventral skin tissues of both control & treated group animals were processed for the extraction and estimation of collagen fractions. Using correlation, the statistical significance of the data was evaluated.

Results

Following results show the correlation between body weight and total collagen content of Duttaphrynus melanostictus in relation to age with the administration of T4 & T3 (0.5 µg/gm).

I. T4 treatment

A. Dorsal skin

There was a significant positive correlation between the body weight and the total collagen content of dorsal skin of controls (r= 0.859, P< 0.01). Initially it increased upto maturation period with increasing body weight. However, the T4 treated (r =0.510; P, NS) animals exhibited a very similar trend as compared to control animals. There was an increase in total collagen of T4 treated animals as compared to controls before the onset of maturity while it declined during the postmaturity period (Table 1 & 2, Fig. 1).

B. Ventral skin

The total collagen content of the control animals (r=0.577; P, NS) declined at the young age. With the onset of maturity, it remained constant and with the increase in age, it shows higher elevation during postmaturity period. However, T4 treated (r=0.020; P, NS) animals, it exhibited biphasic characteristics. It decreased up to the onset of maturity period. Then it increased with increasing body weight subsequently (Table 1 & 2, Fig. 2).

C. Muscle

The body weight and total collagen content of muscle showed a significant positive correlation in

controls (r =0.720; P< 0.02) while it is insignificant in treated animals (r =0.547; P, NS) (Table 1 & 2, Fig. 3).

II. T3 treatment

A. Dorsal skin

There was a significant positive correlation between the body weight and total collagen content of dorsal skin of controls (r =0.859; P< 0.01). The total collagen content increased after the onset of maturity with increase in age. The treated (r =0.892; P< 0.001) animals also showed a significant positive correlation. There was a decrease in total collagen content in treated animals as compared to controls (Table 1 & 3, Fig. 4).

B. Ventral skin

The total collagen content of control animals (r =0.577; P, NS) declined in young age. With the onset of maturity, it remained constant and with increase in age. There was a significant positive correlation between the body weight and total collagen content in T3 treated animals (r = 0.715; P< 0.02) (Table 1 & 3, Fig. 5).

C. Muscle

There was a significant positive correlation between the body weight and total collagen content of muscle of controls (r=0.720; P< 0.02). The total collagen content increases during the post maturity period. The T3 treated animals also showing the significant positive correlation (r =0.785; P< 0.01) between the body weight and total collagen content (Table 1 & 3, Fig. 6).

In vivo effects of thyroxine (T4 & T3) (0.5 µg/gm), i.e. Treated-I & Treated-II on total collagen characteristics in dorsal skin, ventral skin & muscle tissues of Duttaphrynus melanostictus.

CONTROL

Sl No.	Initial Body wt. (gm)	Final body wt. (gm)	Dorsal skin (mg/gm)	Ventral skin (mg/gm)	Muscle (mg/ gm)
1	15	14	252.539	207.388	200.426
2	19	22	271.646	224.605	223.720
3	24	20	280.459	199.430	213.151
4	27	25	264.581	182.522	252.646
5	28	28	251.650	246.677	206.890
6	45	46	269.993	202.762	234.741
7	50	52	281.227	205.200	298.822
8	55	59	323.457	206.368	343.448
9	65	67	350.384	255.129	397.294
10	69	72	394.821	318.510	245.186

Table-1

TREATED-I

Sl No.	Initial Body wt. (gm)	Final body wt. (gm)	Dorsal skin (mg/gm)	Ventral skin (mg/gm)	Muscle (mg/ gm)
1	13	25	286.094	425.008	72.400
2	27	21	319.150	442.367	56.208
3	31	29	302.445	368.189	81.741
4	33	30	335.767	205.399	119.501
5	34	31	322.177	210.869	135.195
6	41	36	171.865	123.139	33.062
7	54	50	240.896	158.649	70.590
8	62	54	289.811	345.646	158.538
9	69	61	473.129	366.816	111.592
10	81	74	441.314	373.994	373.994

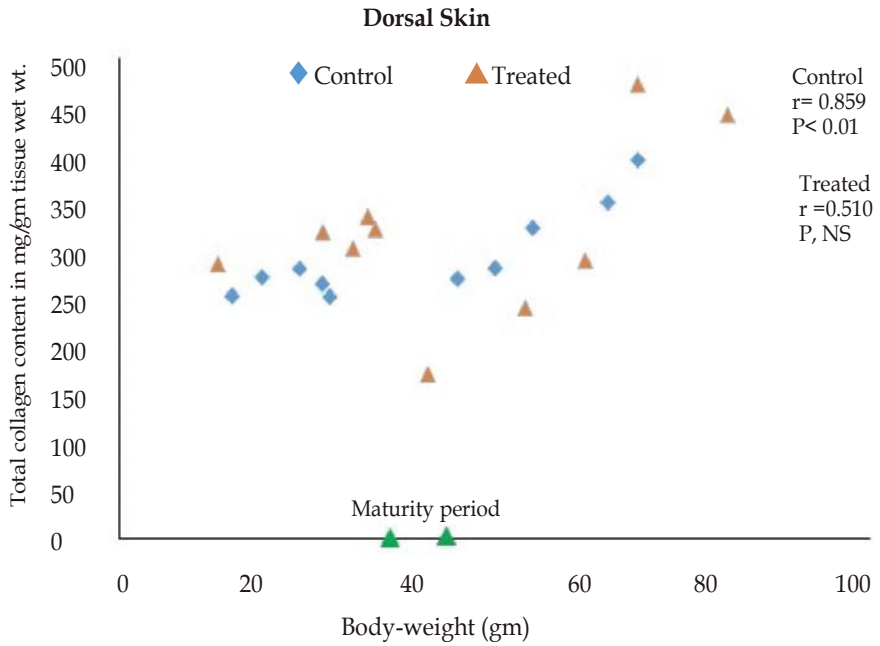

Fig. 1: Correlation of total collagen content in dorsal skin of control (r =0.859398395; P< 0.001) and T4 (r =0.509743145; P, NS) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

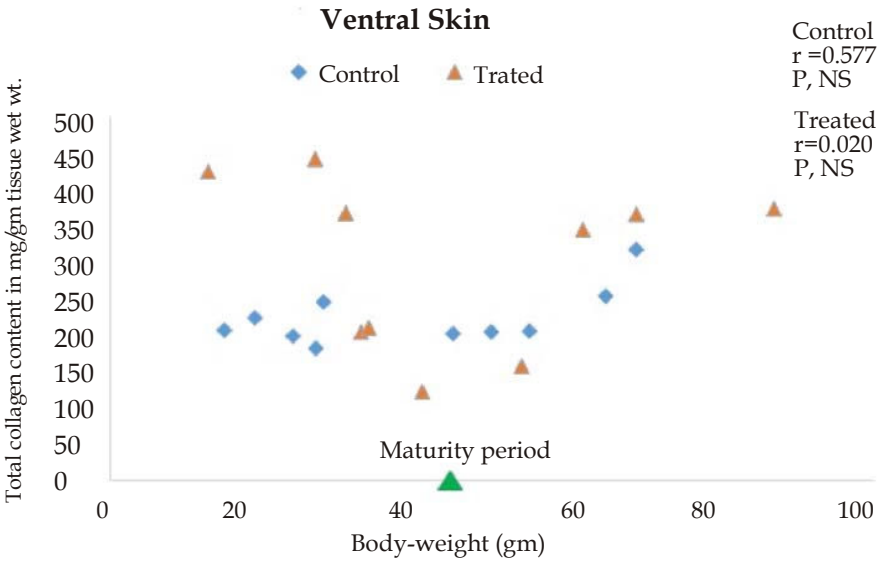

Fig. 2: Correlation of total collagen content in Ventral skin of control (r =0.576702986; P, NS) and T4 (r =0.020572113; P, NS) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

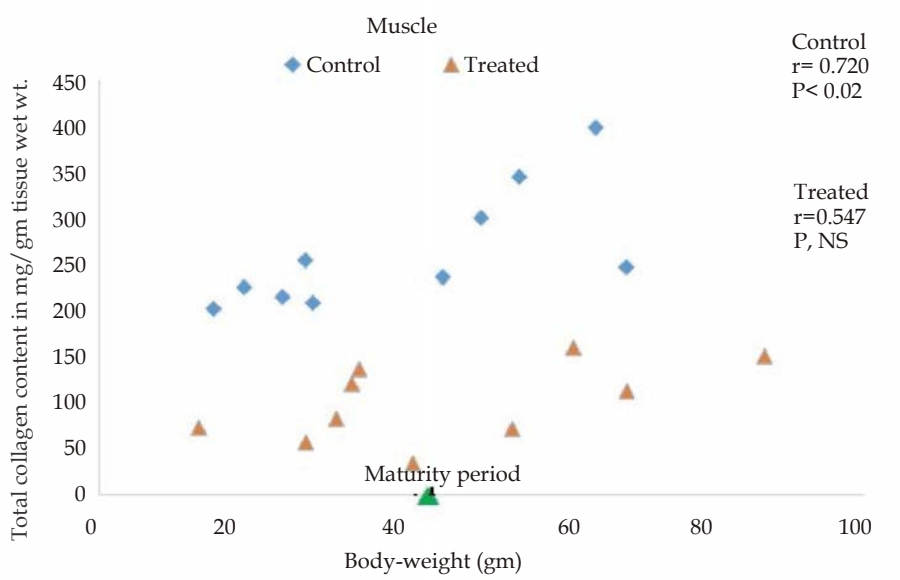

Fig. 3: Correlation of total collagen content in Ventral skin of control (r =0.576702986; P, NS) and T3 (r =0.71509395; P< 0.02) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

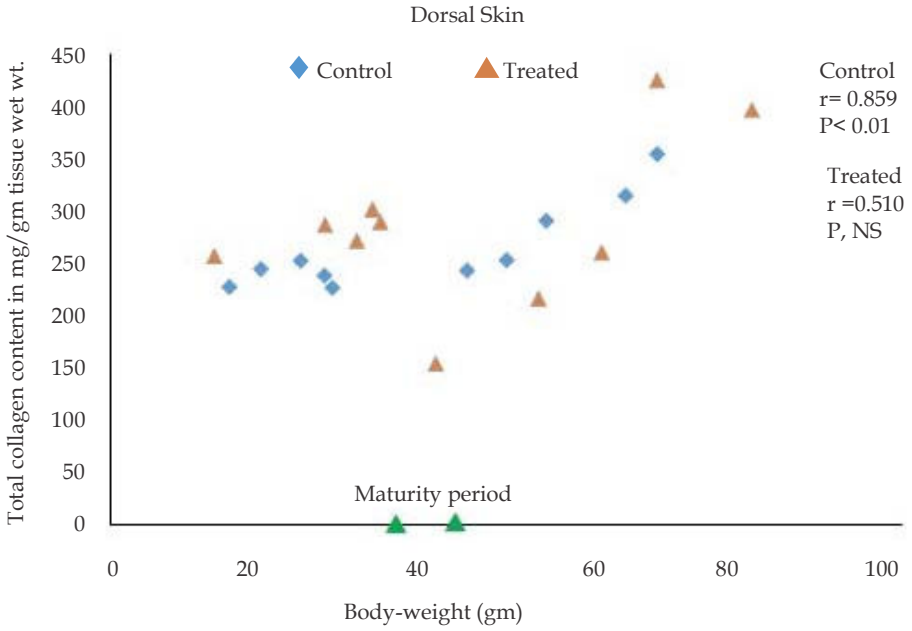

Fig. 4: Correlation of total collagen content in Dorsal skin of control (r =0.859398395; P< 0.01) and T3 (r =0.892517292; P< 0.001) treated toads, Duttaphrynus melanostictus through different ages. Values are µg / g tissue wet weight. Dose - low dose (0.5 µg / gm).

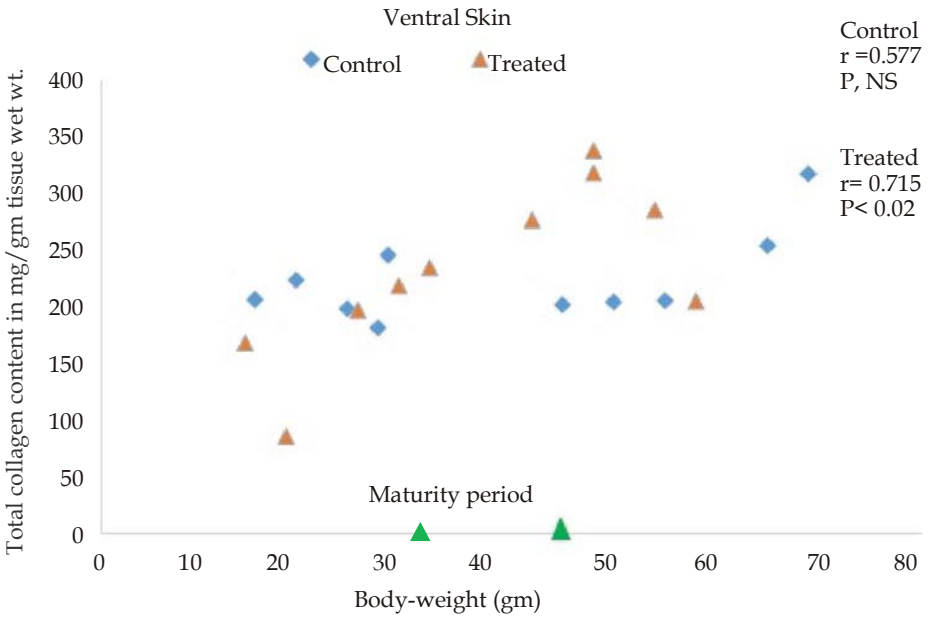

Fig. 5: Correlation of total collagen content in Ventral skin of control (r =0.576702986; P, NS) and T3 (r =0.71509395; P< 0.02) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

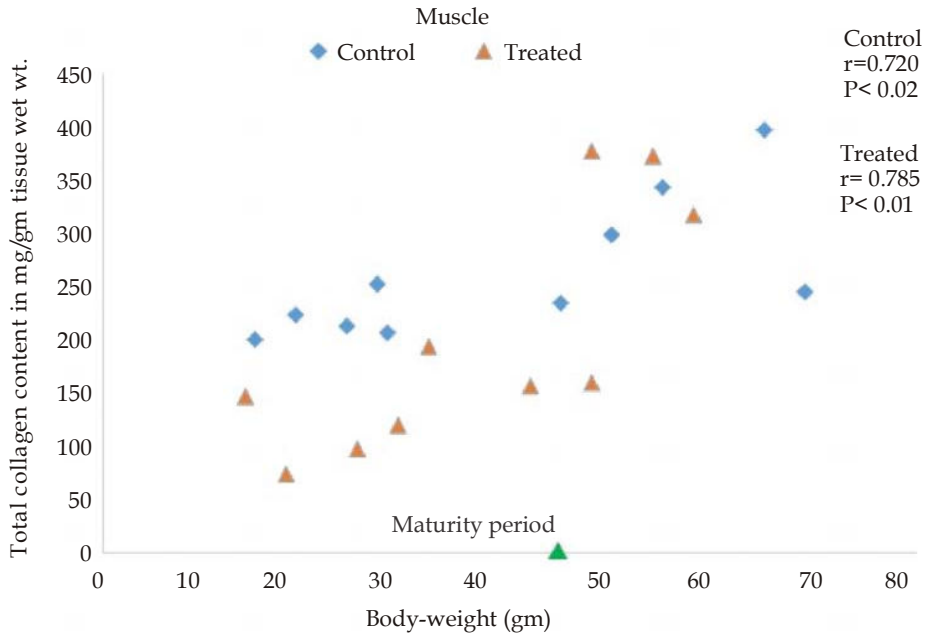

Fig. 6: Correlation of total collagen content in Muscle of control (r =0.720144006; P< 0.02) and T3 (r =0.785391931; P< 0.01) treated toads, Duttaphrynus melanostictus through different ages. Values are µg/g tissue wet weight. Dose - low dose (0.5 µg/gm).

Discussion

This have been reported to influence the metabolism of collagen. Hyperthyroidism potentially can be caused by an increased catabolism of both soluble and insoluble collagen. Hypothyroidism appears to be accompanied by lower rates of collagen catabolism (Kivirikko et al., 1965). The rate of collagen synthesis is declined both in hyperthyroidism and in hypothyroidism (Kivirikko et al., 1967).

One of the well-known parameters to accesses ageing in vertebrates is the changes associated with the characteristics of connective tissue protein collagen (Sinex, 1968). With advancing age, the number of intra and intermolecular cross-link ages increase in collagen molecule. This eventually leads to a derangement in physiological functions of various tissues. Cross-linked collagen in extracellular space may not effectively permit the transport of nutrients and oxygen to tissues. Vital organs like heart and kidney may not function effectively. The contraction mechanism of skeletal muscle may be impaired due to such cross-linkages. The deposition of calcium salts in collagen-containing matrix of bone may be disturbed. Thus, the ageing of collagen might affect the ageing of the organism as a whole. Such changes in the characteristics of collagen during ageing provide excellent support to the "cross-linking theory" of ageing (Panigrahy and Patnaik, 1973). A decrease in solubility (Verzar, 1964; Hall, 1976) and in soluble/ insoluble collagen ratio (Sinex, 1968; Walford et al., 1969) is the consequence of increased number of cross-links in collagen molecule (Mishra, 1987).

Several workers have thoroughly reviewed the hormonal control of mammalian collagen metabolism and its implications for growth and aging (Everitt and Burgess, 1976). Since the collagen characteristics undergo considerable modification during development, growth and aging, it is necessary to verify the involvement of hormones in such processes.

Higher-dose thyroxine is known to reduce the production of collagen in mammalian tissues. Hyperthyroidism appears to increase the catabolism of collagen (Kivirikko et al., 1963, 1967). Fink (1967) also stated that thyroxine causes bone collagen degradation. The total collagen content in the tendon decreases after treatment with thyroxine and increases in lizards treated with thiourea suggest that the hormone induces collagen degradation in garden lizards. Induced anabolic actions on collagen have also been reported of thyroxine (Drozdz et al., 1979).

Total content of collagen indicates approximately the balance between the synthesized and degraded amounts. There was a positive correlation between body weight and total collagen content in relation to age. The total collagen content is increased both in dorsal and ventral skin by the T4 treatment showing the deposition of collagen in youngs while adult toads showing degradation of collagen in dorsal skin and muscle tissue. T^3 treatment resulting in collagen degradation at both young and adults. In a majority of cases the quantity of collagen has been shown to increase with advancing age. In skeletal muscle (Schaub, 1963) and cardiac muscle (Schaub, 1964/5) of rat, the collagen content increases with age. A similar pattern was observed in the skeletal muscle of garden lizard (Haseeb and Patnaik, 1978). On the other hand, the total collagen content in the bone of garden lizard Calotes versicolor increased till sexual maturity and remained constant there after (Panigrahy and Patnaik, 1973) which supports our results. Increased collagen degradation is observed in hyperthyroidism (Kivirikko et al., 1963, 1967). Hyperthyroidism also stated by Fink in 1967 leads to increased degradation of bone collagen (Brahma and Pattnaik, 1982). T4 treatment showing collagen degradation reduces the total collagen content in both dorsal and ventral skin. Retardation of growth by decreasing synthesis of collagen followed by collagen catabolism (Pattnaik and Mishra, 2018).

Conclusion

An analysis of the graphs indicates an age-related response that is dependent on dose and specific tissue. There are several earlier reports that there has been an age-dependent response of these two parameters to thyroid hormones (Andia, 1984; Choudhury, 1992; Pattnaik et al., 2015; Mohanty, 2018). Such a dependency may result possibly due to the simultaneous effect of other hormones such as growth hormone and pituitary secretions, gonadal and adrenocortical hormones, nutritional status and other environmental factors. The correlation results indicated an increase in collagen in response to T4 and a decrease in the same in T3 with tissue-specific action.

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