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Metabolic effect of thyroxine on collagen characteristics in skin tissue of common Indian toad

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Abstract

The effect of thyroxine on alterations of biochemical parameters like collagen characteristics in both dorsal and ventral skin were investigated in our present study. Thyroxine (T_4) treatment decreased the collagen synthesis in dorsal skin at a negligible amount whereas it decreased in ventral skin significantly. Thyroxine induced the crosslinking of collagen fibres by aldimine bonds in both dorsal & ventral skin as compared to the control values. The insoluble collagen decreased in both dorsal & ventral skin preventing intra & intermolecular crosslinking. On administration of thyroxine, there was a decrease in total collagen content in both dorsal & ventral skin as compared to control values.

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Introduction

The major iodinized hormone, thyroxine (T_4) produced by the thyroid gland plays a pivotal role in several developmental processes (Bhargava *et al.,* 2009). The essential role of Thyroid hormones (THs), T_4 (thyroxine) and T_3 (triiodothyronine) in the improvement and metabolism of numerous tissues and organs, both in ahead of schedule and grown-up life, is interceded for the most part through T_3. Thyroid hormone receptors play a pivotal role in regulation of gene expression. Thyroid hormones are fundamental variables for regulation of cells morphology, differentiation and proliferation (Lima *et al.*, 1997; Trentin, *et al.*, 1998; Trentin, 2006) and the synthesis and organization of ECM (Mendes-de-Aguiar *et al.*, 2008; Dezonne, *et al.*, 2013).

Hypothetically, increased collagen degradation of both soluble and insoluble collagen may be resulted in hyperthyroidic conditions whereas in hypothyroidism, decrease in degradation of collagen is shown (Diez *et al.*, 2002; Garrity & Bahn, 2006; Magnan *et al.*, 2014; Berardi *et al.*, 2014).

The impacts of different factors on collagen biosynthesis have been looked into (Miiller *et al.*, 1982). The impacts of thyroid hormones on the biosynthesis of collagen have not yet been deliberately considered. A decreased amount of newly synthesized collagen protein was shown by the administration of T_3 to the cultured skin fibroblasts media (Rycker *et al.*, 1984). Thyroid hormones T_3 and T_4 can preferentially stimulate total collagen, but individual collagen types were not measured quantitatively (Lee *et al.*, 2015; Whitney, 2017).

The biomechanical strength of connective tissue is determined by the collagen concentration. Thyroid gland has regulatory influence on connective tissue metabolism. Collagen is the most abundant protein & constitutes about 70% of dry weight of skin of human beings (Bauer and Uitto, 1979). In the view of above, the present study aims to find out the changes in skin collagen in common Indian toad through thyroxine treatment.

Materials and methods

Animals

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. Each group consists of eight number of animals. The treated group of toads were injected intramuscularly with thyroxine (T_4), Na-salts (Fluka A.G.) at a dose of 2.0µg/gm dissolved in 0.65% NaCl solution, pH 8.3; while the control animals received an equal value of 0.65% NaCl solution, pH 8.3. This injection schedule continued for 7 days at fixed time. The animals were sacrificed on the eighth day for the estimation of biochemical parameters.

Tissue Processing

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.

Statistical analysis

Using student's t-test, the statistical significance of the data were evaluated.

Results

T- test analyses the experimental results suggest that thyroxine has an effect on collagen crosslinking.

Dorsal skin

Statistical analysis showed that there is no significant difference in salt soluble collagen (Fig. 1 & Table 1) whereas, salt soluble/ salt insoluble collagen ratio & % of salt solubility increased in a highly significant way (P<0.001) (Fig. 2 & 3 respectively, Table 1) after the treatment of T_4 at the dose of 2.0µg/gm body wt as compared to the control ones. A statistically nonsignificant increase of acid soluble collagen resulted at T_4 administration at a dose of 2.0µg/gm body wt (Fig. 4, Table 1), but showing highly significant increase in acid soluble/ acid insoluble ratio & % of acid solubility (P<0.001) as compared to the controls (Fig. 5 & 6 respectively, Table 1). Insoluble collagen and total collagen decreased in a significant manner (P< 0.001 & P< 0.002 respectively) as compared to controls (Fig. 7 & 8 respectively, Table 1).

Ventral skin

From the statistical analysis, it is evident that by the administration of T_4 at the dose of 2.0µg/gm body wt, the salt soluble collagen, salt soluble/salt insoluble collagen ratio & % of salt solubility decreased significantly (P<0.001, P<0.05 & P<0.02) as compared to the control ones (Fig. 1, 2 & 3; Table 2). Moreover, the acid soluble collagen, acid soluble/ acid insoluble collagen ratio & % of acid solubility increased in a highly significant manner (P<0.001) (Fig. 4, 5 & 6; Table 2). Insoluble collagen decreased significantly (P< 0.01) (Fig. 7, Table 2) whereas total collagen decreased nonsignificantly (P, NS) as compared to the controls (Fig. 8, Table 2).

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

Experimental Condition	Salt soluble	Acid soluble	Insoluble	Total	Salt soluble/salt insoluble	Acid soluble/ acid insoluble	% of salt solubility	% acid solubility
Control	59.062	63.161	187.434	307.085	0.161	0.172	14.867	12.940
	±	±	±	±	±	±	±	±
	1.820	1.944	3.015	11.753	0.005	0.007	0.005	0.504
	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
P	P, NS	P, NS	P<0.001	P<0.002	P<0.001	P<0.001	P<0.001	P<0.001
Treated	58.934	70.373	120.293	238.782	0.445	0.678	30.756	30.862
	±	±	±	±	±	±	±	±
	3.424	3.099	11.564	12.533	0.028	0.060	0.028	1.113
	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)

Table 2. Effect of thyroxine (T_4) (2.0µg/gm body wt.) on collagen characteristics of Ventral skin in Common Indian toad. The soluble, insoluble and total collagen are mg/gm tissue wet-weight (Mean ± SEM), Numbers in brackets indicate sample size, NS, Not significant, at 0.05 confidence level.

Experimental Condition	Salt soluble	Acid soluble	Insoluble	Total	Salt soluble/salt insoluble	Acid soluble/ acid insoluble	% of salt solubility	% acid solubility
Control	59.322	29.370	196.891	240.107	0.182	0.161	18.525	24.144
	±	±	±	±	±	±	±	±
	3.242	2.099	20.418	6.232	0.006	0.014	0.941	0.379
	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
P	P<0.001	P<0.001	P<0.01	P, NS	P<0.05	P<0.001	P<0.02	P<0.001
Treated	20.591	59.680	121.039	226.335	0.155	0.450	15.383	41.696
	±	±	±	±	±	±	±	±
	1.137	2.318	5.984	7.083	0.008	0.053	0.507	1.473
	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)

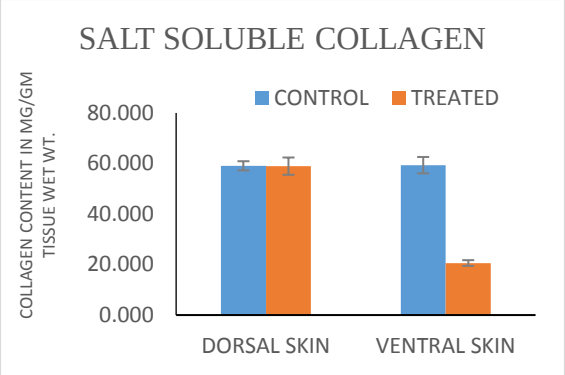

Fig. 1. Salt soluble collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T_4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

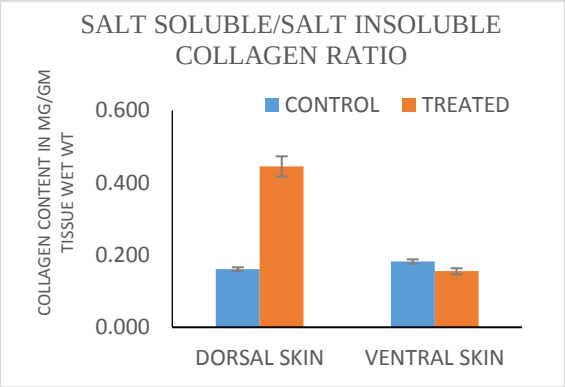

Fig. 2. Salt soluble/ salt insoluble collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

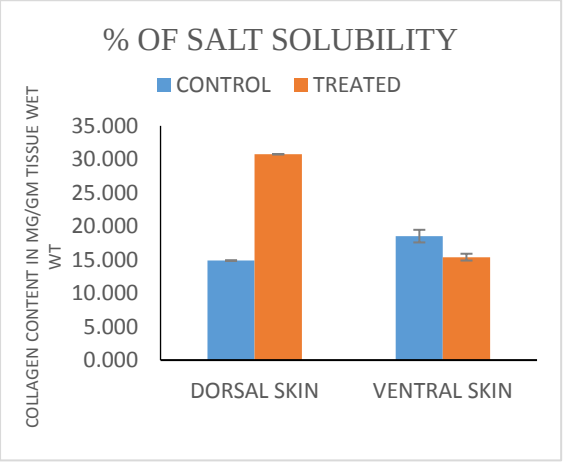

Fig. 3. % of salt solubility collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T_4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

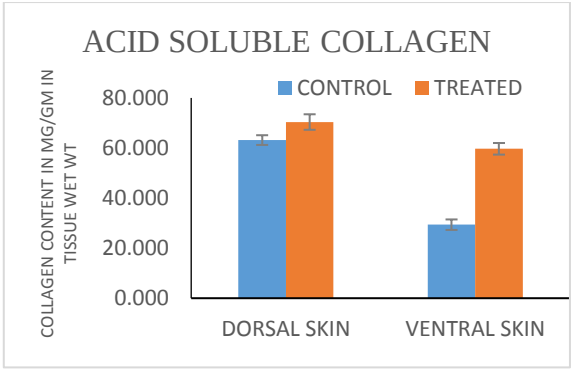

Fig. 4. Acid soluble collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

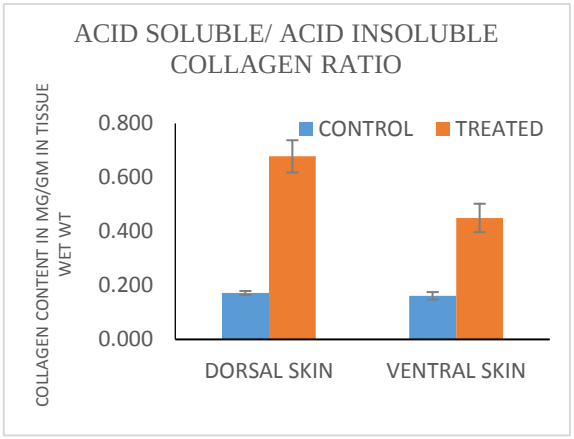

Fig. 5. Acid soluble/acid insoluble collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

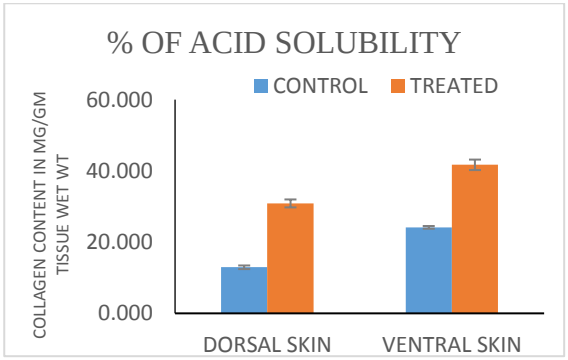

Fig. 6. % of acid solubility in collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

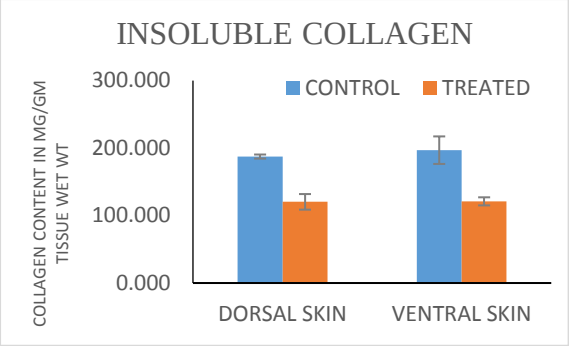

Fig. 7. Insoluble collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T_4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

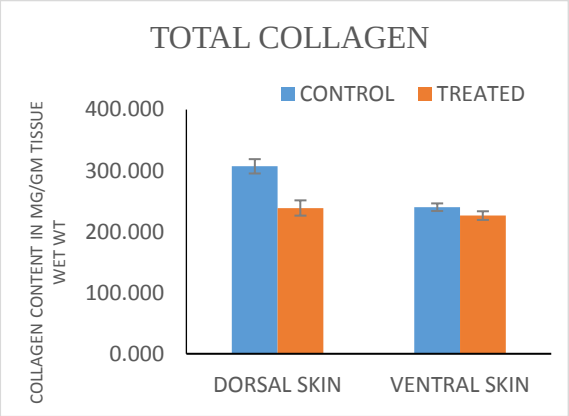

Fig. 8. Total collagen fraction in dorsal skin & ventral skin of common Indian toad by the administration of T_4 (2.0µg/gm). Values are mg/gm tissue wet wt., columns represent the mean values & vertical bars SEM.

Discussion

The significance of TH in skin was first appeared in lower vertebrates. The skin is changed from a bilayered non-keratinized epithelium into a stratified, keratinized epidermis in Amphibian metamorphosis (Furlow & Neff, 2006; Antonini *et al.*, 2014). Thyroid hormone influences the skin by means of the thyroid hormone receptor and all the three isoforms present in skin. TH receptors are available in different zones all through the skin matrix including the skin fibroblasts, vascular endothelial cells, epidermal keratinocytes, sebaceous organ cells, and all through the hair follicles and scalp. The receptor sites in skin respond and function with respect to thyroid hormone secretion (Hnilo, 2017).

Since salt soluble collagen is regarded as newly synthesized collagen, our results showing that salt solubility of ventral skin decreases following thyroxine treatment suggests that thyroxine demotes growth whereas in dorsal skin, slight decrease in it indicating the negligible action of thyroxine on it showing tissue specific action. The collagens are stabilized with covalent crosslinks and get deposited into the extracellular matrix continuously which is fundamental process occurring with the advancement of age (Mays *et al.*, 1991). Crosslinking participates in the increased stability of collagen towards proteolytic degradation. Acid soluble collagens are the more stabilized forms formed by the intramolecular covalent crosslinks which affects the physiology of the concerned tissue which is dependent on degree of crosslink formation. Increase in acid soluble collagen and acid solubility significantly, clearly shows an anti-ageing effect in both dorsal & ventral skin supported by Davison *et al.* (1972) & Singh *et al.* (2016). The insoluble collagen are due to the stabilization of the collagen fibrous by inter and intramolecular cross linking. Thyroxine (both T_4 and T_3) administration accelerated the collagen synthesis and also accelerated the conversion of soluble to insoluble collagen differing to a small degree of fraction contrasting to our results which shows a decrease in insoluble collagen indicating prevention of more cross linkages by intra and intermolecular covalent bonding (Mishra *et al.*, 2018).

Total collagen content roughly indicates the balance between the amounts synthesized and degraded. Thyroxine at higher doses is known to decrease collagen formation in mammalian tissue. In hyperthyroidism, the degradation of collagen is found to be increased (Kivirikko *et al.*, 1963, 1967). Fink in 1967 also reported hyperthyroidism also leads to increase in bone collagen degradation (Brahma and Pattnaik, 1982). Our results showed that the total collagen content is decreased both in dorsal and ventral skin by the T_4 treatment showing the degradation of collagen. For the estimation of degree of cross-link formation in collagen, percentage of solubility and soluble/insoluble ratio are taken as notable indirect measures.

From our experiment it is found that the salt soluble/salt insoluble collagen ratio increased significantly in dorsal skin but decreased in ventral skin showing a contradiction. But acid soluble/ acid insoluble collagen ratio and % of acid solubility increased in both dorsal and ventral skin.

Conclusion

The thyroid hormone plays a crucial role in physiology and development of skin. Various studies regarding the effects of thyroid hormone action on skin demonstrated its involvement in epidermal proliferation, differentiation, along with affecting the functions of dermal fibroblasts. Our study focuses on biochemical parameters like collagen metabolism. From the Experimental results it is concluded that, retardation of growth by decreasing collagen synthesis followed by catabolism of collagen. Results from acid solubility showing the stabilization of collagens by covalent crosslinks which is crucial for normal development of functional tissue and organs.

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