ISSN: 2394-1391

Indian Journal of Biology
Volume 5 Number 1, January - June 2018
DOI: http://dx.doi.org/10.21088/ijb.2394.1391.5118.6

Original Article

Impact of Thyroid Hormone in Liver Collagen of *Duttaphrynus Melanostictus*

Gitanjali Mishra*, Bandita Panda, Subhasmita Pattnaik**, Purnima Maharana****

Abstract

Thyroxine is important for both collagen synthesis and matrix metabolism (Yen, 2001, Oliva *et al.*, 2013). The thyroid hormones T_4 and T_3 are formed in a large prohormone molecule, thyroglobulin, the major component of the thyroid and more precisely of the colloid. Thyroid dysfunction may perturb the liver function and vice versa (N. Kumar, 2013). T_3 stimulates synthesis and post-translational modification of type I collagen (Varga, *et al.*, 2010), induces expression of alkaline phosphatase (Gouveia, *et al.*, 2006) and regulates synthesis and secretion of the bone matrix proteins i. e. osteopoetin and osteocalcin (Gouveia *et al.*, 2001, Varga *et al.*, 2010). Recent study demonstrated the changes in liver collagen in *Duttaphrynus melanostictus* by daily administration of thyroid hormones (both T_4 & T_3) at the dose of 0.5 µg/gm, for 7 days by the method of Newman & Logan (1950) as modified by Leach (1960). The salt soluble, acid soluble, insoluble, total collagen, % of salt solubility, % of acid solubility, salt soluble/ insoluble ratio, acid soluble/insoluble ratio of collagen were statistically found out at 0.05 P confidence level.

Keywords: Thyroid Hormone (T_4& T_3); Collagen; *Duttaphrynus Melanostictus.*

Introduction

Thyroid hormones (THs), T_3 and T_4, play an essential role in the development and metabolism of many tissues and organs, and exert profound metabolic effects in adult life, including changes in oxygen consumption, protein, carbohydrate, lipid, and vitamin metabolism (Oliva *et al.*, 2013). Most circulating T_3 is derived via metabolism of T_4, from which an outer-ring iodine atom is removed by activity of the type 1 iodothyronine deiodinase enzyme (Dio1) principally in liver and kidney (Bianco and Kim,2006, Williams, 2013). The thyroid hormones, triiodothyronine (T_3) and its prohormone, thyroxine (T_4), are regulated by TSH made by the thyrotropes of the anterior pituitary gland that are primarily responsible for regulation of metabolism (Pattnaik, 2014). Limited studies use T_3 and T_4 to specifically improve the functional properties of neocartilage engineered from articular chondrocytes, as existing studies largely focus on understanding hormone effects at the cellular level. For instance, T_3, when applied to salginate-embedded chondrocytes, enhanced the hydroxyproline content per cell (Randau *et al.*, 2013). In the presence of bone morphogenetic protein 2(BMP-2) and insulin, T_3 significantly increased collagen type II mRNA and reduced BMP-2/insulin-induced collagen type X expression (Liu *et al.*,2007). These studies demonstrate the beneficial effects of T_3 in eliciting increased collagen production in articular chondrocytes in three-dimensional culture. However, the effect of T_3 and T_4 hormones on increasing the functional properties of engineered neocartilage is understudied.

Author's Affiliation: *Professor **Research scholars, P.G. Department of Zoology, Berhampur University, Brahmapur, Odisha 760007, India.

Reprint's Request: Bandita Panda, Research Scholars, P.G. Department of Zoology, Berhampur University, Brahmapur, Odisha 760007, India.
E-mail: gmishra.bu@gmail.com, bandita7panda@gmail.com

Received on 12.04.2018, **Accepted on** 05.05.2018

Collagen is one of the most abundant animal proteins, constituting approximately one-third of the total body protein of mammals. It is a major protein of the extracellular matrix and the most profuse protein in humans making up 30% of our skin, bone and connective tissues. During developmental growth, collagens are believed to be continuously deposited into an extracellular matrix which is increasingly stabilized by the formation of covalent crosslinks throughout life (Mays *et al.*, 1991). Collagen formation is an important function of liver parenchymal cells that may be relevant to the pathogenesis of hepatic fibrosis. The hepatic stellate cell (HSC) is the primary cell type in the liver responsible for excess collagen synthesis during hepatic fibrosis.According to the experiment of Tseng *et al.*, 1983 collagen IV along with collagen type I, III, and V was observed from normal rat liver hepatocytes culture. The mechanical properties, biocompatibility, and degradation rate of collagenous materials are profoundly influenced by the method and extent of collagen crosslinking. Crosslinking also further reduces collagen antigenicity (Meade and Silver, 1990). Collagen solubility in weak acids is indirectly related to the degree of cross linkage in the collagen of the tissue under study: a higher solubility index indicates a higher degree of cross linkage of the collagen molecules (Robins *et al.*,1973). Changes in collagen content and cross-linking occur in many organs with a variety of diseases, chronic injury, and aging (Peleg *et al.*,1993).

Materials & Methods

Collection

Animals of both sexes were collected from nature during evening time and were transferred to the laboratory in the next morning. They were maintained in laboratory conditions in wire-netted wooden cages (75× 40× 35 cm in size) containing a moist sand bed for about five days. They were forced-fed with about 1 gm of goat liver (composition mg/gm wet weight: 110±41 protein, 84±16 lipid, 2.3±1.1 glycogen) each on every alternate day and water was provided ad libitum).

Treatment

After laboratory acclimation animals of mixed sexes were divided into control and experimental groups. There were two treated groups named as experiment. One experimental group of toads were injected intramuscularly with thyroxine (T4) Na- salts (fluka AG), and the other group with triiodothyronine (T_3) Na-salts (Fluka AG) at a dose of 0.5 μg/gm dissolved in 0.65% NaCl solution, pH 8.3. The control animals received an equal volume of 0.65% NaCl solution, pH 8.3. These injection periods were maintained for 7 days. On the 8th day, the animals were sacrificed in batches for estimation of biochemical parameters after taking their final body-weight.

Collection of Tissue

The animals were pithed by piercing a pointed needle immediately posterior to the occipital region. The animals were quickly dissected out. The liver tissue was transferred to cold Amphibian ringer (KCl – 140 mg, NaCl – 6.5gm, $CaCl_2$ – 120 mg, $NaHCO_3$ – 100 mg per litre, pH – 7.4). The adherent tissues were cleaned, blotted off in Whatman filter paper. After soaking in filter paper, weighed quantities (25mg) of tissue were taken for extraction of different collagen fractions. Then the different collagen fractions were extracted and estimated following the method of Neuman and Logan (1950) as modified by Leach (1960).

These data were statistically analysed by the student t – test (Abramoff and Thomson, 1966, Bishop, 1966).

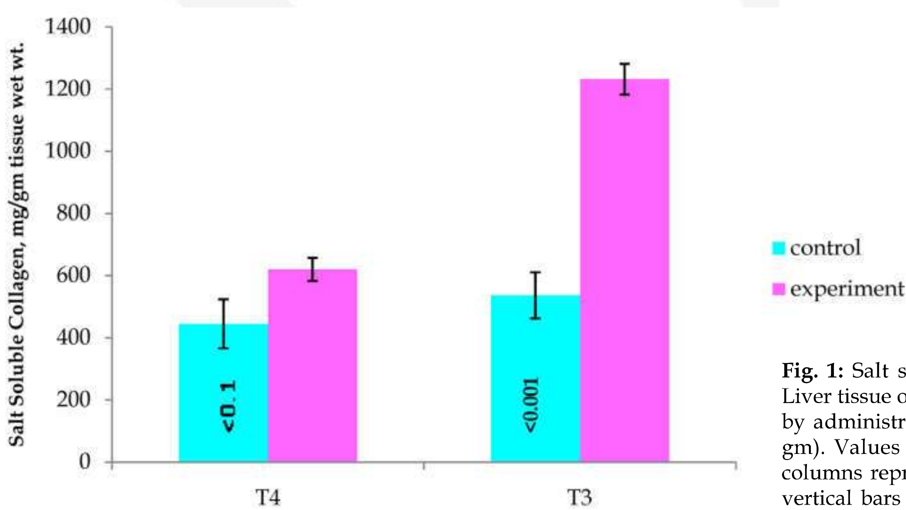

Fig. 1: Salt soluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 μg/gm). Values are μg/gm tissue wet wt., columns represent the mean values and vertical bars SEM

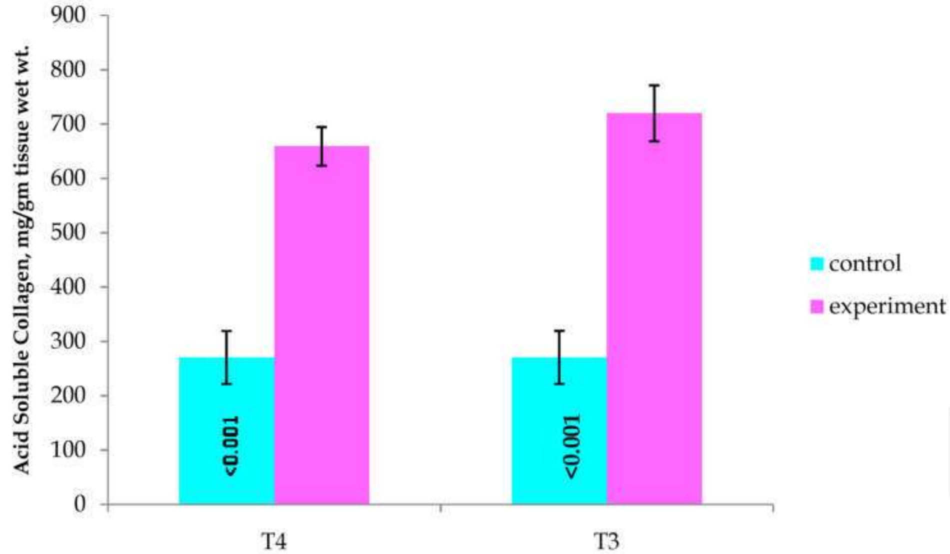

Fig. 2: Acid soluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

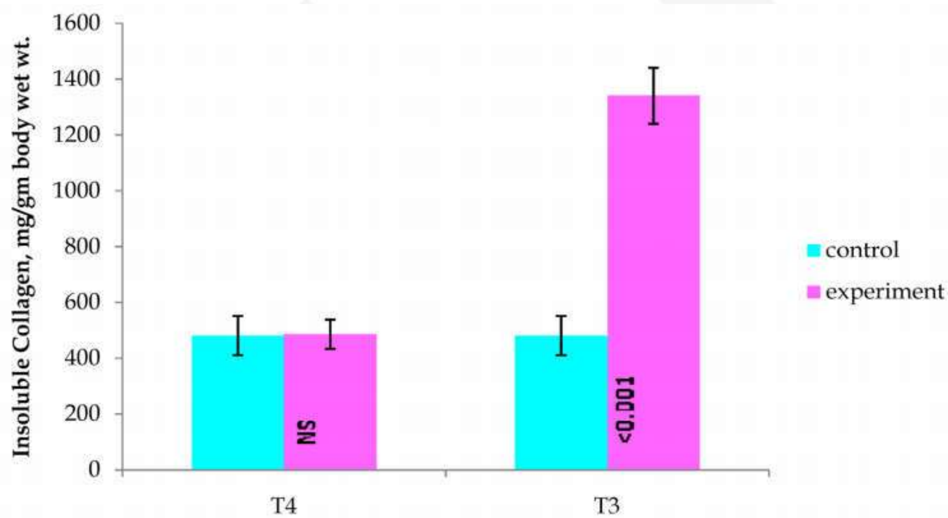

Fig. 3: Insoluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

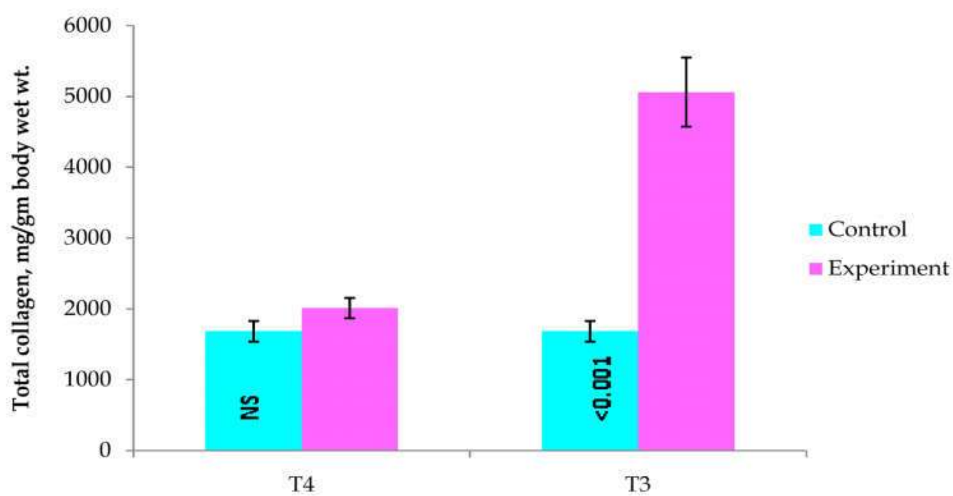

Fig. 4: Total collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3 (0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

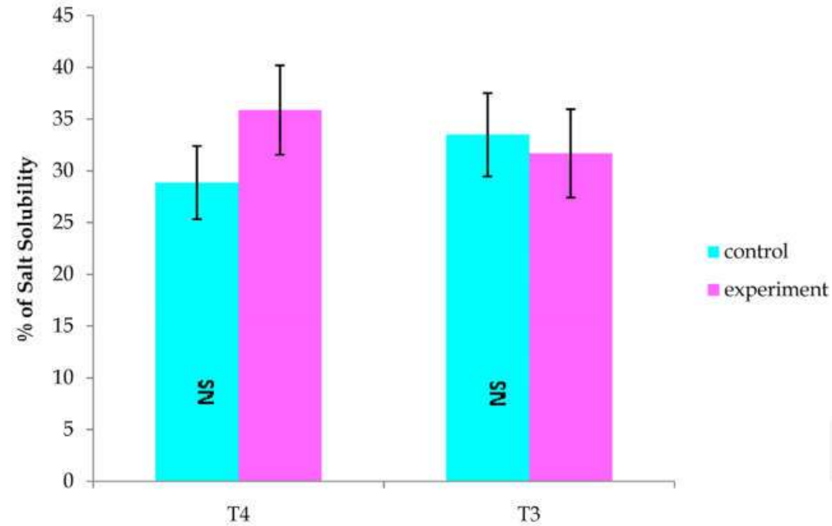

Fig. 5: % of solubility collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3(0.5 μg/gm). Values are μg/gm tissue wet wt., columns represent the mean values and vertical bars SEM

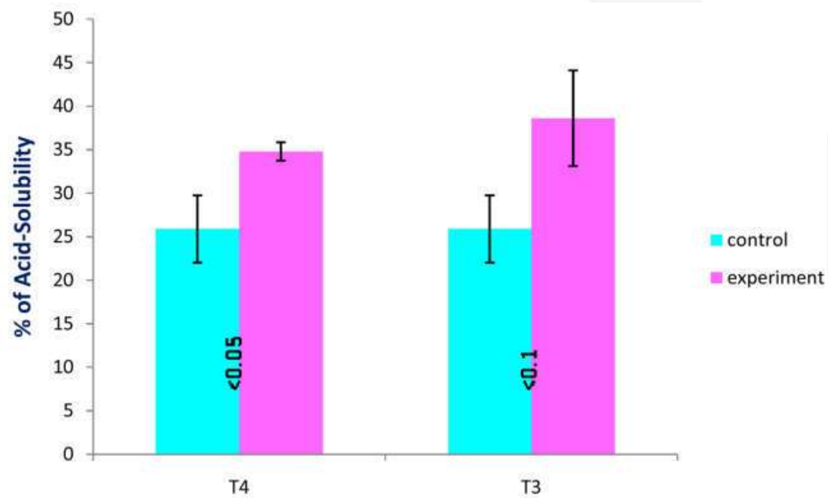

Fig. 6: % of acid solubility collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3(0.5 μg/gm). Values are μg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

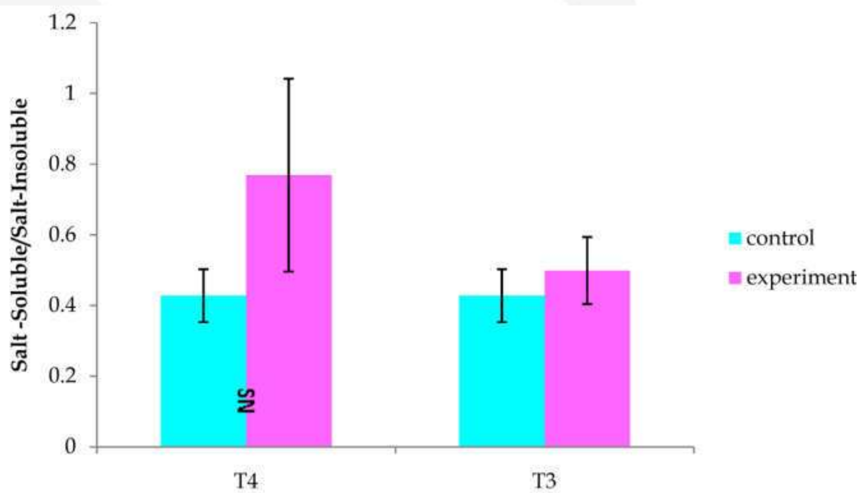

Fig. 7: Salt soluble/ salt insoluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration ofT_4 and T_3 (0.5 μg/gm). Values are μg/gm tissue wet wt., columns represent the mean values and vertical bars SEM.

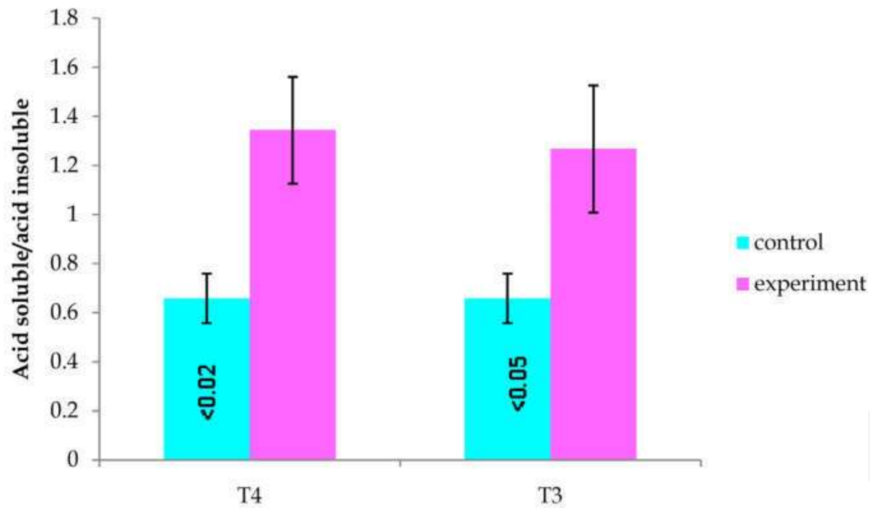

Fig. 8: Acid soluble/ acid insoluble collagen fraction in Liver tissue of *Duttaphrynus melanostictus* by administration of T_4 and T_3(0.5 µg/gm). Values are µg/gm tissue wet wt., columns represent the mean values and vertical bars SEM

Table 1: Effect of thyroxine (T_4) 0.5µg/gm on collagen characteristics of liver tissue of common toad.Values for soluble, insoluble and total collagen are µg/gm tissue wet-weight (Mean ± SEM), numbers in parentheses indicate sample size, NS, not significant at 0.05 P confidence level

Experimental Condition	Salt Soluble	Acid Soluble	Insolu ble	Total	% of Salt Solubility	% of Acid Solubility	Salt Soluble/Salt Insoluble	Acid Soluble/Acid Insoluble
Control	445.069 ± 78.695 (7)	270.673 ± 48.837 (7)	481.880 ± 70.120 (7)	1684.964 ± 146.006 (7)	28.875 ± 3.541 (7)	25.890 ± 3.867 (7)	0.428 ± 0.075 (7)	0.659 ± 0.101 (7)
P	P<0.1	P<0.001	NS	NS	NS	P<0.05	NS	P<0.02
Experiment	620.076 ± 37.154 (7)	659.144 ± 35.621 (7)	486.543 ± 52.108 (7)	2011.625 ± 144.643 (7)	35.892 ± 4.316 (7)	34.794 ± 1.051 (7)	0.769 ± 0.273 (7)	1.344 ± 0.218 (7)

Table 2: Effect of Triiodothyronine(T_3) on collagen characteristics of Liver tissue of common toad. Values of soluble, insoluble and total collagen are µg/gm tissue wet-wt (Mean±SEM), numbers in parentheses indicate sample size, NS, not significant at 0.05 P confidence level

Experimental Condition	Salt-Soluble	Acid-Soluble	Insoluble	Total	% of Salt Solubility	% of Acid Solubility	Salt Soluble/Salt Insoluble	Acid Soluble/Acid Insoluble
Control	536.720 ± 74.270 (7)	270.673 ± 48.837 (7)	481.880 ± 70.120 (7)	1684.964 ± 146.006 (7)	33.512 ± 4.029 (7)	25.890 ± 3.867 (7)	0.428 ± 0.075 (7)	0.659 ± 0.101 (7)
P	P<0.001	P<0.001	P<0.001	P<0.001	NS	P<0.1	NS	P<0.05
Experiment	1232.097 ± 49.725 (7)	720.324 ± 51.551 (7)	1341.164 ± 100.316 (7)	5061.016 ± 488.234 (7)	31.703 ± 4.280 (7)	38.985 ± 5.498 (7)	0.499 ± 0.095 (7)	1.268 ± 0.259 (7)

Results & Discussion

L-Thyroxine accelerated the conversion of soluble to insoluble collagen in adjuvant induced arthritic rats more effectively than prednisolone but was less effective with regard to the inhibition of enhanced catabolism of collagen. However, the synthesis of collagen in adjuvant induced arthritis was improved by both prednisolone and L-thyroxine (Kuberasampath and Bose, 1979). By the administration of T_4, there was a significant increase in salt soluble collagen, acid soluble collagen, in-soluble collagen, % of acid solubility, acid soluble/acid insoluble ratio. The insoluble collagen, total collagen, % of salt solubility, salt soluble/ salt insoluble ratio increased insignificantly.

Salt soluble, acid-soluble, insoluble, total collagen, % of acid-solubility, acid soluble/acid insoluble ratio of collagen increased significantly by the administration of T_3. The salt soluble/salt- insoluble ratio of collagen increased insignificantly. In contrast, the % of salt soluble collagen decreased insignificantly.

L-Thyroxine accelerates the conversion of soluble to insoluble collagen in adjuvant induced arthritic rat more effectively (Kuberasampath and Bose, 1979). Liu *et al.,* 2007 found that in presence of bone morphogenetic protein- 2(BMP-2) and Insulin, T_3 significantly increased collagen type-II mRNA.

The total collagen reflects a balance between a synthesis and degradation. By the administration of T_4 and T_3, the total collagen is increased, more significantly in T3 in comparison to T4. In the preceding sections we have seen that the concentration of salt-soluble collagen increases significantly in liver tissue of *Duttaphrynus melanostictus.* The acid soluble collagen concentration also increased significantly in liver tissue. Salt soluble collagen refers to newly synthesized collagen. Acetic acid extract a form of collagen cross linked into fibers by aldimine bond. The insoluble collagen concentration also increased in both T_4 and T_3. The insoluble collagen are due to the stabilization of the collagen fibrous by inter and intramolecular cross linking. The percentage of salt-solubility, percentage of acid-solubility, salt soluble /salt-insoluble, acid soluble/acid insoluble ratio of collagen increased in both T_4 and T_3 in contrast to % of salt solubility in T_3 which is decreased. The changes in solubility and soluble/ insoluble collagen ration are indirect indicators of alterations in the degree of cross linkages of collagen molecules. This is an indication of impact of thyroxine hormone on the synthesis of collagen. However, the result on % of salt solubility showing a contradiction which is decreased.

Conclusion

Collagen forms a small components of the total protein of normal liver. Liver in turn metabolizes the collagen protein. Crosslinking participates in the increased stability of collagen towards proteolytic degradation. The degree of cross-link formation in collagen affects the physiological functions of the concerned tissue. Thyroxine plays an important role in liver remodelling during metamorphosis. From the findings of the present study, it is concluded that 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 and is showing a tissue specific action.

Acknowledgement

Authors are grateful to authorities of Berhampur University for providing necessary laboratory facilities.

References

1. Yen P.M. Physiological and molecular basis of thyroid hormone action. *Physiological Reviews*. 2001;81:1097–1142.
2. Oliva F., Berardi A. C., Misiti S. and Maffulli N. Thyroid hormones and tendon: current views and future perspectives: Concise review. *Muscles Ligaments Tendons J.*, 2013;3(3):201–03.
3. N Kumar A. The Effect of L–Thyroxine on Metabolic Parameters in Newly Diagnosed Primary Hypothyroidism; Department of Pharmacology, Madras Medical College, Chennai, India; *Clin Exp Pharmacol* 2013;3:128. doi:10.4172/2161-1459.1000128.
4. Varga F., Rumpler M., Zoehrer R., Turecek C., Spitzer S., Thaler R., Paschalis E.P, Klaushofer K. T3 affects expression of collagen I and collagen cross-linking in bone cell cultures. *Biochem Biophys, Res Commun*, 2010;402:180–85.
5. Gouveia C.H., Schultz J.J., Bianco A.C., Brent G.A. Thyroid hormone stimulation of osteocalcin gene expression in ROS 17/2.8 cells is mediated by transcriptional and post-transcriptional mechanisms. *J. Endocrinol*., 2001;170:667–75.
6. Bianco A.C., Kim B.W. Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest*. 2006;116:2571–2579.
7. Williams G.R. Thyroid Hormone Actions in Cartilage and Bone, *Eur Thyroid J*; 2013;2:3-13.

8. Pattnaik S. Calcium and phosphorous metabolism in tissue of thyroxine treated common Indian toad, M.Phil dissertation, Berhampur University. 2014.

9. Randau T. M., Schildberg F. A., Alini M., Wimmer M.D., Haddouti M., Gravius S. The effect of dexamethasone and triiodothyronine on terminal differentiation of primary bovine chondrocytes and chondrogenically differentiated mesenchymal stem cells. *PLoS One*. 2013;8:e72-97.

10. Liu G., Kawaguchi H., Ogasawara T., Asawa Y., Kishimoto J., Takahashi T., Optimal combination of soluble factors for tissue engineering of permanent cartilage from cultured human chondrocytes. *J. Biol. Chem.*, 2007;282:20407–1.

11. Mays P.K., McAnulty R. J., Campa J. S., Laurent G. J. Age related changes in collagen synthesis and degradation in rat tissues. Importance of degradation of newly synthesized collagen in regulating collagen production, *Biochem. J.* 1991;1;276 (Pt 2):30713.

12. Meade K.R., and Silver F.H. Immunogenicity of collagenous implants. *Biomaterials*, 1990;11:176-80.

13. Robins S.P., Shimokomaki M., Bailey A.J. The chemistry of the collagen crosslinks. Age related changes in the reducible components of intact bovine collagen fibres. *Biochem J* 7, 1973;131:771-80.

14. Peleg I., Greenfeld Z., Cooperman H., Shoshan S. Type I and type III collagen mRNA levels in kidney regions of old and young rats. *Matrix* 1993;13:281–87.

15. Abramoff P. and Thomson R.C. In :*An Experimental to Biology*. Freeman and Company, London, 1966.p.251.

16. Bishop O.N. In:*Statistics for Biology*.1st Edn. Longmans Green and Company,London, 1966.p.64.

17. Mikkonen L., Lampiaho K. and Kulonen E. Effect of thyroid hormones, somatotrophin, insulin and corticosteroids on synthesis of collagen in granulation tissue both in vivo and in vitro,*Acta Endocrinol.*, 1966;51:23-31. doi: 10.1530/acta.0.0510023.

18. Kuberasampath T. and Bose S.M. Influence of prednisolone and L-thyroxine on the changes in collagen metabolism in rats with adjuvantinduced-arthritis,*SpringerLink,* 1979;9(5):502–09.

19. Spasov M., Gjorgoski I., Hadzi-Petrushev N., Spasova V. The Liver Parameters In The Collagen-Induced Arthritis Rat Model, *Wulfenia Journal*, Klagenfurt Austria, 2014;21(4):478-97.

20. Liu G., Kawaguchi H., Ogasawara T., Asawa Y., Kishimoto J., Takahashi T., Optimal combination of soluble factors for tissue engineering of permanent cartilage from cultured human chondrocytes. *J. Biol. Chem.*, 2007;282:20407–1.