Effect of Triiodothyronine (T3) on Fast Troponin I, C, & T Protein Levels in Rat Soleus Muscle

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***Abstract*—Troponin complex is essentially situated on slim fiber of striated muscle and control connection of slight and thick fiber and it might be affected by Triiodothyronine (T3). Unfortunately, there is limited study about regulation of T3 on troponin profile. In the present examination, we investigate and distinguish the impact of T3 to troponin proteins. Twelve male rat wistar, 12 weeks old were divided into 2 groups. Hyperthyroidism was induced by injecting T3 20-µg/100-g Body Weight (BW) for 10 days per intraperitoneal (i.p.). After sacrificed using CO2 chamber, rat soleus muscle were dissected out and stored at −80°C until use. T3 stimulated protein level of troponin I, T and and induce but not significant troponin C level in rat soleus muscle. T3 increase significantly the protein expression of Troponin I by 1,67 folds and Troponin T by 1,71 folds compare to control groups. In the other hand, we observed that T3 trend to stimulate the troponin C protein levels although not statistically significant. It seems that troponin C might less sensitive in response to T3 induction. Taken together, T3 alters troponin profile of soleus muscle which it might affect muscle performance.**

***Index Terms*—Triiodothyronine, troponin I, troponin C, troponin T and soleus muscle**

I. Introduction

Thyroid hormones are fundamentally delivered and discharged by the thyroid organ. They are exceptionally fundamental for improvement, development, separation, digestion, and thermogenesis. There are two kinds of thyroid hormones which are triiodothyronine (T3) and thyroxine (T4) [1]. Thyroid hormones have capacity in inciting muscle fiber move, adjusts structure, fiber type and execution of the skeletal muscle [2], [3].

This kind of hormones has a contribution to the plasticity of muscle phenotype which is mainly evident in the case of fiber that is innervated by slow motor neurons. Unlike fast fiber**,** development and maintenance of a slow contractility phenotype is dependent on the almost continuous, low frequency stimulation pattern typical of slow motor innervation. The effect of this stimulation is disputed by thyroid hormone signaling, which not only drives genes expression whereby involved in fast contractility but also stimulates mitochondrial activity and glycolysis [4].

Skeletal muscle strands that are innervated by slow motor neurons can build up a type I, type IIa, type IIx or type IIb phenotype to changing degrees, depending upon the overall quality of these restricting forces. The adjustment of contractile and metabolic properties of skeletal muscle because of changes in thyroid hormone availability embodies the phenotypic adaptability of skeletal muscle by controlling protein turnover and metabolic levels. This function shows that thyroid hormones are in adynamic balance with other outside prompts to drive muscle structure and performance partly via gene expression involved [1], [5].

In skeletal muscle, there is a concert regulation by Sox6 as transcriptional repressor which is depending to myofiber-specific gene expression and determine to the muscle performance. Sox6 regulates myofiber-specific isoforms of sarcomere and calcium regulatory proteins that couple action potentials to the generation of contractile force [6]. During skeletal muscle contraction, role of troponin, tropomyosin, and actin interaction in the Ca^{2+} ion regulation is determine its optimum performance. Tropomyosin obstructs the connection site myosin crossbridge when the muscle is loose. Troponin C ties Ca^{2+}, which balances out the enacted state, where troponin I is never again bound to actin. Troponin T stays the complex on tropomyosin [7]. Troponin complex is essentially situated on slim fiber of striated muscle and control connection of slight and thick fiber stimulated by increasing of intracellular Ca^{2+} . For the most part, troponin complex is comprised of three components, for example, Troponin C, Troponin I, and troponin T which indispensable to muscle withdrawal in skeletal muscle and heart muscle. Troponin is fundamentally joined to the protein tropomyosin and situated inside the depression between actin fibers in muscle tissue [3].

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Stan *et al*. 2016 had reported that skeletal muscle mechanic function closely related with contraction and relaxation whereby it is defined by acting if consisted of actin and myosin. These actin and myosin play important role in skeletal muscle contraction which supported by some other proteins which are troponin and tropomyosin [8].

Unfortunately, there is limited study about regulation of T_3 on troponin profile and it's important for skeletal muscle contraction and relaxation. Therefore, in the present study, we investigate and explore the effect of Thyroid Hormones (TH) on troponin protein stimulation.

II. Materials and Methods

A. Animal

Twelve male Rat wistar, 12 weeks old were obtained from P.T. BioFarma (Bandung, Indonesia) and housed in hanging polycarbonate confines under a 12-hour light, 12-hour dark cycle. Rats were kept up as per the Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the Institutional Board of Ethical Committee at Faculty of Medicine Universitas Padjadjaran no 1462/UN6.KEP/EC/2019.

Hyperthyroidism was induced by injecting T_3 (Sigma-Aldrich; no lot T-074) 20-µg/100-g body weight (BW) in PBS for 10 days per intraperitoneal (i.p.) Control rats (euthyroid) were injected with PBS. Animal models were euthanized in CO2 chambers followed by strangulation. Soleus muscle were dissected out and solidified in Liquid Nitrogen and stored at -80°C for further study.

B. Western Blotting Analysis

Soleus muscle sample were weight and lysed using Radioimmunoprecipitation examine (RIPA) lysis buffer. Lysate samples were centrifugated and supernatant were taken for western blot analysis. Protein concentrations were measured using Lowry method and denaturized at 96°C for 5 minutes. Ten microliter (10 ul) equal with 25 ug of each protein sample were separated using 10% SDS Page Gel Electrophoresis. Protein sample were blotted into PVDF membrane using iBlot (Thermo Scientific). Immunoblotting were performed using Rat polyclonal antibody Troponin I (1:1000; Santa Cruz Biotech), Troponin C (1:1000; Santa Cruz Biotech), Troponin T (1:1000; Santa Cruz Biotech), ß-tubulin (1:1000; #2146, Cell Signaling), Anti rabbit IRDye ® secondary antibodies (1: 10.000; LiCor Biosciences) and anti goat IRDye® secondary antibodies (1: 10.000; LiCor Biosciences). Bands were visualized using Odissey Clx (LiCor Biosciences) and band densitometries were quantified using Image J (NIH, USA).

C. Statistical Analysis

Animal experiments were performed using matched controls, and the data were pooled. Data were presented as average mean ± standard error of the mean (SEM). The statistical significance of differences was performed using one-way ANOVA followed by a Newman-Keuls multiple comparison test. $p < 0.05$ is considered as significant.

III. Result

A. Effect of Thyroid Hormone (T_3) on the Troponin

Triiodothyronine stimulated protein level of troponin I, T and induce but not significant troponin C level in rat soleus muscle. T_3 administration increased significantly the protein expression of Troponin I by 1.67 folds and Troponin T by 1.71 folds compares to control groups. In the other hand, we observed that T_3 trend to stimulate but not significant the troponin C protein levels (Fig. 1).

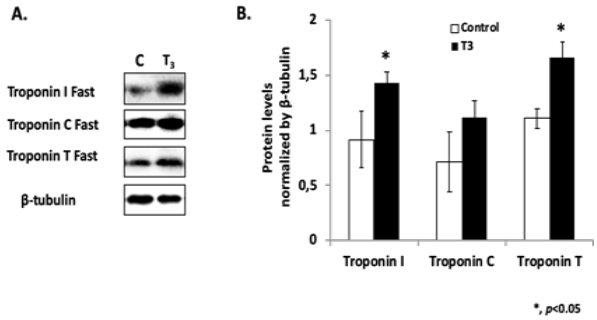

Figure 1. Thyroid hormone (T3) induced protein level of Troponin I and T whether Troponin C showed no significant changes in rat soleus muscle. (A) Representative immunoblot membrane of Troponin I, C and T; (B) Protein levels densitometry is counted by Image J and presented as bar graph. All troponin protein levels is normalized by b-tubulin. Data was presented by Mean Average and Standard Error Minimum (SEM) with p <0.05 will be considered significant (*).

IV. Discussion

Skeletal muscles not only produce various myokines associated with metabolism, growth, and disease but also response to hormones like thyroid hormone (TH) which also have important role in development, growth, differentiation, metabolism and thermogenesis [1], [9], [10]. There are three types of muscles: skeletal, cardiac, and smooth muscle. Among them, skeletal muscle is the major proportion of muscle tissues type and is functioned by contraction-relaxation activation involving actin, myosin, and tropomyosin and troponin communication. Globular actin monomers can polymerize become filamentous actin oriented with tropomyosin (Tm) and it is connected with troponin complex which has three type of regulatory proteins: troponin C, troponin I, and troponin T. [3], [11], [12]. These troponins are encoded by three genes, each of which is predominantly expressed in either fast skeletal, slow skeletal or cardiac muscle [13]. Troponin T and troponin I exist abundantly in skeletal muscle, Troponin T is generated further by alternative RNA splicing process [14]. Troponin is very important protein controlling muscle contraction, thus any changes of the troponins level may influence the muscle performance [3]. Thyroidal status and neuronal control are correlated to ratio and isoforms proportion of the myosin of the muscle, especially thyroid hormone stimulates isoforms transition in rat developing muscles [15], [16]. Hypothyroidism in developing rats also retards the isoform transitions of troponin T but does not inhibit this process completely [16]. Triiodothyronine (T_3) and Tetraiodothyronine (T_4), TH, regulates the of skeletal

muscle protein such as actin and myosin which are important for contraction process like the presence of the TH transporters, and function of TH receptors (THR Alpha or THR Beta) [1]. TH controls bioenergetic metabolism and energy demand in the cell, supplied by substrate oxidation in the mitochondria [17]. In addition, TH stimulates the myogenesis and TH is also essential for skeletal muscle cell's organelle maintenance like mitochondria via autophagy induction [18]. Hyperthyroid or hypothyroid will affect to the muscle performance sarcopenia and myopathies [19]-[21]. TH status might modulate response of skeletal muscle and could be an important determinant and predictor of their response to exercise training [22]. Therefore, homeostasis of TH level is important for regulating muscle performance physiologically.

In the present study, we had observed that TH stimulated Troponin I, C, T in rat soleus muscle (Fig. 1) which may explain the skeletal performance change in thyroid myopathies and Sarcopenia. Flavia Bloise *et al*. had reported that imbalance of thyroid hormone level could cause and related with sarcopenia and different myopathies [21]. The structural and functional roles of the Tn subunits in striated muscle contraction have been recently associated with mutations related with clinical symptom and diseases (example: skeletal myopathies) [23].

Comparison protein level among troponin I, C, and T whereby they have significant increment in protein level which is normalized by B tubulin with the involvement of Thyroid Hormone (T_3) compared to control group. The data shows that troponin T has the highest increment of protein level. THs regulate glycolytic and oxidative pathways in skeletal muscle. T_3 induces an overall shift to faster muscle fibers, displaying a reduced mitochondrial density with the predominant glycolytic metabolism [1], [5]. Troponin I and T (Fig. 1) were more expressed when they were induced by Thyroid Hormone (T_3) if compared to control. Previous study explained that the major effect of thyroid hormones is mediated by modulation of gene transcription. Most of the thyroid response elements in target genes are positive cis-acting elements at which gene transcription is repressed by unliganded thyroid hormone receptors and activated by T_3 occupied thyroid hormone receptors in skeletal muscle. Activation of this process will stimulate thyroid hormone-dependent gene expression connected with a wide array of genes in skeletal muscle[1], [10] and it will concert an effects of thyroid hormone signaling on both contractile and metabolic properties of muscle.

Thyroid hormone influences the time course of ssTnI expression and the life span of cTnI null mice probably via a genomic regulation of ssTnI in the heart [24]. Troponin I bind to actin in thin myofilaments and hold the actin-tropomyosin complex in place. Two different isoform of Troponin I (fast and slow) were existed in skeletal muscle Troponin I by increasing the expression of the gene coding whereby it leads the increment of formation of actin which has a contribution in process of contraction and relaxation in heart [25]. Other factors like chronic low-frequency stimulation in fast-twitch muscles might induce progressive increases in the slow isoforms of TnC and TnI at the expense of their fast isoforms. However, those changes, the fast-to-slow transition of TnI, were more pronounced at the mRNA level [26]. Interestingly, Low-frequency pacing (stimulation) combination with thyroid hormone supplementation defines the neonatal rat cardiac tissues arrangement. It involves significant changes of skeletal structure and cytoplasmic organelle, thus it will define speed and force of skeletal muscle contractions [27].

T_3 administration alters fast Troponin isoform levels in soleus muscle, our results showed that T_3 administration stimulates fast Troponin I protein level by 1,67 folds and Troponin T protein level by 1,71 folds. However, we did not observe any significance increment of fast troponin C protein level after T_3 stimulation. Troponin C is a part of troponin complex whereby has a function in binding to Ca+ in order to produce a conformational change in Tn I. K.T. Hatner and D. Pette had reported that there is different distribution troponin I and C in response to chronic stimulation. In addition, smaller increasement of fast troponin C compared with Troponin I and T after treatment was due to isoform capability to change from slow to fast isoform. In the process of changes and modulation of Tn isoforms transition, TnT has more effectiveness to response to unloading stimuli than for TnI and TnC and it is related to the mechanical activity of muscle. However, slow and fast isoforms of the different Tn are not affected in the same manner by mechanical activity, hindlimb unloading [28]. Troponin T is one of protein integral to the contraction of the skeletal and cardiac muscle. They are mostly expressed in skeletal and cardiac myocytes. It has a capability in binding to tropomyosin and helping position it on actin. Troponins also are affected by alteration of metabolic rate levels like metamorphosis and these conditions correlated with changes of thyroid hormone levels. Troponin T (Tn) isoform is also modulated in metamorphosis process to support capability body or muscle development [29]. Taken together, T_3 plays a role in controlling Tn isoforms proportion in skeletal muscle, unfortunately its specific molecular mechanism remains unclear.

V. Conclusion

Triiodothyronine (T_3) stimulates a significant increment the level of troponin I and T protein levels in soleus muscle. It shows a trend to stimulate the troponin C protein levels; however Troponin C might less sensitive in response to (T_3) induction. Taken together, our study might explain association of abnormal of skeletal muscle performance (muscle weakness) or myopathies in hyperthyroidism via troponin levels alteration.

Conflict of Interest

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

RL, HG had conducted and designed the Invivo research; RL and AH had performed the western blot experiment, RL, RA and US had analized the data and drafted the manuscript; all authors had approved the final version.

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