# DISCUSSION

The experimental model of PTU - induced hypothyroidism was used in the present study. The animals were intraperitoneally injected with PTU (12 mg/kg BW) every other day for 12 weeks. PTU, an antithyroid drug, generally acts to inhibit thyroid hormone production by inhibiting the iodination of tyrosine and the coupling of two iodinated tyrosines to form triiodothyronine ($T_3$) or thyroxine ($T_4$) (Cooper, 1984). The results showed that PTU effectively suppressed the circulating levels of thyroid hormones (Figures 6,7). It was also found that no significant changes ($P>0.05$) in serum $T_4$ concentrations were observed in control animals through the entire 12 weeks of experimental period, while the serum $T_3$ levels tended to be lower ($P<0.05$) than its baseline control. This is consistent with the studies of Westgren, et al. (1976) and Nishikava, et al. (1981) who demonstrated that serum $T_3$ concentration showed an inverse correlation with age. From the knowledge that monodeiodination of $T_4$ to either $T_3$ or $rT_3$ (reverse $T_3$) is the major pathway of $T_4$ metabolism, it may be inferred that the prolonged turnover of $T_4$ as a function of age is a main cause of the decreased serum $T_3$ concentrations with age.

Previous studies concerning the influence of exercise training on thyroid gland have yielded inconsistent results. Balsam and Leppo (1975) reported that normal men undergoing 6 weeks of track running had a decreased plasma $T_4$ concentration while plasma $T_3$ concentration was not changed. In contrast, experimental and human studies showed that only the serum $T_3$ concentration obviously declined during exercise period (Nicod, et al., 1976 ; Boyden, et al., 1982 ; Krotkiewski, et al., 1984). This finding is compatible with the results

obtained from the present study. Up to the present time, the mechanism responsible for the reduction of circulating $T_3$ level during exercise training has not been explained precisely. It has been ascribed that the circulating level of $T_3$ is proportional to the quantity of calories ingested (Katzeff, et al., 1986 ; Katzeff, Bovbjerg and Mark, 1988). According to the study of Loucks and Collister (1993), they found that some reproductive disorders which occurred in exercising women, i.e. amenorrhea, were related to a low circulating $T_3$ level despite normal thyroid function. In addition, this athletic amenorrhea could be prevented or reversed by increasing dietary intake without reducing exercise quantity or intensity. Thus, the decreased serum $T_3$ concentration observed in exercise animals in the present study may be assumed to be associated with a negative caloric balance during exercise.

It was clear from the present study that growth rate had been delayed in hypothyroidism since the final body weights and the weight gain in hypothyroid animals (group II) were significantly less ($P<0.05$) than those seen in control animals (group I) (Figure 4). Additional data also supported the failure of growth in hypothyroid state were the soleus and plantaris muscle weights. It was found that the total muscle weights obtained from hypothyroid animals (group II) were significantly lower ($P<0.05$) than that observed in controls ($P<0.05$), however, no significant difference ($P>0.05$) was noted when expressed as normalized weight (mg/100 g BW) (Figures 8,9). According to the previous studies, the reduction in the growth rate in hypothyroidism may be due to a) a decreased protein synthesis capacity in hypothyroid animals (Crispell, et al., 1956) and b) a decrease in synthesis and secretion of GH caused by hypothyroidism which are involved in the growth retardation (Utiger, 1989).

The effects of exercise on body growth in the present study were found to be similar to those which have been previously reported (Oscai, 1973). The data revealed that male rats subjected to swimming exercise gained weight more slowly and had significantly lower final body weight than the controls. Food consumption is one of the variables which must be considered when discussing about body growth. Oscai, Mole' and Holloszy (1971) reported that exercise was associated with the reductions in food intake and weight loss. This was in agreement with Crews, et al. (1969) who reported that in addition to an increased caloric expenditure, exercise had an appetite - suppressing effect in male rats. Though a number of factors which may be related to the reduced food - comsumption following exercise have been suggested (Russek and Pina, 1962 ; Crews, et al. ,1969 ; Mazzeo and Horvath, 1986), it seems reasonable to deduce that appetite suppressing induced by swimming program in this present study is mediated by the increased level of catecholamines associated with the stress of exercise (Vendsalu, 1960 ; Russek and Pina, 1962).

As expected, the weights of both soleus and plantaris muscles in comparison with the body weight were similar in exercise and control animals. These findings clearly indicated that there were not hypertrophy of these muscles directly involved in the swimming exercise. The interpretation is based on the view that the effect of exercise on muscle size is determined by the forcefulness rather than by the frequency of muscle contraction. Therefore, the endurance exercise, involving exercise of relatively low intensity but being maintained for long period, does not alter the fiber diameter of skeletal muscle (Edstrom and Grimby, 1986). In contrast to normalized muscle weight (mg/100 g BW), the total weights of soleus and plantaris obtained from exercise animals were significantly

lower ($P<0.05$) than those observed in the controls. This may be attributed to the reduction in weight gain noted in exercise animals.

It has long been declared that a decrease in contractile protein resulting in a decrease in tension per gram of muscle. However, from the fact that an increase muscle fiber size is accompanied with a proportional increase in myofibrillar material and a quantitative change in myofibrillar content of the muscle fiber. It can also be stated that the alteration of muscle cross - sectional area has affected the tension that capable of exertion (Edstrom and Grimby, 1986). The present study revealed that the minor decreases in absolute twitch tensions of both hypothyroid soleus and hypothyroid plantaris compared with those of controls were observed while twitch tensions per gram of muscles were found no significant differences ($P>0.05$) (Figures 11,18). Although the muscle cross - sectional areas of both muscles are waiting for further investigation, the results implied that hypothyroidism produces a severe body weight loss but the contractile protein apparatuses of muscles may be well preserved.

Whether exercise training will have influence on muscle tension is dependent on the type of training. This may be related to the changes in myofibrillar content with the corresponding alteration in muscle cross - sectional area of the exercise muscles. In contrast to heavy - resistance training, endurance training does not induce an increase in muscle fiber size as well as muscle weight (Helander, 1961; Seiden, 1976). This may support the finding in the present study illustrating that twitch tensions of soleus and plantaris muscles, expressed as absolute tension or tension per gram of muscles, were similar in both exercise and control animals.

Thyroid hormones are important for the biochemical and functional developments of skeletal muscles. There have been observed that the interconversion of contractile properties of fast and slow muscles can result from alterations in the levels of thyroid hormones. In addition, slow muscle is considerable more responsive to thyroid hormone alterations than fast muscle (Fitzsimons, Herrick and Baldwin, 1992). This was supported by the results concerning the twitch characteristics of the present study. The time to peak twitch (Figures 12,19) and one - half relaxation time (Figures 13,20) of isometric twitch of the hypothyroid - soleus muscle were significantly prolonged ($P<0.05$) while only one - half relaxation time was prolonged in hypothyroid - plantaris. One feasible explanation for these results may be due to a shift in muscle fiber composition. A number of studies have demonstrated that thyroid hormones affect the phenotypic expression of myosin heavy chain (MHC) genes (Fitzsimons, Herrick and Baldwin, 1990 ; Caiozzo, Herrick and Baldwin, 1992). As a consequence, an increase in the expression of slow myosin (SM) isoforms and slow type I MHC genes have been found in hypothyroid muscles (Izomo, Nadal - Ginard and Mahdavi, 1986). This was compatible with the report that the number of slow twitch fibers of hypothyroid muscle increased whereas the proportion of fast - twitch fibers decreased (Nwoye, et al. ,1982). As the speed of muscle contraction is related to characteristics of the myosin ATPase in each fiber and the relative proportion of fibers with faster vs. slower myosin ATPase in the muscle as a whole (B'ara'ny, 1967), an increase in slow myosin in hypothyroid muscle which results in the changes of myosin ATPase activity may be accounted for a prolonged time to peak tension of muscle, especially slow twitch soleus. In addition, from the knowledge that the sarcoplasmic reticulum, by its ability to

ptake calcium from the vicinity of contractile myofilament, plays an important role on excitation - contraction coupling and regulates the rate of muscle relaxation, the prolonged one - half relaxation time of hypothyroid - soleus and plantaris may be due to a decrease in the activity of sarcoplasmic reticulum. Validation of these rationales are supported by the clinical and experimental studies illustrating that there are changes of the myosin phenotype and ATPase activity (Ianuzzo, et al. ,1980) and calcium uptake by isolated sarcoplasmic reticulum in the direction of fast to slow in hypothyroid muscle (Fangburg, 1968).

The results obtained from the present study showed that swimming exercise tended to cause a slow relaxation of muscle as shown by the significant increases ($P<0.05$) in one - half relaxation times of both soleus and plantaris muscles. These findings were in accordance with the report that the fast - twitch white (type IIb) fibers had been converted to fast - twitch red (type IIa) fibers after prolonged endurance exercise (Jansson and Kaijser, 1977 ; Chi, et al. ,1983). In addition, the experimental study of Fitzsimons, et al. (1990) demonstrated that endurance training had an influence on the isomyosin expression in skeletal muscles by eliciting a fast to slower myosin tranformation. Consequently, the changes in both biochemical and contractile properties toward a slow - twitch muscle characteristics were observed after endurance training. These changes included the sarcoplasmic reticulum activity. Therefore, to the extent that rate of relaxation reflects the speed at which $Ca^{2+}$ is taken up by the sarcoplasmic reticulum, the present data suggested that a prolonged one - half relaxation time in trained muscles was supposed to be due to some changes in the sarcoplasmic reticulum. This assumption can be supported by the experimental studies with isolated sarcoplasmic reticulum from trained muscle exhibited that there were

some decreases in major $Ca^{2+}$ binding protein of the sarcoplasmic reticulum (Heilmann and Pette, 1979). Moreover, an increase of several typical membrane proteins of sacroplasmic reticulum in slow - twitch muscle was also observed.

It was noted from the data of the present study that hypothyroidism and endurance training had the influences on the twitch contraction times, in particular, one - half relaxation time. This may be secondary to the similar effects of both hypothyroidism and exercise on the relative changes in myosin isoform and sarcoplasmic reticulum activity toward the direction of fast to slow fiber characteristics. Thus, if hypothyroid animals were subjected to exercise training, the contraction and relaxation time should be markedly increased. Interestingly, no significant differences ($P>0.05$) in the twitch contraction times (time to peak tension and one - half relaxation time) of both soleus and plantaris muscles were observed between exercise and hypothyroid - exercise animals and between hypothyroid and hypothyroid - exercise animals. In addition, the value of one - half relaxation time of hypothyroid - exercise soleus seemed to be shorter than that noted in hypothyroid soleus. Considering the results together, it can be inferred that swimming exercise may in part prevent the slowness of contraction and relaxation of hypothyroid soleus muscle. In order to elucidate the mechanism, the further experiment focussing on the myosin ATPase activity and the calcium uptake by isolated SR should be performed.

From the force - frequency curves of soleus and plantaris, it could be deduced that the development of tetanic tension was depressed in hypothyroidism (Figures 14,21). Previously, Gold, et al. (1970) reported that both the rate of tension development and velocity of muscle contraction decreased in hypothyroid state. Thus, the decrement in tetanic tension of hypothyroid muscles

at all stimulating frequencies could be attributed to a decrease in sarcoplasmic reticulum activity as indicated by a prolonged one - half relaxation time. This also implied that during repetitive stimulation, the amount of $Ca^{2+}$ released into the cytosol which participated in the cycling between the sarcoplasmic reticulum and cytosol was reduced in the hypothyroid muscles. The reduction in tetanic tension resulting from thyroid hormone deficiency in the present study may explain, at least in part, the muscular weakness in hypothyroid patients. In contrast to hypothyroid muscles, trained muscles exhibited a considerable increase in tetanic tension with increasing frequency of stimulation. Everts and Hardeveld (1987) have demonstrated that the developed muscle tension is directly proportional to the rise in cytosolic calcium concentration. From this view the greater development in tetanic tension of exercise muscles may be, in part, attributed to some changes in $Ca^{2+}$ managment in cytosol induced by endurance training. This may be related to an increase in the amount of $Ca^{2+}$ which binds to troponin C in excitation - contraction coupling. Furthermore, muscles were observed hypertrophy in neither soleus nor plantaris. In this regard, the possible explanation for the greater tetanic tension of trained muscles than that of controls may be an increase in oxidative capacity in muscle fiber which is due to biochemical changes in response to training. This rationale is based on the fact that the capability of the muscle fibers to keep up tension associates with the content of oxidative enzymes (Burke, et al., 1973). Comparison of force - frequency curves of soleus and plantaris illustrated that the increase of tetanic tension along with the frequency of stimulations in hypothyroid - trained muscles was the same magnitude as those observed in the controls. As a matter of the results, it may be suggested that some biochemical adaptations in muscle fiber

with exercise training can prevent the defects in the development of tetanic tension induced by the lack of thyroid hormones. Regarding to the results of serum thyroid hormone concentrations, it can also be inferred that this muscle adaptation is independent on the normal thyroid function.

The fatigue index was used in this study as the indicator of the muscle fatigue resistance or muscular endurance properties. According to the criteria of Burke, et al. (1973), the fatigue index of the soleus was closed to the range associated with good endurance properties whereas the fatigue index of the plantaris was much lower than that of the soleus (Figures 16,23). The result of the present study, in accordance with the study of Argov, et al. (1981), clearly showed that hypothyroidism depressed fatigue resistance of both soleus and plantaris muscles. This may explain the clinical report that many hypothyroid patients complain of exercise intolerance and fatigue (Ruff, 1986). The causes of the reduced endurance capacity of hypothyroid muscles are considerably complex. The depletion of muscle glycogen appears to be rapid during exercise in hypothyroidism. It has long been known that enhancing free fatty acid avilability increases the energy supply from lipid during prolonged exercise, thereby, decreasing the rate of muscle glycogenolysis. The capacity of muscle to oxidize free fatty acids is directly proportional to the mitochondrial content of the muscle (Oscai, Mole' and Holloszy, 1971). The observation of the reduction in the capacity of hypothyroid muscle for β- oxidation is consistent with the fact that mitochondrial components and associated oxidative enzymes are actually reduced in thyroid - deficient muscle (Baldwin, et al., 1980 ; Argov, et al., 1988). This may lead to the acceleration of muscle glycogenolysis and hence a faster depletion of the limited stores of muscle glycogen. In addition, a reduced muscle blood flow

resulting from an impaired cardiovascular function in thyroid deficiency probably contributes to the poor endurance capacity of hypothyroid muscles (McAllister, Ogilvie and Terjung, 1991).

The fatigue index of a muscle is a function of the relative contributions of the muscle fiber types to total force production (Burk, et al., 1973). A number of studies have documented an increase in slow - twitch type I fiber or slow myosin expression in the hypothyroid muscle (Izomo, Nadal - Ginard and Mahdavi, 1986 ; Fitzsimons, Herrick and Baldwin, 1990 ; Caiozzo, Herrick and Baldwin, 1992). It might be expected that this could theoretically elevate the levels of oxidative enzymes with an attendant increase in muscle fatigue resistance. However, thyroid - deficient muscle exhibits the reduction of mitochondrial contents despite an increase in the proportion of slow - twitch muscle fibers (Tata, et al., 1963 ; Baldwin, et al., 1980 ; Argov, et al., 1988). Indeed, hypothyroidism brings about an uncoupling of customary relationship between fiber composition and the oxidative capacity of the muscle.

The results obtained from the present study demonstrated that the endurance of both soleus and plantaris muscles increased as a result of swimming exercise. It has been well known that exercise training induces a number of adaptations in skeletal muscles. This enhanced performance appears to be primarily related to the mitochondrial changes (Fitts and Holloszy, 1977 ; Holloszy and Coyle, 1984). The increase in oxidative capacity in muscle fiber as a response to exercise training has been well documented in both animal experiments and humans. This may be attributed to an increase in the oxidative enzyme activities. The changes in oxidative enzyme activities correlated with an increase in total mitochondrial protein which can be demonstrated in exercise

muscle (Cooper and Eccles, 1930 ; Schersten, 1982 ; Friden and Sjo"stro"m, 1983). One consequence of the adaptations induced in muscle by endurance training is the change in the metabolic profile. Endurance training results in an increased capacity to hydrolyze triglyceride to free fatty acids by stimulating the lipoprotein lipase activity in muscle fibers (Borensztajn, et al., 1975). Therefore, muscle decreases carbohydrate utilization during repeated muscle contractions at the expense of increased utilization of fat. This can be explained by the slow depletion of muscle glycogen and the low level of lactate in blood and muscle (Holloszy and Coyle, 1984). It may be suggested that the glycogen - sparing effect of the increased fat oxidation probably plays an essential role in increasing muscle endurance that occurs with training. Other adaptations as a consequence of exercise training that associate with endurance are the change in muscle vascularization. In more recent studies, the increase in capillary density has been reported to occur with training (Mai, Edgerton and Barnard, 1970). It has also been reported that there has been completed conversion of fast - white (type IIb) to fast - red (type IIa) fibers in trained muscles (Jansson and Kaijser, 1976 ; Chi, et al., 1983). This was substantiated when linked to the study of Gregory, Low and Stirewalt (1986) who illustrated that the expression of slow and intermediate myosin isoform in muscle was increased after endurance training. From these findings and the results obtained from the present study, it can be inferred that the corresponding changes of contractile properties from fast - twitch to slow - twitch fibers may be another consequences that muscle fibers adapt to increase endurance training.

The training program of swimming in the present study brought about the

increase in fatigue indexes of both soleus and plantaris muscles of hypothyroid animals to the values essentially the same as those found in the controls. As a matter of the results, it may be suggested that the impaired mitochondria and reduction in associated oxidative enzymes in hypothyroid muscles can be ameliorable after training. This interpretation is based on the previous works of Baldwin, et al. (1980) demonstrating that the decrement in both maximal $O_2$ consumption and oxidative capacity induced by thyroid deficiency can be reversed by physical training.

In conclusion, the results of the present study revealed that hypothyroidism causes a reduction in growth rate as shown by the decreases in both body weight and weight gain in hypothyroid animals. The results also demonstrated that a reduction in weight gain during exercise period may confirm a report of an appetite suppressing effect of swimming exercise. The contractile speeds of muscles were affected by hypothyroidism. In soleus, hypothyroidism led to considerable increases in both time to peak tension and one - half relaxation time. In plantaris, one - half relaxation time was prolonged by hypothyroidism whereas time to peak tension was not altered. This alteration in one - half relaxation times provides an evidence supporting the idea that hypothyroidism produces the functional impairment of sarcoplasmic reticulum. Tetanic tension (normalized for weight) at all stimulating frequencies of both plantaris and soleus muscles are depressed by hypothyroidism. The decreased fatigue resistance found in hypothyroid soleus and plantaris is suggested to be due to the reduction in skeletal muscle oxidative capacity. A training program of swimming effectively improves the reduction in tetanic tension of both hypothyroid plantaris and soleus at all stimulating frequencies except at

5 Hz. The fatigue resistance of hypothyroid muscles, both soleus and plantaris, can be increased by swimming exercise. In addition, compared with hypothyroid muscles, one - half relaxation time of exercise - hypothyroid muscles, especially of soleus tends to decrease. Finally, it may be proposed that endurance training in hypothyroidism can improve the impaired contractile properties of slow - soleus and fast - twitch plantaris muscles.