



## BIOCHEMICAL BASIS OF MITOCHONDRIAL DYSFUNCTION IN HYPOTHYROIDISM

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**ABSTRACT**

Thyroid hormones are required for normal function of most tissues of the body, playing essential roles in growth, development, differentiation and metabolism with major effects on O<sub>2</sub> consumption and metabolic rate. Mitochondria by virtue of their biochemical function are natural candidates as the target for the calorogenic effects of thyroid hormone. Thyroid hormone might act at several sites in the

mitochondria to regulate cell respiration. Site 1: Activity of ADP/ATP translocase. Site 2: Citric acid cycle intermediates. Site 3: Phosphate transporter. Site 4: F<sub>0</sub>F<sub>1</sub> ATP<sub>ase</sub> and Site 5: Electron transport chain. ANT, which exerts major control over the rate of oxidative phosphorylation maybe a direct target of T<sub>3</sub> action on the mitochondria. Biochemical basis of the mitochondrial dysfunction related with low level of ANT in hypothyroidism is due to low level of cardiolipin. The activity of the crab cycle is not specifically affected by the hypothyroidism. There is a decrease in phosphate transporter activity in hypothyroidism and it can be due to an effect of thyroid hormones on lipid microenvironment of the phosphate carrier. The hepatic mitochondrial lipid composition is altered significantly in hypothyroidism. It is probably a nuclear effect of thyroid hormone, as it is on phosphate transport in kidney and liver. The electron transport chain complexes and F<sub>0</sub>F<sub>1</sub> ATP<sub>ase</sub> activity are decreased in many tissues in thyroid deficient conditions. Hypothyroidism affects the expression of mitochondrial proteins from respiratory chain. In state 3 respiration, the major

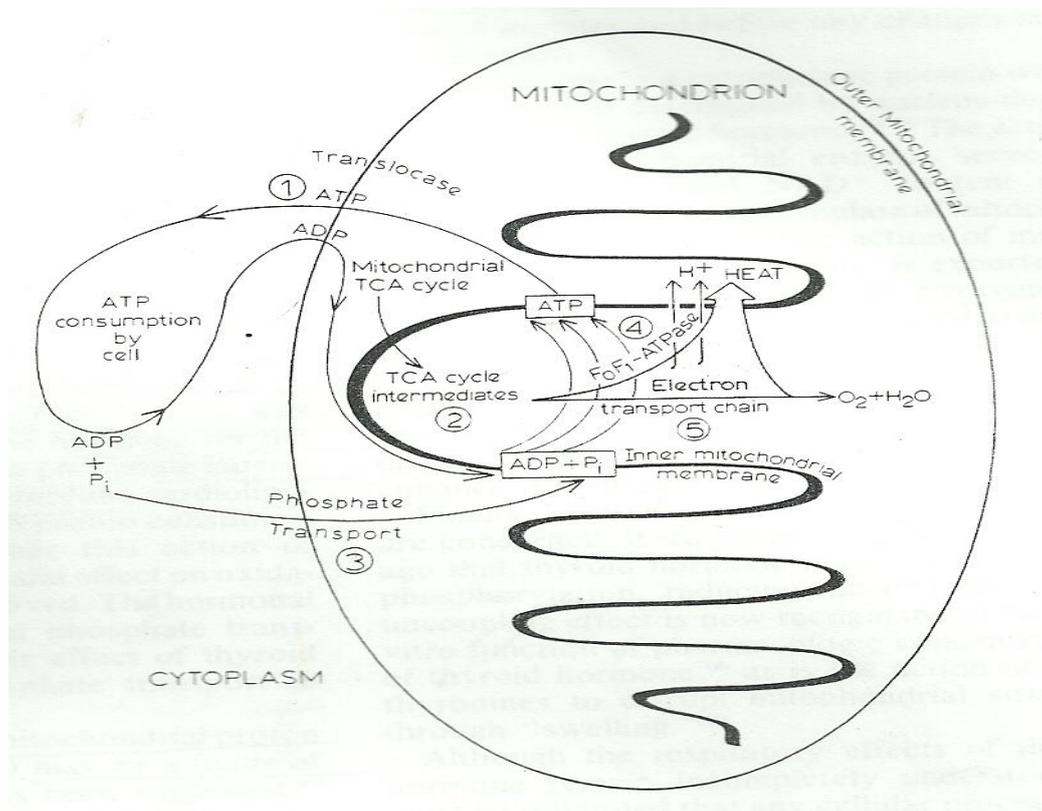
effect of decrease in thyroid hormones is via changes in the reactions involved in the phosphorylation of ADP and the export of ATP, probably at the adenine nucleotide carrier. In state 4 respiration, the rate is controlled very largely by the proton leak across the mitochondrial inner membrane. Leak is depressed in hypothyroid mitochondria due to alterations in membrane fatty acid composition primarily in n-3 content. Fatty acids are not only inducers of uncoupling but they have also a regulatory function in this process. Thyroid hormone has an impact on membrane fluidity and lipid composition. Hypothyroidism causes decreased membrane rigidity and greater extent of mitochondrial membrane lipid damage. Chronic state of hypothyroidism is characterized with impairments in the redox potential leading to free radical chain reactions and to metabolic suppression of antioxidant capacity. Accumulation of ROS and loss of membrane integrity are two primary factors that potentially trigger apoptosis.

**KEYWORDS:** ANT(Adenine nucleotide translocase), ROS(reactive oxygen species), Mitochondria, Hypothyroidism.

## INTRODUCTION

Mitochondria have a particular status in the cell. They possess their own genome, a specific genetic code and a specific apparatus involved in DNA replication, expression and protein synthesis.<sup>[1]</sup> The modern day mitochondrion is believed to have evolved over a billion or more years originating as an invading eubacterium in early eukaryotic cells. Of the 1000 or so mitochondrial proteins, mitochondrial genome encodes only 13 enzymatic subunits of the respiratory chain, two rRNAs and 22 tRNAs, while the remainder are transcribed and translated from the nuclear genome and transported into the inner mitochondrial membrane.<sup>[2]</sup> Cellular respiration consists of the oxidation of glucose, fatty acids and amino acids to produce ATP. The oxidation of these substances occurs mainly in the mitochondria of cells through the citric acid cycle. The oxidation of citric acid cycle intermediates in mitochondria results in the reduction of nicotinamide adenine dinucleotide (NAD) to NADH and flavin adenine dinucleotide (FAD) to FADH<sub>2</sub>.<sup>[3]</sup> Oxidation of reducing equivalents from NADH by mitochondria occurs by way of an electron transport chain that involves several proteins, including cytochromes, heme proteins that accept electrons from NADH and FADH<sub>2</sub>, and shuttle electrons to molecular oxygen. Electron transport system produces a proton gradient across the inner mitochondrial membrane whose energy is harnessed to synthesize ATP by a mitochondrial ATPase (F<sub>0</sub>F<sub>1</sub>ATPase) from intramitochondrial ADP and inorganic phosphate

( $P_i$ ).<sup>[3]</sup> Thyroid gland primarily produces two hormones, L-thyroxine (T<sub>4</sub>) and triiodo-L-thyronine (T<sub>3</sub>). These hormones are well known to exert profound effects on the energy metabolism. Thyroid hormones (TH) are required for normal function of the most tissues of the body, playing essential roles in growth, development, differentiation and metabolism, with major effects on O<sub>2</sub> consumption (QO<sub>2</sub>) and metabolic rate.<sup>[4]</sup> Current available data indicate that TH calorogenesis is achieved by both: (i) a short-term nongenomic signaling mechanism mediated by 3,5-diiodothyronine and 3,3,5-triiodothyronine (T<sub>3</sub>) leading to the allosteric activation of cytochrome c oxidase<sup>[5]</sup>, and (ii) a long-term pathway upregulating nuclear and mitochondrial gene transcription through T<sub>3</sub>-signalling.<sup>[6,7]</sup> Influence of thyroid hormones on metabolic process has been widely recognized particularly the profound changes in ATP metabolism. ATP production is primarily given by mitochondria along with other relevant processes including intracellular calcium ion regulation, redox signaling and apoptosis triggering. In this respect it is known that hypothyroidism changes oxygen consumption, temperature regulation, growth and development, the metabolism of proteins, fats, carbohydrates, nucleic acids, vitamins and inorganic anions and cations. In the present study an effort was made to see the biochemical changes that occur in mitochondria due to hypothyroidism



**Fig 1: Showing various sites in mitochondria where thyroid hormones can act**

### **Actions and effects of thyroid hormones in mitochondria**

Early experiments showing cellular mechanism of action of thyroid hormone were performed by J.R. Tata and coworkers in 1960s. These authors showed that administration of thyroid hormone to hypothyroid rats induces an increase in their basal metabolic rate. Mitochondria by virtue of their biochemical function are natural candidates as the target for the calorogenic effects of thyroid hormones.

Thyroid hormones might act at several sites in the mitochondria to regulate cell respiration.

Site 1: Activity of ADP/ATP translocase.

Site 2: citric acid cycle Intermediates.

Site 3: Phosphate transporter.

Site 4: F<sub>0</sub>F<sub>1</sub> ATPase

Site 5: Electron transport chain.

Involvement of skeletal muscles is among the most prevalent consequences of hypothyroidism. Symptoms such as fatigue, exercise intolerance, exertional myalgia and cramps are frequent hallmarks of hypothyroidism and sometimes represent the prodromic signs of hypothyroidism. It is widely accepted that mitochondrial respiration represents one of the main targets of thyroid hormone deficiency.

#### **Site 1: Thyroid Hormones and ADP/ATP translocase**

Adenine nucleotide translocase (ANT) plays a key role in energy metabolism by exchanging adenine nucleotide between the cytosol and the mitochondrial matrix and in so doing provides the cytosol with adenosine triphosphate (ATP), to drive energy requiring reactions.

Translocase is the most abundant mitochondrial protein. Its isoforms appear to be tissue-specific and it has been reported to be the mitochondrial T<sub>3</sub> binding protein.<sup>[8]</sup>

It is a protein of particular interest in the understanding of the mitochondrial energy coupling process because (i) it exerts high control on mitochondrial respiration<sup>[9,10]</sup> (ii) it is involved in the mitochondrial permeability transition<sup>[11]</sup> and (iii) it mediates uncoupling of oxidative phosphorylation by non-esterified long chain fatty acids.<sup>[12,13]</sup>

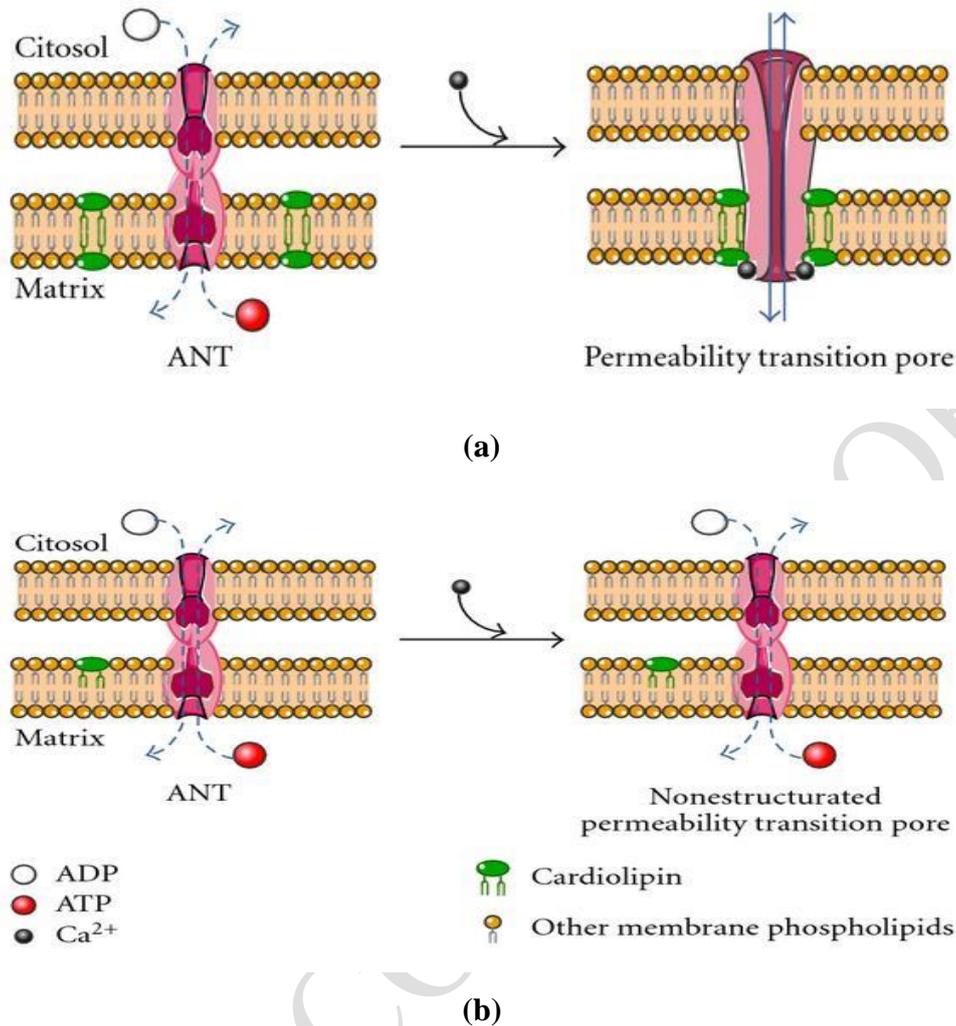
Hoopner and Rasmussen (1988)<sup>[14]</sup> investigated the effect of T<sub>3</sub> on rat liver, heart and kidney ANT gene expression. Hypo and Hyperthyroid rats showed no differences in size nor in the amounts of heart, liver and kidney ANT-mRNA. According to them, the long term action of

thyroid hormones on increasing the carrier mediated ADP/ATP translocation cannot be ascribed to an effect of T3 on ANT gene expression.

Mak, Shrago and Elson (1983)<sup>[15]</sup> saw the effect of thyroidectomy on the kinetics of ADP-ATP translocation in liver mitochondria. Both ANT and respiratory activities in liver mitochondria of thyroidectomized rats were 30% below normal. Mitochondrial ANT activities were determined by the back-exchange method of Pfaff and Klingenberg(1968).<sup>[16]</sup> ANT in hypothyroid rat liver mitochondria exhibited a 25-35% lower  $V_{max}$  and 75% higher  $K_m$  when assayed over temperature range 0 to 37degrees c. The ADP-ATP translocase in hypothyroidism was more resistant than in control carrier to bongkrekate inhibition. The decrease in transport of ADP, which is consistent with the decreased oxidative activity associated with hypothyroidism, occur secondary to changes in the lipid matrix of the inner mitochondrial membrane.

Experiments of Kenneth sterling(1995)<sup>[17]</sup> showed that mitochondria of hypothyroid rats has a markedly diminished ANT(adenine nucleotide translocase) activity which is restored to normal levels within 72 hrs by intraperitoneal injection of 10 to 20 $\mu$ g triiodothyronin T3/100gm body wt. Quantitatively similar results on ANT activity were obtained in liver mitochondria within 30 to 60 minutes following intravenous injection into hypothyroid rats of a more physiological dose of T3 (40ng/100gm body wt). These findings support the view that ANT which is considered to exert major control over the rate of oxidative phosphorylation may be a direct target of T3 action on the mitochondria.

Biochemical basis of the mitochondrial dysfunction related with low level of ANT in hypothyroidism can be explained on the basis of low level of cardiolipin (a dimeric phospholipid synthesized by the mitochondrial enzyme cardiolipin synthase) in hypothyroidism.<sup>[18-20]</sup> Normal ADP/ATP exchange activity of ANT depends on cardiolipin.<sup>[21]</sup> Paradies et al<sup>[22]</sup> demonstrated that cardiolipin content in liver mitochondria from hypothyroid rat was 24nmol/mg versus 40nmol/mg in euthyroid rats. These values correlated with a diminution in ANT levels, from 58nmol/mg in control rats to 40 nmol/mg hypothyroid rats. In addition to its normal function, ANT forms the inner membrane channel of the mitochondrial permeability transition pore (MPTP) which allows the movement of matrix ions and metabolites through the mitochondrial membrane.



**Figure 2: Conversion of the adenine nucleotide translocase (ANT) to permeability transition pore. (a) Under euthyroid conditions, ANT allows the selective exchange (dotted lines) of ADP and ATP between the citosol and the mitochondrion matrix.  $\text{Ca}^{2+}$  triggers the assembling of ANT with cardiolipin and other proteins (not shown in the figure) to integrate the permeability transition pore. (b) In hypothyroidism, the low content of cardiolipin in the mitochondrion membrane limits the pore formation, thus conserving the ANT function and matrix content unaltered.**

Binding of cyclophilin-D (cyp-D) to ANT matrix surface facilitates a calcium-triggered conformational change converting it from a specific transporter to a nonspecific pore. Both proteins alongwith the voltage- dependant anion channel(VDAC) and cardiolipin probably represent the minimum MPTP configuration.<sup>[23-25]</sup> Once integrated, MPTP opening is favoured by  $\text{Ca}^{++}$  accumulation in the matrix or by inhibiting the ADP/ATP translocase activity with the specific inhibitor carboxyatractyloside(CAT).<sup>[26]</sup> As it is already mentioned,

translocase activity in the membrane depends on the lipid constitution of the bilayer (cardiolipin).<sup>[27,28]</sup> In hypothyroidism mitochondria has low cardiolipin level and low expression of ANT<sup>[22]</sup>, MPTP formation is impaired thus conserving the ANT function and matrix content unaltered.

**Site2:** Regulation of the availability of kreb cycle intermediates in the mitochondria by actions on the mitochondrial tricarboxylic acid cycle is a theoretical effect of thyroid hormone. The activity of the crab cycle is not specifically affected by the hypothyroidism.<sup>[31]</sup>

**Site3:** Phosphate transport is influenced by thyroid hormone. Activity of mitochondrial phosphate transporter is increased by hyperthyroidism.<sup>[29]</sup> and decreased by hypothyroidism. Rate of phosphate transport is reduced (around 40%) in mitochondria from hypothyroid rats compared to that obtained in mitochondria from normal rats. Treatment of hypothyroid rats with thyroid hormone reverses this effect completely. There is no significant difference either in the respiratory control ratios or in the ADP/O ratios between these two types of mitochondria. Decrease in phosphate transporter activity can be attributed to an effect of hormones on the lipid microenvironment of the phosphate carrier. The hepatic mitochondrial lipid composition is altered significantly in hypothyroid rats. Total cholesterol increases, the phospholipid decreases and cholesterol/ phospholipid molar ratio increases(around 40%). Among the phospholipids, cardiolipin shows the greatest alteration (30% decrease) in hypothyroid rats. Phosphotidylethanolamine/phosphatidyl choline ratio also decreases. Alterations were also found in the pattern of fatty acids.<sup>[30]</sup> Whether this action of thyroid hormone has a substantial effect on oxidative phpsphorylation is unproved. The hormonal action on the mitochondrial phosphate transporter is probably a nuclear effect of thyroid hormone, as it is on phosphate transport in kidney and liver.

#### **Site 4 And Site 5**

The reduced cofactors generated during fuel oxidation donate their electrons to the mitochondrial electron transport chain (ETC). ETC transfers the electrons to O<sub>2</sub>, which is reduced to water. As electron travel through the electron transport chain, protons are transferred from the mitochondrial matrix to the cytosolic side of the inner mitochondrial membrane. The asymmetric distribution of protons across the inner mitochondrial membrane generates an electrochemical gradient across the membrane which consists of a change in pH ( $\Delta\text{pH}$ ) across the membrane and also a difference in charge ( $\Delta\psi$ ) across the membrane. Proton entry into the mitochondrial matrix is energetically favorable and drives the synthesis

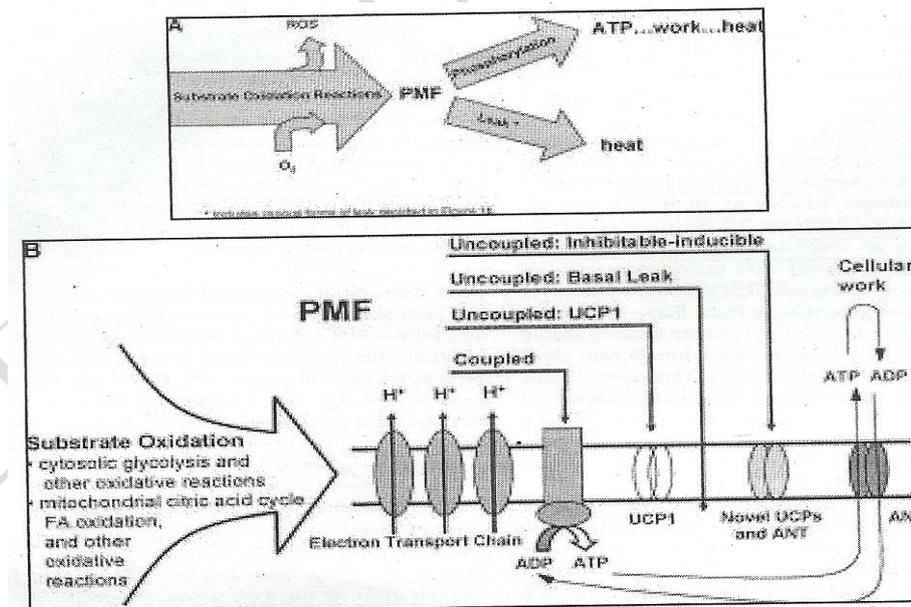
of ATP via the ATP synthase, a process called oxidative phosphorylation (state 3 respiration). Uncouplers allow respiration to continue in the absence of ATP synthesis because the energy inherent in the proton gradient is released as heat (state 4 respiration).

Mitochondrial uncoupling plays an important role in thermoregulation in brown adipose tissue (BAT). It is dependent on the presence of uncoupling protein -1 (UCP-1) in this tissue.

### Thyroid hormones and Uncouplers

Over the years, several biochemical processes have been put forward to explain the mechanism by which THs stimulate thermogenesis.<sup>[31]</sup>

THs, in synergism with the sympathetic nervous system, are involved in BAT cold induced thermogenesis.<sup>[32]</sup> Hypothyroid rats do not survive cold well and fail to increase BAT recruitment in response to nor-adrenaline. Since the discovery of UCP2 and UCP3 in most tissue in mammals, including humans and the proposal that they play a putative role as uncouplers, many studies have been performed to try to disclose an involvement of these proteins in the effect exerted by THs on energy expenditure. Administration of T3 to rodents leads to increase in the expressions of UCP2 and UCP3 in heart and skeletal muscle.<sup>[33,34]</sup>



**Fig 3: (A) The oxidative phosphorylation system, showing coupled and uncoupled routes of the dissipation of proton motive force (PMF). (B) Components of the oxidative phosphorylation system potentially affected by thyroid hormone status.**

Alteration in the tissue respiratory activity is one of the most characteristic metabolic changes observed in thyroid dysfunction. Pathologically low amounts cause low levels of respiration with a general slowing of metabolic activity. The affect on respiration is primarily the result of changes in the expression of respiratory genes and modulation of inner membrane structure. Thyroid hormone influences the expression of a number of nuclear encoded respiratory genes at the level of mRNA and enhances expression of mitochondrially encoded respiratory genes.<sup>[35]</sup> Several mitochondrial key-enzymes (tricarboxylic krebs cycle enzyme complexes of the respiratory chain) are inhibited by experimental thyroid hormone deficiency or activated by experimental thyroid excess.<sup>[36 - 42]</sup>

Role of mitochondria in cellular energy metabolism and the possibility of direct control of mitochondrial function by thyroid hormone has been the subject of much study.<sup>[43,44]</sup> Mitochondria isolated from hypothyroid rats show respiration rate lower than those of mitochondria isolated from euthyroid rats. Injection of nanomolar quantities of T3 into hypothyroid rats in the presence of inhibitor of DNA transcription and protein synthesis can rapidly increase the respiration rate of the subsequently isolated mitochondria.<sup>[45,46]</sup>

The electron transport chain complexes and F<sub>0</sub>F<sub>1</sub> –ATPase activities are decreased in many tissues in thyroid deficient condition.<sup>[47,48]</sup> Hypothyroidism affects the expression of mitochondrial proteins from the respiratory chain. Coenzyme Q<sub>10</sub> levels and the antioxidant capacity of mitochondria are found to be decreased in hypothyroidism.<sup>[49,50]</sup>

Martinez et al (2001)<sup>[51]</sup> analysed the effect of hypothyroidism on mitochondrial activity in the brain of neonatal rats by studying oxidative phosphorylation and the activity of the respiratory complexes in different brain areas. They showed that the rate of ATP synthesis in the presence of NADH-generating substrates was decreased only in isolated mitochondria from the cerebral cortex and straitum of hypothyroid rats. They also observed a decrease in the activity of complex I and complex III in cerebral cortex and straitum of same animals.

Effect of thyroid hormone on the expression of mitochondrial proteins was evaluated during development by measuring cytochrome c oxidase (CYTox) activity and cytochrome c protein and mRNA levels in heart and skeletal muscle of control hypothyroid rats. Cytochrome c oxidase activity was reduced in hypothyroid animals throughout development in heart compared to controls by 50 % at 56 days. But in muscles no effect of hypothyroidism was observed. Hypothyroidism reduced cytochrome c in muscles by 30-35% at 56 days but had

no effect in heart. Hypothyroidism also reduced cytochrome c mRNA in heart, without a change in cytochrome c protein.<sup>[52]</sup>

The hypothesis that thyroid hormones (initially T4 and then T3) exerted their hypermetabolic effects by “uncoupling an oxidative phosphorylation” was given by Lardy and colleagues over 50 years ago.<sup>[53,54]</sup> They demonstrated that enzyme preparations from liver of hyperthyroid rats were less efficient in coupling phosphorylation with oxidation than were preparations from normal rats. Seitz et al found that within 2 hrs of addition of T3 to the perfusate, the mitochondrial ATP/ADP ratio in hypothyroid rat liver was significantly decreased.<sup>[55]</sup> It was also seen that the ATP/ADP ratio in livers from T4 treated hyperthyroid rats was significantly decreased in mitochondria and increased in the cytosol. Thus thyroid hormones increase mitochondrial respiration and ATP regeneration, which is associated with accelerated adenine nucleotide translocator(ANT) activity i.e. increased translocation of ADP and ATP. Another study showed that the addition of 10pM T3 to hypothyroid rat liver mitochondria doubles ANT activity at low ADP concentrations.<sup>[56]</sup> The authors explained that the covalent modification of ANT to form its externally facing c-conformation increases its leakiness to cations (e.g. Ca<sup>++</sup>, H<sup>+</sup>, and K<sup>+</sup>) and increasing its capacity for ADP import.<sup>[57]</sup>

Although mitochondrial oxidative phosphorylation is responsible for the synthesis of about 90% of a cell's ATP, the oxidation of fuel substrates (e.g. glucose and fatty acids) is uncoupled from ATPsynthase activity most of the time. This uncoupling occurs through a process called “basal mitochondrial proton leak”.<sup>[58]</sup>

Basal proton leak is physiologically important. In isolated hepatocytes it accounts for 20-30% of oxygen consumption.<sup>[59]</sup> In perfused resting skeletal muscles in rats it accounts for approx. 50% of oxygen consumption.<sup>[60]</sup> Rolfe and Brand found that the contribution of basal mitochondria proton leak to resting energy expenditure at whole body level could be in the range of 20-25%.<sup>[60,61]</sup>

It was observed that when succinate was employed as a respiratory substrate for mitochondria incubated in a mannitol/sucrose/phosphate buffer, and measurements were performed during initial additions of ADP, the magnitude of state 3 and state 4 respiration was not different between mitochondria from the hypothyroid and those from the control rats. During the course of repetitive additions of ADP and consequently of sequential transitions from state 4 to state 3 and back to state 4. Mitochondria from hypothyroid animals showed a gradual

decline in the rate of both state 3 and state 4 respiration whereas those from normal animals did not. The total succinate dehydrogenase activity was not different between the two types of mitochondria, and the decline in state 3 and state 4 respiration was not accompanied by any change in the apparent  $K_m$  for ADP or in the corrected ADP/O ratio. The amount of oxygen consumed during the state 4  $\rightarrow$  state 3  $\rightarrow$  state 4 transition was lower in the hypothyroid than in the control mitochondria.<sup>[57]</sup>

In isolated mitochondria there are two separate effects of thyroid hormones on oxygen consumption. One is dominant in state 3 respiration and other is dominant in state 4 respiration.<sup>[44,62]</sup> In state 3, control over the respiration rate is shared approximately equally between the reactions that produce proton motive force and those that consume it to make ATP.<sup>[63]</sup> Most of the control is in substrate transport and in the adenine nucleotide carrier.<sup>[64]</sup> The major effect of decreases in thyroid hormones is via changes in the reactions involved in the phosphorylation of ADP and the export of ATP.<sup>[62]</sup> probably at the adenine nucleotide carrier.<sup>[65,8]</sup> In state 4 the respiration rate is controlled very largely by the proton leak across the mitochondrial inner membrane<sup>[63,64,66]</sup>, and thyroid hormones act predominantly by altering this proton leak. The leak is depressed in hypothyroid mitochondria and stimulated in the hyperthyroid mitochondria.<sup>[44,67,68]</sup> A significant portion of the effect of thyroid hormone status on proton leak is due to alterations in membrane fatty acid composition, primarily changes in n-3 content. Both the hypothyroid state and dietary effects appear to be mediated in part by inhibition of the  $\Delta^6$ - and  $\Delta^5$ - desaturase pathways.<sup>[69]</sup>

Regarding the mechanism responsible for basal proton leak and the thyroid hormone induced changes in leak, it was demonstrated that half to two-thirds of the basal proton leak of mitochondria is catalyzed by ANT.<sup>[70]</sup> while this uncoupling was shown to be independent of the ATP/ADP exchange function of ANT and of the known fatty acid induced leak function of ANT, further research is needed to re-examine the role of thyroid hormone effects on ANT-mediated basal proton leak.

### **Membrane Fatty Acids and Hypothyroidism**

Long chain fatty acids are weak acids that can cross the membrane in both protonated and deprotonated forms. Effects of fatty acids are interrelated to (i) increased uncoupling (ii) increased reactive oxygen species production (iii) opening of mitochondrial permeability transition pore.<sup>[71,72]</sup>

Fatty acids can act as like the Protonophoric oxidative Phosphorylation uncouplers with Protonophoric action on the inner mitochondrial membrane and/ or interaction of fatty acids with adenosine – 5- diphosphate carrier, cytochrome C oxidase and adenosine – 5- triphosphate synthase are presumed.<sup>[13]</sup> A recent study suggests that fatty acids are not only inducers of uncoupling but they have also a regulatory function in this process.<sup>[73]</sup> The affects of thyroid hormones in a diverse range of animal species and experimental approaches, demonstrate with little exception that almost every membrane system shows increase in linoleic acid content in hypothyroidism.<sup>[74]</sup> The opposite effects are observed for arachidonic acid i.e. decreases with hypothyroidism.

Physical characteristics of membranes and their fatty acid composition are also affected by thyroid hormone status and such changes have been proposed as mechanism through which oxidative Phosphorylation could also be altered by thyroid hormones.<sup>[74,75]</sup> It is clear that thyroid status has an impact on membrane fluidity and lipid composition. It was observed that mitochondrial membranes as well as mitoplasts (mitochondria lacking the mitochondrial outer membrane, but retaining the inner membrane) isolated from livers of hypothyroid rats have decreases membrane rigidity compared to euthyroid mitoplasts.<sup>[76]</sup>

### **Reactive oxygen species production in mitochondria and hypothyroidism**

Both oxygen consumption and free radical production take place in mitochondrial inner membrane. The sensitivity of these membranes to oxidative damage is strongly dependent on their unsaturated fatty acid content. The lipid environment can directly affect membrane function, including mitochondrial electron transport and ROS Production.<sup>[77]</sup> Available data on lipid peroxidative processes and oxidative status in hypothyroid liver are controversial. It was reported that levels of LPx products in liver from hypothyroid rats<sup>[78]</sup> and mice<sup>[79]</sup> do not differ from euthyroid values. On the other hand, Mogulkoc et al<sup>[80]</sup> reported reduced MDA levels in hepatic tissue from hypothyroid rats. It was asserted that hypothyroidism provided protection against LPx.<sup>[81]</sup> It further prevented increase in LPx as well as tissue damage induced by intracolonic administration of trinitrobenzene sulphonic acid and decreased susceptibility to oxygen radical- induced lung damage in newborn rats exposed to prolonged hyperoxia.<sup>[82,83]</sup> The lower toxicity of arsenic In hypothyroid animals was associated with prevention of arsenic induced LPx in liver and kidneys.<sup>[84]</sup> Furthermore, hypothyroidism was able to protect against acetaminophen hepatotoxicity.<sup>[85]</sup> Serum TBARS (thiobarbituric acid-reactive substances) concentration did not show differences between euthyroid, overt

hypothyroid and subclinical hypothyroid women.<sup>[86]</sup> On the contrary, hypothyroidism did not provide protection from LPx in testicular mitochondria as evident from augmented TBARS levels in SMP (sub mitochondrial particles).<sup>[87]</sup>

There is also evidence of increased LPx in erythrocytes, heart and plasma of PTU-treated rats(6-n-propyl-2-thiouracil)<sup>[88,89]</sup> and serum of hypothyroid patients.<sup>[90-92]</sup> Similarly, sutapa Mukharjee et al,2014, revealed greater extent of mitochondrial membrane lipid damage in hypothyroidism which was alleviated upon T3 treatment. In a study in hypothyroid patient before and after treatment, Baskol et al<sup>[93]</sup> reported that MDA levels were higher in patients with hypothyroidism before treatment, which subsided after thyroxin replacement.

Chronic state of hypothyroidism is characterized with impairments in the redox potential leading to free radical chain reactions and to metabolic suppression of antioxidant capacity. It is understandable that thyroid hypo function in patients with intellectual disability in some way is linked to the low levels of the major antioxidant molecules found in these patients. The depletion of antioxidants observed in hypothyroidism individuals may reflect the increased free radicals production at the electron transport chain on the mitochondrial inner membrane. These authors suggested a very high production of ROS and oxidative stress in patients with hypothyroidism with enhanced lipid peroxidation and concomitant failure of antioxidant defense mechanisms.<sup>[94]</sup>

### **Hypothyroidism And Apoptosis**

Accumulation of ROS and loss of membrane integrity are the two primary factors that potentially trigger apoptosis. Sutapa et al(2014) found that histological analyses of liver samples showed recovery of cellular integrity in hypothyroid rats supplemented with T3. This findings is well justified by the fact that T3 powerfully induced hepatocytes to enter S phase 24 h after injection.<sup>[95]</sup> T3-induced cyclin D1 expression enhanced phosphorylation of pRb and increased expression of transcription factor EzF mediated hepatocyte proliferation.<sup>[96]</sup> Besides, Alisi et al.<sup>[97]</sup> found that, both in control and in partially hepactomized animals, hyperthyroidism increased cyclins D1, E and A levels and the activity and of cyclin-cdk complexes and decreased the levels of cdk inhibitors such as p16 and p27. These authors further showed that hypothyroidism caused downregulation of the activity of cyclin-cdk complexes decreasing cyclin levels.

Morphological hallmarks of apoptosis in the nucleus are chromatin condensation and nuclear fragmentation.<sup>[98]</sup> The condensation starts peripherally along the nuclear membrane, forming a crescent or ring like structure. During later stages of apoptosis the nucleus further condenses, and finally it breaks up inside a cell with an intact cell membrane, a feature described as karyorrhexis.<sup>[99]</sup> DNA strand breaks can be detected by incorporating labeled dUTP by the TUNEL method.

The presence of apoptotic cells in hypothyroid liver sections as revealed from TUNEL assay clearly indicates activation of apoptotic pathways, which was later reversed upon administration of T3. It is suggested that hypothyroidism induced cytochrome c release to cytosol during early development might contribute to initiation of apoptosis through formation of apoptosomes and activation of caspase cascade. Moreover congenital hypothyroidism increased not only the extent but also the duration of apoptosis following similar mitochondrial mechanism in hippocampal neurons of developing rats.<sup>[100]</sup>

The antiapoptotic effects of T3 are well supported by both in vivo and in vitro reports. Farnandez et al.<sup>[101]</sup> showed that administration of 0.1 mg T3/kg body weight for 68-72 h caused upregulation in expression of the antiapoptotic protein Bcl-2. T3 inhibited TNF- $\alpha$ /Fas-induced apoptosis in mouse hepatocytes.<sup>[102]</sup> It is relevant to mention in this context that T3 protected cardiac myocytes against ischemia- induced apoptosis via Akt signaling.<sup>[103]</sup> Thus T3 can initiate hepatoprotective mechanism in pathological conditions by suppressing apoptosis and enabling hepatocyte survival.

## CONCLUSION

From the above study, it can be concluded that due to hypothyroidism mitochondria has low cardiolipin level and low expression of ANT. There is a change in lipid microenvironment of phosphate carrier which results in decrease of phosphate transport. This may have a substantial effect on oxidative phosphorylation.

Hypothyroidism brings about metabolic suppression through oxidative damage to mitochondrial membrane lipids and inhibition of the electron transport chain complexes. Quite aptly, a recent clinical study suggested that antioxidant therapy should be advised along with thyroid hormone replacement therapy to diminish further complications.

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