Journal of Bioenergetics and Biomembranes, Vol. 12, Nos. 1/2, 1980

The Effect of Thyroid Hormone on Mitochondrial Biogenesis and Cellular Hyperplasia

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Received May 18, 1979; revised Sept. 17, 1979

Abstract

The purpose of this investigation was to study the effects of thyroid hormone treatment on the levels of DNA, RNA, and protein in hepatocytes and hepatocyte mitochondria. A preliminary investigation was conducted to establish an effective dosage of thyroid hormone. Male Sprague-Dawley rats were given daily subcutaneous injections of L-thyroxine (20, 40, or 60 μg/100 g body weight) and the following determinations made over a 14-day period: (1) body weight; (2) total body respiration; and (3) the activities of the mitochondrial enzymes, succinate dehydrogenase and α-glycerophosphate dehydrogenase. Dosages of 20 and 40 μg L-thyroxine/100 g body weight produced significant stimulation of (a) total body respiration and (b) succinate dehydrogenase and α-glycerophosphate dehydrogenase activities without any inhibitory effects on normal weight gain of the animals. Injections of 40 μg L-thyroxine/100 g body weight were utilized for subsequent studies. Hepatic DNA levels of treated animals were greater than age-paired control values by 28% on day 7 and 43% by day 14. Total liver RNA levels of thyroid-treated animals were 17% greater than those of controls by day 7 and 47% greater by day 14. Analyses were also performed on mitochondria quantitatively collected by rate zonal centrifugation. Total liver mitochondrial DNA levels in thyroid-treated animals were greater than age-paired controls by 79% at 7 days but only 67% at 14 days since a small gain occurred in control animals and no further increase occurred in treated rats during the second week. Mitochondrial RNA and protein from treated livers were 26% and 16% higher, respectively, than age-paired controls at day 7 and 40% and 58% higher, respectively, at day 14. The results of this study indicated that thyroid hormone treatment produces hyperplasia and an increase in mitochondrial number and mass in rat liver.

0145-479X/80/0400-0001$03.00/0

Introduction

This investigation was undertaken to study the influence of thyroid hormone on proliferation of rat hepatocytes and hepatocyte mitochondria.

It is generally agreed that thyroid hormone stimulates mitochondrial, cellular, and organismic respiration [1–3]. Furthermore, many workers have demonstrated the stimulatory effects of thyroid hormone on the specific activities of certain mitochondrial enzymes: α-glycerophosphate dehydrogenase, succinate dehydrogenase, enzymes of the fatty acid oxidizing system, cytochrome *c* and cytochrome oxidase [1, 4–7]. This would suggest that thyroid hormone treatment results in a stimulation of mitochondrial synthesis and, as a result, an increase of mitochondrial respiratory activity.

Estimates of the numbers and volumes of hepatic mitochondria in control and treated rats by electron and light microscopic analyses have been inconsistent. De Leo et al. [8] reported increases in mitochondrial populations due to thyroid hormone treatment. Paget and Thorp [9] found a "moderate increase in the numbers of mitochondria" and Jacovcic [10] found a nonsignificant (7%) increase in mitochondria per cell. Last of all, Reith [11] reported a decrease in the number of mitochondria per unit volume of tissue, although examination of the data reveals that there was not a significant change. All of these investigations have consistently found increases in the total cristae and inner membrane volumes per mitochondrion.

Measurements of hepatocyte and mitochondrial DNA, RNA, and protein have also provided contradictory evidence. Tata and Widnell [12] found no changes in hepatic DNA and nuclear or soluble RNA per gram of tissue, but rRNA levels were increased by thyroid hormone treatment. King and King [13] reported increased rRNA synthesis, in agreement with the stimulation of Mg^{2+}-activated RNA polymerase reported by Tata and Widnell [12]. Gross [14] found that thyroid hormone had no effect on the level of mitochondrial DNA per milligram mitochondrial protein, whereas De Leo et al. [8] found this quantity to increase significantly. The latter result is in agreement with the stimulation of mitochondrial DNA polymerase observed in hyperthyroid animals [15]. De Leo et al. [8] and Gross [14] reported mitochondrial protein levels per gram of liver to remain constant during hormone treatment. However, Roodyn et al. [16] and Volfin et al. [17] demonstrated stimulation of mitochondrial protein synthesis by thyroxine. The increase in mitochondrial RNA levels [16, 18] and its synthesis [15, 19–21] suggests that thyroid hormone is involved in increasing mitochondrial translational capacity.

The contradictory evidence listed above does not resolve whether thyroid hormone produces increased levels of total hepatic and mitochondrial components or whether there is only a qualitative alteration of the cells and their

organelles. Most of the previous studies were conducted on mitochondria collected by differential centrifugation. This isolation procedure results in contamination of the mitochondrial pellet with other subcellular constituents and a loss of a significant fraction of the mitochondrial population [22]. Furthermore, liver mitochondria have been shown to be biochemically and functionally heterogeneous [23–36]. To complicate matters further, the loss of mitochondria may not be restricted to specific biochemical or functional types since thyroid hormone may alter the heterogeneity [27, 28]. Therefore, quantitative estimates based on a component or marker enzyme activity from preparations obtained by differential centrifugation could be in error. However, a procedure was developed recently for quantitative isolation of rat liver mitochondria utilizing rate zonal centrifugation [29]. Mitochondria collected in this way show minimal contamination by lysosomes, microsomes, and plasma membranes, and no detectable nuclear contamination [29].

Another complicating feature is that many, if not all, of the previous studies conducted analyses per unit volume, or quantity, of tissue. If quantitative increases occurred in parallel in a variety of elements (for example, mitochondrial DNA and mitochondrial protein), then the ratios of these components might remain constant despite the fact that alterations had indeed occurred in each. Such quantitative analyses should be made for the entire organ [30].

For these reasons, the specific objectives of this study were: (1) to measure hepatic DNA and RNA for the whole organ to determine if cellularity and the synthetic capacity of liver are increased by thyroid hormone treatment, and (2) to quantitate whole organ mitochondrial DNA, RNA, and protein for rat liver (using rate zonal centrifugation) as a means of determining if mitochondrial number and mass are increased by thyroid hormone treatment.

Materials and Methods

Effect of Thyroid Hormone Dosage

Male Sprague-Dawley rats weighing 215 to 235 g were divided into four groups and given the following daily doses of L-thyroxine (μg/100 g body weight) by subcutaneous injections for two weeks: (1) control, 0; (2) 20; (3) 40, and (4) 60. The rats were fed Purina Rat Chow and water *ad libitum*. Whole body respiration, measured with a small animal spirometer, and body weight were recorded over the two-week period. The rats were then killed by decapitation and liver succinate dehydrogenase and α-glycerophosphate dehydrogenase activities were determined as follows. All operations were

performed at 0–4°C. The excised livers were transferred to 0.9% (w/v) NaCl, weighed, and a 10% (w/v) homogenate prepared in 0.044 M potassium phosphate buffer, pH 7.4, with a Waring blender. After being filtered through fine mesh cheesecloth, the homogenate was further diluted in the buffer to 1% (w/v) with a Potter-Elvehjem homogenizer. Succinate dehydrogenase was assayed by the method of Veeger et al. [31]. The reaction mixture contained 0.044 M potassium phosphate buffer, pH 7.4 (1.7 ml), 30 mM KCN (100 μl), 1 ml of 0.5% (w/v) tissue homogenate diluted in the buffer immediately prior to the assay, and 100 μl of $K_3Fe(CN)_6$ at 51 mM, 34 mM, 22 mM, or 15 mM concentrations. The reduction of $FE(CN)_6^{3-}$ was followed at 420 nm with a Cary 15 split-beam recording spectrophotometer. Lineweaver–Burk plots were computed and the results extrapolated to V_{max} with respect to oxidant. Assays for α-glycerophosphate dehydrogenase were performed by the method of Ringler and Singer [32]. The reaction mixture contained 0.044 M potassium phosphate buffer, pH 7.4 (1.6 ml), 30 mM KCN (100 μl), 300 mM $MgCl_2$ (100 μl), 1 ml of 0.5% (w/v) or 0.25% (w/v) homogenate diluted in buffer immediately prior to the assay, and 15mM $K_3Fe(CN)_6$ (100 μl). The reduction of $Fe(CN)_6^{3-}$ was followed at 420 nm with a Cary 15 split-beam recording spectrophotometer.

Isolation of Mitochondria

Four to six rats were weighed, decapitated, and the livers quickly removed and placed in ice-cold 0.9% (w/v) NaCl. All subsequent steps were performed at 0 to 4°C. The livers were weighed and a 15% (w/v) homogenate in 0.25 M sucrose, 0.01 M Tris, 0.1 mM EDTA, pH 7.4, was prepared utilizing a Potter-Elvehjem homogenizer and a motor-driven Teflon pestle. The homogenate was then filtered through medium and fine-mesh cheesecloth. A 20-ml sample of homogenate was fractionated with the SZ-14 reorienting gradient zonal rotor in Sorvall RC2-B centrifuge according to the method of Wilson et al. [29]. Fractions of 40 ml were collected, mixed by inversion, and aliquots taken to determine turbidity at 700 nm with a Beckman spectrophotometer and sucrose concentration with an Abbe refractometer. The fractions were also analyzed for marker enzymes as modified by Wilson et al. [29]: cytochrome oxidase (mitochondria), acid phosphatase (lysosomes), 5′-nucleotidase (plasma membranes), and glucose-6-phosphatase (microsomes).

Since turbidity was found to coincide with cytochrome oxidase distribution, mitochondrial fractions were chosen on the basis of this measurement and were centrifuged at 21,000 $\times$ g_{av} for 15 min in a Sorvall RC2-B centrifuge with an SS-34 rotor. The pellets were washed in 0.154 M KCl, 0.01 M Tris buffer, pH 7.6, and recentrifuged at 21,000 $\times$ g_{av} for 15 min. The pellets were washed again in 0.154 M KCl, 0.01 M Tris buffer, pH 7.6, and

all fractions were combined. The sucrose-free mitochondrial suspension was mixed by inversion and two 100-μl samples were taken to determine mitochondrial protein by the method of Lowry et al. [33].

Quantitation of Nucleic Acids

DNA and RNA were extracted by the method of Schmidt and Thannhauser [34] as modified by Fleck and Munro [35]. The mitochondrial suspension was centrifuged at 21,000 $\times$ g_{av} for 15 min, and the pellet was resuspended in 0.154 M KCl, 0.01 M Tris buffer, pH 7.6, and brought to final concentrations of 0.05% (v/v) Triton X-100 and 0.22 N $HClO_4$. Whole liver homogenates (15% w/v) were diluted 1:3 in 0.154 M KCl, 0.01 M Tris buffer, pH 7.6, and brought to a final concentration of 0.22 N $HClO_4$. After precipitation on ice for 15 min, the whole homogenate and mitochondrial suspensions were centrifuged at 9,200 $\times$ g_{av} for 10 min. The pellets were resuspended in 0.22 N $HClO_4$ and centrifuged at 9,200 $\times$ g_{av} for 8 min. Removal of the supernatants left pellets containing nucleic acids. Alkali hydrolysis of the RNA in 0.3 M NaOH at 37°C was carried out for 1 hr. Hydrolysis was stopped by addition of 3.0 N $HClO_4$ and, after a 15-min precipitation on ice, the mixture was centrifuged at 9,200 $\times$ g_{av} for 8 min. The supernatant was saved and the pellet washed in 0.22 N $HClO_4$ and recentrifuged as above. This second supernatant was pooled with the first for RNA analysis by the orcinol reaction [36]. The pellets were resuspended in 1.0 N $HClO_4$ and acid hydrolysis of the DNA was carried out at 70°C for 15 min. The mixtures were centrifuged at 37,100 $\times$ g_{av} for 10 min and the supernatants collected for DNA analysis by Burton's diphenylamine method [37] as modified by Giles and Meyers [38].

Analytical Ultracentrifugation of Nuclear and Mitochondrial DNA

Isolation of nuclear and mitochondrial DNA was carried out by the method of Marmur [39] with the modifications described by Wilson et al. [29].

Results

In devising an experimental system with which to study the effects of thyroid hormone on rat hepatocytes and hepatic mitochondria, it was necessary to determine the dosage of thyroxine that produced maximal physiological responses to treatment. It was also important to avoid the thyrotoxic effects caused by excessive dosages. Therefore, body weight, whole body respiration, and the activities of two liver mitochondrial enzymes, succinate

Table I. Changes in Body Weight with Thyroid Hormone Treatment[a]

	Days treated				
Group	0	4	7	11	14
Control (4)	222 ± 5.11	241 ± 4.15	270 ± 6.61	286 ± 3.89	302 ± 5.80
Treated					
20 μg (4)	225 ± 4.80	—	269 ± 3.90	—	300 ± 3.20
40 μg (4)	225 ± 3.49	238 ± 4.44	268 ± 2.86	282 ± 4.72	295 ± 5.91
60 μg (4)	225 ± 2.59	—	252 ± 4.85	—	271 ± 7.63[b]

[a]Treated animals were given daily subcutaneous injections of L-thyroxine (20, 40, or 60 μg/100 g body weight) and animal weights recorded on the days indicated. Results are given as mean body weights, expressed in grams, ± S.E.M. The number of animals used for each group is given in parentheses.
[b]Significantly different from 14-day controls, $P < 0.05$.

dehydrogenase and α-glycerophosphate dehydrogenase, were monitored to study the effects of daily injections of 20, 40, and 60 μg L-thyroxine/100 g body weight. The body weights of all animlas increased over the 14-day period, without significant differences from controls for animals treated with either 20 or 40 μg/100 g body weight doses (Table I). Weight gain of animals treated with 60 μg/100 g body weight was significantly lower than that of the control group at 14 days. However, whole body respiration of hormone-treated animals was significantly greater than controls for all experimental groups (Table II). Similarly, the mitochondrial enzyme activities measured were significantly increased by thyroid hormone treatment at 14 days (Table III). Since a treatment dosage of 40 μg/100 g body weight resulted in a greater (although not significant) stimulation of mitochondrial responses to thyroid hormone with no inhibitory effects on body weight increases, this dosage of thyroxine was used to study the effects of the hormone on proliferation of rat hepatocytes and hepatic mitochondria.

Table II. Changes in Total Body Respiration with Thyroid Hormone Treatment[a]

	Days treated				
Group	0	4	7	11	14
Control (4)	385 ± 19.3	384 ± 17.5	453 ± 14.2	473 ± 17.1	486 ± 12.7
Treated					
20 μg (4)	422 ± 12.1	—	595 ± 9.96[b]	—	654 ± 33.1[b]
40 μg (4)	414 ± 8.63	530 ± 20.0[b]	599 ± 6.00[b]	650 ± 37.5[b]	721 ± 24.2[b]
60 μg (4)	425 ± 25.1	—	591 ± 22.5[b]	—	685 ± 34.6[b]

[a]The treated animals were given daily subcutaneous injections of L-thyroxine (20, 40, or 60 μg/100 g body weight). The measurements were carried out as described in the Materials and Methods section on the days indicated. Results are expressed as mean systemic O_2 uptake, expressed in ml O_2/hr, ± S.E.M. The number of animals in each group is given in parentheses.
[b]Significantly different from controls of the same age, $P < 0.05$.

Table III. Changes in Mitochondrial Enzyme Activities with Thyroid Hormone Treatment[a]

Group	Total liver α-glycerophosphate dehydrogenase activity	Total liver succinate dehydrogenase activity
Control (4)	36.2 ± 1.41	102.7 ± 7.32
Treated		
20 μg (4)	82.3 ± 4.08[b]	151.2 ± 9.89[b]
40 μg (4)	102.0 ± 7.05[b]	146.7 ± 9.43[b]
60 μg (4)	97.2 ± 2.53[b]	149.3 ± 3.01[b]

[a]Treated animals were given daily subcutaneous injections of L-thyroxine (20, 40, or 60 μg/100 g body weight) and after 14 days, total liver succinate dehydrogenase and α-glycerophosphate dehydrogenase activities were determined as described in the Materials and Methods section. Succinate dehydrogenase and α-glycerophosphate dehydrogenase activities are expressed as μmoles succinate oxidized per minute and μmoles α-glycerophosphate oxidized per minute, respectively, for the total organ. Results are expressed as mean values ± S.E.M. The number of determinations is given in parentheses.
[b]Significantly different from controls, $P < 0.05$.

Quantitative analyses of total liver DNA and RNA were performed on control and treated animals over a 14-day period. Total hepatic DNA measurements were used to provide an index of liver cellularity, and determinations of total liver RNA were made as an assessment of the synthetic capacity of the tissue. Both total liver DNA and RNA were significantly increased in the treated animals (Table IV). It was also noted that despite the rise in total organ quantities of these components, no increase in liver weight of treated animals was evident (Table V).

Total levels of mitochondrial DNA, RNA, and protein were determined from a quantitative isolation of rat liver mitochondria collected by rate zonal centrifugation. Total mitochondrial DNA levels were used to assess changes

Table IV. Changes in Total Liver DNA and RNA with Thyroid Hormone Treatment[a]

	Days treated			
Determination	0	4	7	14
DNA, mg/liver				
Control	22.6	22.1	23.3	27.9
Treated	22.6	27.2	29.8	39.9
RNA, mg/liver				
Control	112	113	124	133
Treated	112	126	145	195

[a]Treated animals were given daily subcutaneous injections of 40 μg L-thyroxine/100 g body weight. DNA and RNA levels in liver homogenates were determined on the days indicated as described in the Materials and Methods section. DNA and RNA are expressed as milligrams per organ. Livers from four animals were pooled for each determination. Data for control and treated animals were subjected to least-squares linear regression analysis and the slopes were found to be significantly different, $P < 0.01$.

Table V. Changes in Liver Weight with Thyroid Hormone Treatment[a]

Group	Days treated			
	0	4	7	14
Control	10.6 ± 0.29 (4)	11.7 ± 0.34 (4)	12.6 ± 0.47 (4)	13.5 ± 0.85 (4)
Treated	10.6 ± 0.29 (4)	10.9 ± 0.47 (4)	12.2 ± 0.40 (4)	12.8 ± 0.56 (4)

[a]Treated animals were given daily subcutaneous injections of 40 μg L-thyroxine/100 g body weight. The livers were removed and organ wet weights determined on the days indicated. Results are expressed as mean values ± S.E.M. The number of determinations is given in parentheses.

in mitochondrial number, total mitochondrial RNA levels for changes in synthetic capacity, and total mitochondrial protein for changes in organelle mass. In thyroid-hormone-treated rats total hepatic mitochondrial DNA was 79% greater than levels in age-paired controls at day 7 of treatment (Table VI). The amount of mitochondrial DNA was not increased further in experimental animals at day 14. The increase in total hepatic mitochondrial DNA was more dramatic than the rise in nuclear DNA (Table IV).

The results of the mitochondrial RNA and protein quantitations (Table VI) indicate that total mass of hepatic mitochondria was significantly increased during thyroid hormone treatment. For 14-day animals the elevation above controls of 40% and 58% for mitochondrial RNA and protein levels, respectively, paralleled the increases in total liver DNA and RNA.

In doing a quantitative analysis of mitochondrial DNA, it is imperative to avoid nuclear contamination since there is a much greater quantity of

Table VI. Changes in Total Liver Mitochondrial (mt) DNA, RNA, and Protein with Thyroid Hormone Treatment[a]

Determination	Days treated			
	0	4	7	14
mt DNA, μg/liver				
Control	174	—	211	225
Treated	174	295	378	376
mt RNA, mg/liver				
Control	8.5	—	13.4	13.9
Treated	8.5	11.1	16.9	19.5
mt Protein, mg/liver				
Control	321	—	405	376
Treated	321	395	469	595

[a]Treated animals were given daily subcutaneous injections of 40 μg L-thyroxine/100 g body weight. Mitochondria were quantitatively harvested by rate zonal centrifugation and determinations of mitochondrial DNA, RNA, and protein levels were made on the days indicated as described in the Materials and Methods section. Livers from four animals were pooled for each determination. Data for control and treated animals were subjected to least-squares linear regression analysis and the slopes were found to be significantly different, $P < 0.01$.

nuclear DNA relative to organelle DNA [40]. It was previously shown [29] that there was no detectable contamination of mitochondria collected by rate zonal centrifugation with nuclear DNA. However, the possibility existed that the sedimentation characteristics of cellular organelles might be changed with thyroid hormone treatment [27, 28] and that contamination with nuclear meterial might be altered. In order to demonstrate that the mitochondria from thyroxine-treated animals were not contaminated with nuclear DNA, the DNA extracted from the isolated mitochondria was analyzed by CsCl gradient centrifugation after denaturation–renaturation to separate nuclear and mitochondrial DNA. As was previously found for control mitochondria, no detectable contamination of the mitochondria with nuclear DNA was obtained with thyroxine treatment. Therefore, the observed increase in total hepatic mitochondrial DNA was a result of thyroid hormone treatment and not an artifact of the isolation procedure.

Although there was no contamination of the mitochondrial fractions with nuclear DNA, it was also important to determine (1) if mitochondria from thyroid-treated animals were being quantitatively collected and (2) if contamination by lysosomes, plasma membranes, and microsomes was altered. For this purpose rat liver homogenate fractionated by zonal centrifugation was analyzed for the following marker enzymes: cytochrome oxidase (mitochondria), acid phosphatase (lysosomes), 5′-nucleotidase (plasma membranes), and glucose-6-phosphatase (microsomes). Distribution of these marker enzymes in the fractionated material did not differ with thyroid hormone treatment and was the same as that previously reported by Wilson et al. [29] for control animals. Therefore recovery of mitochondria and contamination by other subcellular constituents was the same in control and experimental animals.

Discussion

It was important in this *in vivo* study to use a dosage which could give maximal stimulation of processes known to be enhanced by thyroid hormone without producing thyrotoxic conditions. It was found that daily treatment of 40 μg L-thyroxine/100 g body weight resulted in increases in mitochondrial oxidative capacity as determined by succinate dehydrogenase and α-glycerophosphate dehydrogenase activities and by whole body respiration of the animals. Furthermore, the body weights of these animals did not differ from untreated controls, indicating that this dosage did not produce a thyrotoxic effect. With a dosage of 60 μg thyroxine/100 g body weight, weight gain was significantly less than controls, suggesting that a toxic dose was reached. The

importance of characterizing the psysiological responses of the thyroid hormone doses used was previously stressed by Tata [2].

To distinguish between quantitative and qualitative responses of mitochondrial components to thyroid hormone, it was necessary to harvest mitochondria quantitatively, free from contamination by other cellular structures. This was accomplished in this investigation by the use of rate zonal centrifugation. This technique allows mitochondria to be collected so that greater than 90% of the cytochrome oxidase activity is recovered and yet the contamination by components of plasma membranes, lysosomes, and microsomes is less than 10%, with no detectable nuclear contamination [29]. Elimination of contaminating nuclear DNA is critically important in determining whether the number of mitrochondria is altered by thyroid hormone since the amount of nuclear DNA per cell is nearly 100 times that of mitochondrial DNA [40]. Since there was no detectable nuclear DNA in the CsCl analytical centrifugation of mitochondrial DNA isolated from thyroxine-treated animals, the apparent proliferation of mitochondria observed is not an artifact of contamination.

Previously investigators have relied on either differential or isopycnic centrifugation to collect liver mitochondria. Neither of these methods allows for a quantitative recovery of the organelles. It is also possible that mitochondria selectively harvested by these procedures may represent an enrichment of a particular class of organelles due to heterogeneity. Ruh et al. [27] have shown that mitochondria from thyroid-treated animals form two bands with densities different from controls when isolated by isopycnic centrifugation; this may be due to an alteration of the membrane phospholipid:protein ratio caused by thyroid hormone treatment. Also, Katyare et al. [41] and Satav et al. [28] have shown that of the heavy, light, and fluffy mitochondrial fractions obtained by differential centrifugation, the light fraction responds to a greater extent to thyroid hormone in terms of amino acid incorporation. Therefore, thyroid hormone treatment could alter sedimentation characteristics of mitochondria and also selectively affect a segment of a heterogenous mitochondrial population.

The contradictory report of Gross [14] showing no significant changes in mitochondrial protein and DNA could be explained by the differences in mitochondrial isolation procedures and quantitation methods. Total mitochondrial protein was estimated by extrapolating from marker enzyme activities (succinate–cytochrome *c* reductase was reported) of mitochondria isolated by differential and isopycnic centrifugations and on organ homogenates. The assumption that these enzyme activities are proportional to total mitochondrial protein may be invalid, since both succinate dehydrogenase and cytochrome *b* were shown to exhibit heterogeneous distributions in liver mitochondria [24]. In addition, the quantitation of mitochondrial DNA

involved recovery of DNA from organelles isolated by differential and isopycnic centrifugations.

The differences between our results and those of other investigators could be due to the manner in which the data were expressed. For example, the nonsignificant decrease in mitochondrial population after thyroid hormone treatment reported by Reith et al. [42] could be the result of expressing data as ratios of mitochondrial number or volume per volume of tissue or cell. Also, the determinations of levels of hepatic and mitochondrial constituents obtained by most investigators were generaly reported as mass per gram tissue or per milligram mitochondrial protein (8, 12, 14–16, 18, 20, 43]. Organ weights were not always reported. As demonstrated in this study, cellularity and total mitochondrial DNA and protein in treated livers were significantly increased. It has been shown that data expressed as ratios of quantities can lead to erroneous interpretations since both quantities may change [30]. For example, the ratio of mitochondrial DNA to mitochondrial protein in this study was not appreciably different for controls or treated animals at day 14, despite the greater total organ quantities for each component in experimental animals.

This study has demonstrated that thyroid hormone increases total hepatic DNA and RNA, suggesting that liver hyperplasia is produced in the hyperthyroid state. Thyroid hormone also increases total levels of hepatic mitochondrial DNA, RNA, and protein. It is concluded that *in vivo* thyroxine treatment induces mitochondrial proliferation in rat liver, and may serve as a model system for studying mitochondrial biogenesis in mammalian cells.

Acknowledgment

This work was supported by University of California Grant No. 2278.

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