

Biochemical Studies in Experimentally Induced-hyperthyroid Rats Treated with Folic and Ascorbic Acid

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Abstract Hyperthyroidism is a relatively prevalent condition, and is one of the most common endocrine problems seen in clinical practice, high serum cholesterol and increased oxidative stress are common associated risk factors of hyperthyroidism. Multivitamin deficiencies in folate and vitamin C are also one of the hyperthyroidism complications. Individually or combined Folic acid and vitamin C were introduced to hyperthyroid induced rats. A relative enhancement in the thyroid hormones with a normal lipid profile was noticed. Previous researches confirmed that the depletion on the lipid in hyperthyroidism will be gained after treatment.

Keywords: Hyperthyroidism, lipid profile, cortisol, oxidative stress, folic acid, vitamin C

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1. Introduction

Many websites publish information about hyperthyroidism and how to deal with it. We should be aware about its beneficial role or its hazards. Many of these remedies are prescribed for both hyperthyroidism and hypothyroidism at the same time, so we tested two of the vitamins usually prescribed and there is depletion in the information about them, the vitamin C as a natural vitamin is found in most of our food like Blackcurrants (200 mg 100 g⁻¹), Oranges (50 mg 100 g⁻¹) and Green peppers (100 mg 100 g⁻¹) [1].

Folate as a natural antioxidant vitamin occurs naturally in orange juice, strawberries, dark green leafy vegetables and peanuts [2]. Decreased hepatic stores of folic acid have been observed in experimental animals made hyperthyroid by the administration of thyroid hormone) [3]. There is a depletion of the information dealing with the effects of folic acid and vitamin C on hyperthyroid rats and their effect on the thyroid hormones and the cortisol level.

Both lipogenesis and lipolysis are increased in hyperthyroidism, but the net effect is lipolysis, as reflected by an increase in the plasma concentration of free fatty acids and glycerol and a decrease in the serum cholesterol level; triglyceride levels are usually slightly decreased. The enhanced mobilization and oxidation of free fatty acids in response to fasting or catecholamines is a result of enhancement of lipolytic pathways by thyroid hormones [4].

Natural remedies and vitamins may replace the antithyroid drugs at one day, antithyroid drugs act

principally by interfering with the organification of iodine, thereby suppressing thyroid hormone levels. Methimazole (Tapazole) and propylthiouracil (PTU) are the two agents available in the United States. Remission rates vary with the length of treatment, but rates of 60 percent have been reported when therapy is continued for two years. Relapse can occur in up to 50 percent of patients who respond initially, regardless of the regimen used. A recent randomized trial indicated that relapse was more likely in patients who smoked, had large goiters, or had elevated thyroid-stimulating antibody levels at the end of therapy) [5]. Increased aspartate aminotransferase and alanine aminotransferase are observed in nearly one third of patients treated with propylthiouracil, and the rise is usually dose-related) [6]. Agranulocytosis is the most serious complication of antithyroid drug therapy and is estimated to occur in 0.1 to 0.5 percent of patients treated with these drugs [7]. This research aimed to study the protective effects of folic acid and ascorbic acid as natural vitamins in hyperthyroidism by assessing many biochemical markers as thyroid hormones, cortisol, oxidative stress markers and lipid profile.

2. Materials and Methods

The experiments were performed on 70 male albino rats (Sprague dawley) weighing 125±10 g and of 9-10 week's age. They were obtained from our laboratory farms in the Zoology Department, Faculty of Science, Tanta University, Egypt. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (20% casein, 15% corn oil, 55% corn

starch, 5% salt mixture and 5% vitaminized starch obtained from Egyptian Company for Oils and Soap; Kafr-Elzayat, Egypt) and water available ad libitum) [8]. The temperature in the animal room was maintained at $23\pm 2^{\circ}\text{C}$ with a relative humidity of $55\pm 5\%$. Light was on a 12:12 hr light-dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research and was approved by the faculty of science. The rats were randomly and equally divided into seven groups (10 animals each).

GI a: Control group in which animals never received any treatment.

GI b: Folic acid group in which animals received 8 mg/kg of body weight folic acid 2nd week to 6th week [9,10].

GI c: Vitamin C group in which animals received 240 mg/kg of body weight vitamin C daily from 2nd week to 6th week [11].

GII a: Hyperthyroid group in which rats received 100 $\mu\text{g}/\text{Kg}$ L-Thyroxin sodium administration as a chewable lab chow for 4 weeks [8,12].

GII b: Co-treated hyperthyroid with folic acid.

GII c: Co-treated hyperthyroid with vitamin C.

GII d: Co-treated hyperthyroid with folic acid and vitamin C.

At the end of the experimental period rats were euthanized with intravenous injection with sodium pentobarbital and subjected to a complete necropsy. Blood samples were individually collected from the inferior vena cava of each rat in non heparinized glass tubes, random blood samples were collected from rat's tail at approx 8 am for cortisol estimation. Serum was separated by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at -20°C until analysis. Serum was analyzed to determine the T3, T4, TSH, AM - Cortisol and some lipid profile levels.

1. Estimation of thyroxine (T4), triiodothyronine (T3) and thyrotropin (TSH) according to the methods of Thakur et al. [13], Maes et al. [14] and Mandel et al. [15] respectively. The kits for these hormones were obtained from Calbiotech INC (CBI), USA.

2. Determination of serum cortisol was performed in serum collected from rat's tail at approximately 8 am (from 8 to 9.45 in our experiment) and then stored at -20°C till analyzed by ELISA technique using MyBio Source, Inc. Catalog No. MBS701698 by Assay designs

following the manufacturer instruction using competitive inhibition enzyme immunoassay technique.

3. Some Lipid Profile parameters were performed using Bioassay systems Kits catalog no ECCH-100 and EHDL-100 by using quantitative colorimetric determination of Cholesterol, LDL and HDL. Triglycerides was estimated by BioVision Kit catalog no k622-100.

2.1. Determination of Tissue Oxidative Stress

The adrenal glands collected at the end of the experiment and the adrenal homogenate (10%; w/v) was prepared in an ice-cold 0.067 M phosphate buffer. Then, the homogenate was centrifuged at 3000 rpm for 10 min at 4 C. The resulting supernatant was used to determine the adrenal catalase, MDA, total protein, reduced glutathione and nitric oxide.

1. Catalase activity was estimated according to the method of Xue et al. [16].

2. MDA was estimated by the method of Mesbah et al. [17].

3. Determination of total protein was performed by using Diamond Diagnostic kit using a Biuret reagent as described by Beyer [18].

4. Reduced glutathione was determined by the method of Ellman [19].

5. Nitric oxide level was determined following the procedure described by Miranda et al. [20].

2.2. Statistical Analysis

Data (n = 10) were expressed as mean values + SEM. All data were subjected to one-way ANOVA test to determine differences among groups. P values less than 0.05 were considered significant. Results were analyzed and graphed using Graphpad prism 6 software (Graphpad, San Diego, CA).

3. Results

The rat weight was measured at the beginning of the experiment and before the rat euthanasia and was expressed in Table 1 as the mean of the weight difference \pm SEM. The folic acid group (GI b) and the vitamin C group (GI C) had a normal rat weight values as compared to the control group.

Table 1. Changes in the % of body weight level in different group under study

Groups	Body weight (g) Mean \pm SEM		% of body weight difference
	At start	At sacrifice	
GI a	123.2 \pm 1.267	153.7 \pm 6.593	30.48 \pm 6.714 \uparrow
GI b	123.2 \pm 1.161	151.8 \pm 10.42	28.62 \pm 10.49 \uparrow
GI c	123.2 \pm 1.403	156.0 \pm 7.073	32.79 \pm 7.211 \uparrow
GII a	120.4 \pm 0.8532	115.2 \pm 5.343	-5.220 \pm 5.411 \downarrow
GII b	121.7 \pm 1.088	161.3 \pm 5.151	39.62 \pm 5.265 \uparrow
GII c	119.9 \pm 1.207	136.3 \pm 7.415	16.34 \pm 7.513 \uparrow
GII d	123.1 \pm 1.476	140.4 \pm 3.305	17.26 \pm 3.619 \uparrow

The hyperthyroid group (GII a) showed a weight loss as compared to the control group. The co treatment with folic acid (GII b) gained rats more weight as compared to the control group. The co treatment with vitamin C or both vitamins (GII c or GII d) gained rats more weights as compared to the hyperthyroid group but didn't reach the normal value as compared to the control group.

The T3, T4 and TSH levels in different groups were shown in Table 2. T3, T4 and TSH values in the negative control groups (GI b & GI c) were at the normal values as compared to the control group.

The hyperthyroid group (GII a) showed a higher T3 & T4 values as compared to the control group. The co treatment with folic acid and / or vitamin C (GII b, GII c &

GII d) reduced the T3 & T4 levels as compared to the hyperthyroid group but didn't reach the normal values as compared to the control group.

Table 2. Changes in T4, T3 and TSH levels in different group under study. (mean \pm SEM)

Group	T4 (ng/mL)	T3 (ng/dl)	TSH (ng/mL)
GI a	3.037 \pm 0.0656 ^b	31.31 \pm 0.79 ^b	1.343 \pm 0.02343 ^b
GI b	3.077 \pm 0.031 ^b	31.92 \pm 0.68 ^b	1.348 \pm 0.03176 ^b
GI c	2.950 \pm 0.10 ^b	29.37 \pm 0.73 ^b	1.357 \pm 0.02777 ^b
GII a	5.900 \pm 0.09 ^a	93.50 \pm 5.19 ^a	0.0300 \pm 0.006831 ^a
GII b	4.220 \pm 0.16 ^{ab}	71.00 \pm 5.30 ^{ab}	0.0420 \pm 0.005925 ^a
GII c	4.602 \pm 0.12 ^{ab}	72.10 \pm 5.28 ^{ab}	0.0350 \pm 0.0050 ^a
GII d	3.950 \pm 0.10 ^{ab}	66.20 \pm 5.20 ^{ab}	0.1910 \pm 0.027 ^{ab}

a Significantly different from control (GI a) (P < 0.05).

b Significantly different from hyperthyroid (GII a) (P < 0.05).

As in Table 2 the hyperthyroid group (GII a) showed a reduced TSH value as compared to the control group, the TSH value was slightly increased in the group treated with both vitamins (GII d).

Graph 1 shows the AM – cortisol values of different groups, The group treated with vitamin C (GI c) and the group treated with folic acid (GII b) slightly reduced the AM – cortisol value as compared to the control group.

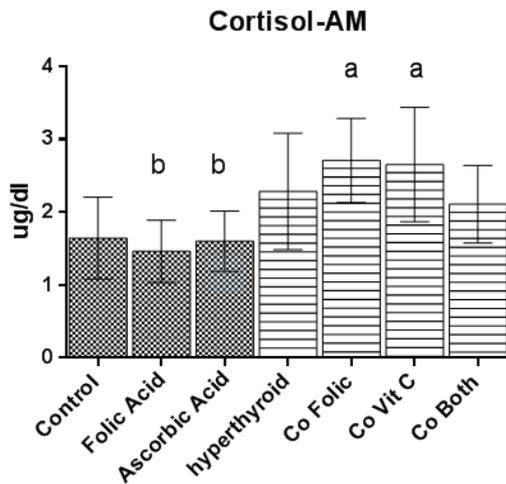


Figure 1. Changes in AM- cortisol level in different group under study. ^aSignificantly different from control (GI a) (P < 0.05). ^bSignificantly different from hyperthyroid (GII a) (P < 0.05).

The hyperthyroid group (GII a) had a more AM – cortisol value as compared to the control group. The co treatment with folic acid or vitamin C (GII b or GII c) increased the AM – cortisol value as compared to the hyperthyroid group and the co treatment with both vitamins (GII d) reduced the AM – cortisol value as compared to the hyperthyroid group but it didn't reach the normal value as compared to the control group.

Table 3 shows the total cholesterol, LDL, TG and HDL in different groups. Treatment with folic acid (GI b) or vitamin C (GI c) reduced the total cholesterol level as compared to the control group; treatment with vitamin C

reduced the total cholesterol value more than the treatment with folic acid. Hyperthyroid group (GII a) had less cholesterol level as compared to the control level, co treatment with folic acid (GII b) or vitamin C (GII c) increased the total cholesterol value as compared to the hyperthyroid group but it didn't reach the normal value as compared to the control group. Co treatment with both vitamins (GII d) reduced the cholesterol level as compared to the control level or the hyperthyroid group.

The groups treated with folic acid (GI b) or vitamin C (GI c) had higher TG values as compared to the control group. The hyperthyroid group (GII a) had less TG value as compared to the control group. The co treatment with folic acid (GII b) increased the TG level as compared to the hyperthyroid group but it didn't reach the normal level as in the control group. The co treatment with vitamin C (GII c) or both vitamins (GII d) reduced the TG value as compared to the hyperthyroid group or the control group.

The folic acid treated group (GI b) had a nearly normal LDL level as compared to the control group. The treatment with vitamin C (GI c) reduced the LDL value as compared to the control group. The hyperthyroid group (GII a) had less LDL value as compared to the control group. The co treated groups with folic acid (GII b) or both vitamins (GII d) showed increased LDL value as compared to the hyperthyroid group but only co treatment with both vitamins (GII d) exceeded the normal value and the other groups was below the normal value as compared to the control group.

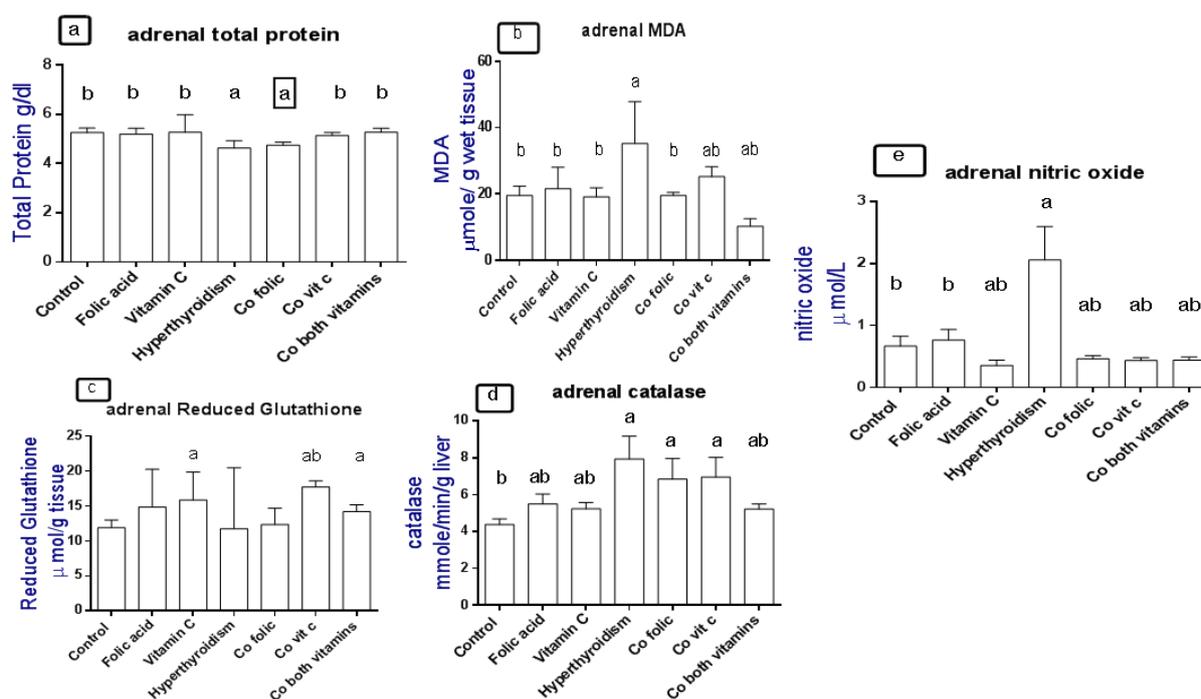
Serum HDL value in folic acid group (GI b) or vitamin C group (GI c) was lower than the normal value as compared to the control group. The HDL value of the hyperthyroid group was lower than the control group. Co treatment with folic acid (GII b) or vitamin C (GII c) increased the HDL value as compared to the hyperthyroid group and exceeded the normal value. The co treatment with both vitamins (GII d) reduced the HDL value as compared to the hyperthyroid or the control group. The co treatment with vitamin C (GII c) had a higher value than the co treatment with folic acid (GII b).

Table 3. Determination of some lipid profile parameters in induced hyperthyroid rats as well as those treated with folic acid and / or vitamin C as compared to control (mean \pm SEM)

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
GI a	58.00 \pm 1.783 ^b	83.33 \pm 1.04 ^b	30.34 \pm 0.92 ^b	11.10 \pm 1.100
GI b	52.80 \pm 2.347	84.16 \pm 1.25 ^b	29.28 \pm 0.77 ^b	6.100 \pm 0.45 ^a
GI c	43.90 \pm 3.39 ^a	110.2 \pm 12.04 ^{ab}	17.80 \pm 1 ^{ab}	4.300 \pm 0.372 ^{ab}
GII a	50.10 \pm 2.111 ^a	78.50 \pm 1.80 ^a	25.40 \pm 0.81	8.700 \pm 1.45
GII b	56.50 \pm 3.481	82.50 \pm 1.25	27.23 \pm 1.16	12.50 \pm 2.12
GII c	50.60 \pm 1.507 ^a	56.80 \pm 4.27 ^{ab}	18.00 \pm 1.76 ^{ab}	21.45 \pm 0.98 ^b
GII d	47.60 \pm 1.368 ^a	49.60 \pm 1.59 ^{ab}	33.30 \pm 1.45 ^b	4.350 \pm 1.71 ^a

a Significantly different from control (GI a) (P < 0.05).

b Significantly different from hyperthyroid (GII a) (P < 0.05).



Graph 2 a represents the adrenal total protein, 2-b represents the adrenal MDA, 2-c represents the adrenal reduced glutathione, 2-d represents the adrenal catalase, 2-e represents the adrenal nitric oxide in induced hyperthyroid rats as well as those treated with folic acid and / or vitamin C. ^aSignificantly different from control (GI a) ($P < 0.05$). ^bSignificantly different from hyperthyroid (GII a) ($P < 0.05$).

Graphs 2-a, 2-b, 2-c, 2-d and 2-e represents total protein, MDA, reduced glutathione, catalase and nitric oxide in the adrenal gland tissue.

There was no significant change in total protein levels in the adrenal gland tissue as shown in graph 2-a.

In graph 2-b, the hyperthyroid group shows an increased MDA level as compared with the control groups. Treatment with folic acid during the hyperthyroid state reduced the MDA level in a more sufficient way than treatment with vitamin C but treatment with both vitamins reduced the MDA level more sufficiently than the individual treatment.

In graph 2-c the rate of reduced glutathione in the hyperthyroid group was lower than the negative control groups, co vitamin C or co treatment with both vitamins increased the glutathione level as compared to the hyperthyroid group.

In graph 2-d, the catalase activity was increased in the hyperthyroid group more than the control group. The treatment with folic acid or vitamin C reduced the catalase activity as compared to hyperthyroid group. Treatment with both vitamins reduced the enzyme activity more efficiently than the single vitamin treatment.

In graph 2-e, the nitric oxide level in the hyperthyroid group was higher than all other folic acid and /or vitamin C treated groups.

4. Discussion

Hyperthyroidism is reported to cause high oxidative stress and to decreased anti-oxidant metabolites in hyperthyroid patients, these changes are corrected in euthyroidism, without any influence of thyrostatic drugs per se. Nutritional support with antioxidant agents, which are defective during hyperthyroidism, is warranted [21,22].

As markedly from the results obtained in Table 1, Hyperthyroid rats (GII a) were the only rats that showed decreased body weight. This decrease can be explained by the concept stated that hyperthyroidism is commonly associated with increased food consumption, parallel with a loss of body weight and decreased serum cholesterol level and increased the levels of lipid peroxidation products in metabolically active tissues [23,24].

Interestingly, from the data obtained in Table 2 hyperthyroid rats (GII a) showed elevation in T3 & T4 levels by incredible increases reach to about 3 and 2 folds, respectively. While TSH concentration showed a dramatic significant decrease reaches to about 20% of the negative control concentration, we may conclude that the TSH level was decreased as a result of negative feedback inhibition mechanism. T3 level might be increased as a result of deiodinase enzyme which converts T4 into T3 [25]. The treatment with folic acid and/or vitamin C managed to reduce the T4& T3 levels but the TSH level was still below the normal value, suggesting an ameliorating potential of these vitamins in hyperthyroidism.

Hyperthyroidism was reported by Gallagher et al. [26] to increase the cortisol level and increase cortisol clearance, he reported that there was an almost certain increased functional adrenal capacity to nearly double normal as evident from greater cortisol production per unit time. In Table 3 there is increased AM- cortisol level which is consistent with the previous paper. We may assume that the use of folic acid or vitamin C as a co treatment affected the cortisol production/clearance balance in the hyperthyroid state and increased the cortisol level. The use of both treatments as a co treatment decreased the cortisol level.

The hyperthyroid state established in the selected group decreased the body weight gain in the hyperthyroid group

and treatment with vitamins increased the body weight gain.

Thyroid hormone is reported to increase HMG-CoA reductase which is an integral membrane protein of the smooth ER, it is the major point of regulating the pathway of cholesterol synthesis, and much of the cholesterol synthesis in vertebrates takes place in the liver. A small fraction of the cholesterol made there is incorporated into the membranes of hepatocytes, but most of it is exported in one of three forms: biliary cholesterol, bile acids, or cholesteryl esters. Cholesterol and cholesteryl esters, like triacylglycerols and phospholipids, are essentially insoluble in water, yet must be moved from the tissue of origin to the tissues in which they will be stored or consumed. They are carried in the blood plasma as plasma lipoproteins [27]. The total cholesterol level in the hyperthyroid group is lower than the cholesterol level of negative control group or the folic acid group; we may suppose that the thyroid hormone may increase both cholesterol synthesis and cholesterol degradation as a result of the high total body catabolic rate of the hyperthyroidism. Hyperthyroidism is reported to decrease the HDL level and after treatment the HDL level increases [28] and these findings is consistent with our results.

Vitamin C is important in 7α -HYDROXYLASE activity and the 7α hydroxylation of cholesterol is the first and principal regulatory step in the biosynthesis and degradation of cholesterol, 7α -HYDROXYLASE activity is also enhanced by the thyroid hormone, vitamin C deficiency would deactivate this step [29,30]. In our results, the cholesterol level in the vitamin C group was lower than the control group or the hyperthyroid group. We may suppose that the co treatment with folic acid or vitamin C would reduce the T3 & T4 levels so the cholesterol level is slightly more elevated than the hyperthyroid group.

It was reported that there is a relation between hyperthyroidism and deteriorations of free radicals and antioxidant systems that increase lipid peroxidation and oxidative stress [22]. The present study indicated that, by showing a highly significant increase in MDA, catalase and NO and a noticeable decrease in and GSH levels in the hyperthyroid rats group in comparing to the control group. Treatment with folic acid and / or vitamin C managed to reduce the oxidative stress in the adrenal gland as shown in graph 2-b, 2-c, 2-d and 2-e, the hyperthyroid group showed increased MDA, catalase, and nitric oxide and decreased adrenal reduced glutathione which indicates that there is increased oxidative stress in the adrenal tissue as compared to the control group, by treatment with vitamins the oxidative stress was reduced as compared to the hyperthyroid group.

In conclusion, folic and ascorbic acids especially if co-administered together have highly protective effects on hyperthyroidism.

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