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Riboflavin Metabolism in the Hypothyroid Human Adult (42459)

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Abstract. It had been shown that thyroxine regulates the conversion of riboflavin to riboflavin mononucleotide and flavin adenine dinucleotide (FAD) in laboratory animals. In the hypothyroid rat, the flavin adenine dinucleotide level of the liver decreases to levels observed in riboflavin deficiency. We have shown that in six hypothyroid human adults, the activity of erythrocyte glutathione reductase, an accessible FAD-containing enzyme, is decreased to levels observed during riboflavin deficiency. Thyroxine therapy resulted in normal levels of this enzyme while the subjects were on a controlled dietary regimen. This demonstrates that thyroid hormone regulates the enzymatic conversion of riboflavin to its active coenzyme forms in the human adult.

It has been shown that the flavin adenine dinucleotide (FAD) content of livers of hypothyroid rats was reduced to levels found in riboflavin-deficient rats (1, 2). The latter investigators (2) demonstrated that the activity of flavokinase, the enzyme which converts riboflavin to flavin mononucleotide (FMN) is reduced in these animals, and both FMN and FAD levels in the liver were markedly below those observed in normal rats (3). The role of the thyroid in regulating FAD formation and its implications have been reviewed (4). Recent studies have shown that this regulation involves biosynthesis of flavocoenzymes rather than degradation (5).

Application of these findings to humans has been difficult to demonstrate. However, with the demonstration that erythrocyte glutathione reductase (EGR), a FAD-containing enzyme, is reduced in the riboflavin deficient rat (6) and human (7) and is a sensitive index of riboflavin deficiency, such studies became feasible. In this study we report the EGR activity in hypothyroid patients and the effect of thyroxine therapy on this activity.

Materials and Methods. *Study Subjects.* Six adults, ranging in age from 59 to 88, who visited the outpatient department were the subjects. The purpose of the study was explained to each subject and consent forms were signed. Hypothyroidism was assessed on the basis of low serum free thyroxine (T4) and elevated thyrotropin (TSH). None had other complicating illnesses. All had been consuming normal diets without supplemental vitamin preparations. In order to rule out nutritional deficiencies, serum levels of a representative water-soluble vitamin, folic acid, and a fat-soluble vitamin, retinol, were determined initially and after therapy with thyroxine. The subjects were hospitalized and maintained on a low-salt dietary regimen. No vitamin preparations were given during the 2-week study period. Each patient received 50 μg of synthetic thyroxine (Synthroid) daily. An intravenous blood sample was collected in a tube containing heparin during the initial day of hospitalization and after 2 weeks of thyroxine therapy.

Laboratory methods. Free thyroxine was measured by the method of Ekins *et al.* (8) and TSH was measured with the Corning Immo Phase kit. The preparation of bloods and methods of assay for EGR, serum folic acid, and vitamin A were previously described (9–11). The results of the EGR assay are expressed in AC (activity coefficient) units and are a measure of the degree of saturation of the apoenzyme with FAD. Normal values are 1.0 to 1.2. Values above 1.2 are considered evidence of riboflavin deficiency.

Results. All the subjects had hypothyroidism based on low free T4 and elevated TSH

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TABLE I. FREE T4, TSH AND EGR AC VALUES IN SIX HYPOTHYROID ADULTS INITIALLY AND AFTER THYROXINE THERAPY

Patient No.	Sex	Age	Initially			After 2 weeks thyroxine, 50 μg/day		
			Free T4 (ng/dl)	TSH (μIU/ml)	EGR AC	Free T4 (ng/dl)	TSH (μIU/ml)	EGR AC
1	F	88	0.5	63.7	1.36	1.2	11.2	1.07
2	F	78	0.2	35	1.24	0.9	10.2	1.14
3	M	64	0.1	51	1.36	0.7	21	1.19
4	F	59	0.5	47	1.27	1.0	11.6	1.18
5	F	80	<0.1	77.2	1.24	1.1	9.8	1.09
6	F	71	<0.1	35.5	1.28	0.6	22.7	1.11
					1.29 ± 0.05[a]			1.13 ± 0.05[a]
Euthyroid levels			0.6–1.7	1–10				

[a] Mean ± SD.

serum levels (Table I). In each case the EGR assays revealed abnormally elevated AC values, signifying a depletion of FAD.

After 2 weeks of daily therapy with 50 μg of thyroxine, the T4 levels increased to within normal levels and the TSH levels decreased markedly. Initially the mean for the AC values was 1.29 ± 0.05 SD. After treatment the mean was 1.13 ± 0.05 SD, within normal limits. These differences were statistically significant ($P < 0.01$).

Each of the subjects listed in Table I had serum folic acid and vitamin A levels within normal range, limiting the probability of nutritional deficiency. In this regard one subject with hypothyroidism (low T4, elevated TSH) and low serum folic acid and vitamin A was not included in Table I because of the possibility of nutritional deficiency complicating the interpretation of the results. The subject was given thyroxine therapy only since the results of the vitamin assays were not yet available. His initial AC value was 1.31. After thyroxine therapy, the AC value declined to 1.16. However, his serum folic acid and vitamin A levels were still below the normal range. Subsequent vitamin therapy corrected these levels.

Discussion. The effect of thyroid hormone in regulating the conversion of riboflavin into its functional coenzyme forms has been shown amply in the livers of animals (4, 5). This regulation in the human has been difficult to demonstrate. In one study only two FAD-containing enzymes, succinic dehydrogenase and mitochondrial α-glycerophosphate dehydrogenase, were found to be reduced in liver biopsy specimens obtained from hypothyroid subjects. Neither of these enzymes was increased in thyrotoxic subjects, and no attempt was made to determine the enzyme levels in the hypothyroid subjects after correction of this condition with thyroxine (12).

With the observation that erythrocyte glutathione reductase, a FAD-containing enzyme, sensitively reflects riboflavin nutritional status, it became possible to study the role of thyroid regulation on a more easily accessible FAD-containing enzyme. Such studies have been reported in the hypothyroid rat where it was shown that the EGR activity was decreased (13).

In our study, six hypothyroid subjects, as determined by low free T4 and elevated TSH serum levels, had decreased EGR activity. This resulted in AC values above the normal range. Elevated AC values can result from the inability of the cell to make FAD from riboflavin in the absence of thyroid hormone. It may also result from a nutritional deficiency of riboflavin. In order to eliminate the latter, the subjects were hospitalized and maintained on a diet essentially similar to the regimen they were on before the start of the study. None appeared malnourished, and none received riboflavin supplements.

The subject who had signs of malnutrition was not included in this study. It is, nevertheless, of interest that in this hypothyroid indi-

vidual the decreased EGR activity reverted to normal after therapy with thyroxine even though the other indices of malnutrition did not improve during this period on the dietary regimen.

In each of the six study subjects, therapy with thyroxine for 2 weeks resulted in increased T4 and decreased TSH serum levels and the return of EGR activity to normal.

In the biochemical test for EGR, full activity could be restored to the test tubes containing erythrocyte preparations of the hypothyroid subjects by the addition of FAD. This is further evidence that the FAD content had been reduced, presumably by the inability to convert riboflavin to its coenzyme forms in the absence of thyroid hormone.

It has been demonstrated that in the human adult with hypothyroidism, the activity of erythrocyte glutathione reductase, a FAD-containing enzyme, is reduced to levels observed in subjects with riboflavin deficiency. After 2 weeks of therapy with thyroxine and without supplementation with riboflavin, the enzyme activity reverted to normal. This indicates that as in laboratory animals, thyroxine regulates the conversion of riboflavin to its coenzyme forms.

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