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# Effect of Triiodothyronine on Human Jejunal Glycolytic Enzymes (39046)

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Thyroidectomy decreases glycolytic and gluconeogenic enzyme activities in rat liver (1, 2). On the other hand, thyroxine increases hepatic phosphoenolpyruvate carboxykinase (3) and gluconeogenesis (4) in the rat. It is also known that human and rat jejunal glycolytic enzymes are influenced by dietary carbohydrates (5). In order to determine the effect of thyroid hormone on human intestinal enzymes, we studied four normal young men and three hypothyroid male patients who were given triiodothyronine together with different types of carbohydrate diets.

*Materials and methods.* Four normal young men*, ages 19–22, served as the control group. They were housed on a metabolic ward and fed diets containing 3000 calories as shown in Table I. During fasting, only calorie-free liquids were allowed. The carbohydrate-free diet was a part liquid, part solid formula containing 70% of calories as fat (corn oil) and 30% as protein (sodium caseinate) together with meat and eggs. The glucose and fructose diets consisted of 50% of calories as the sugar, 30% as fat, and 20% as protein, together with a standard mineral supplement. Nonavitamins,** one capsule, were given daily including the fasting periods. Periods 1 through 4 included no added thyroid hormone. Periods 5 through 8 included triiodothyronine ($T_3$, Cytomel) 100 μg orally every 8 hr. Symptoms of hyperthyroidism occurred in three of the four subjects, including variable tachycardia, loose stools, and irritability. Jejunal biopsies were obtained after overnight fasting at the end of each dietary period, using the Crosby–Kugler intestinal biopsy capsule. The tissue thus obtained was prepared as described previously (5) and the activities of glycolytic enzymes were determined spectrophotometrically: Pyruvate kinase (PK) (6), fructose-1-phosphate aldolase (F1PA), and fructose-1,6-diphosphate aldolase (FDPA) (7) and the gluconeogenic enzyme, fructose diphosphatase (FDPase) (8).

Three hypothyroid men were also studied. Their clinical data is shown in Table II. Informed consent was obtained from all subjects and patients. Each patient was rendered hypothyroid by discontinuing thyroid medication ($T_3$, 75 μg daily) for three weeks before study. The sequence of

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** Vitamins in each tablet: A, 5000 USP units; D, 400 USP units; C, 75 mg; calcium pantothenate, 5 mg; $B_{12}$, 2 mcg; niacinamide, 20 mg; $B_6$, 2 mg; $B_2$, 3 mg; $B_1$, 2 mg.

TABLE I. OUTLINE OF STUDIES IN FOUR NORMAL SUBJECTS.

| Period | Duration (days) | Diet |
|---|---|---|
| 1, 5 | 1 | Fasting |
| 2, 6 | 3 | Carbohydrate-free |
| 3, 7 | 3 | Glucose |
| 4, 8 | 3 | Fructose |

TABLE II. CLINICAL DATA IN THREE HYPOTHYROID PATIENTS.

| Patient | Age | Weight (kg) | Diagnosis | Thyroid function at onset of study: Resin $T_3$ uptake (Normal 25–35%) | Thyroid function at onset of study: Serum thyroxine (Normal 2.9–5.9 μg%) |
|---|---|---|---|---|---|
| 1 | 45 | 112 | Thyroid carcinoma, treated by surgery and $^{131}I$ | 19 | <0.5 |
| 2 | 40 | 101.5 | Graves disease, treated by $^{131}I$ | 30 | 2.9 |
| 3 | 42 | 95.6 | Idiopathic primary myxedema | 19 | <0.5 |

studies and composition of diets was identical to that of the normal subjects, except that daily caloric intake and $T_3$ dosage was set lower (2000 Calories and 25 μg, three times daily) because of hypothyroidism. The duration of each dietary period was two days.

Enzyme assays for normal subjects were analyzed via four three-way factorial analyses of variance, one for each enzyme studied. According to the model used, an observation, enzyme level, is assumed to be influenced by three main effects, the first and second order interactions between the main effects and random error. The main effects are presence or absence of hormone ($T_3$), type of diet (Diet) and Subject. The first order interactions are diet–$T_3$, subject–$T_3$, and diet–subject. The second order interaction is diet–subject–$T_3$. The model used is a mixed model, the effects of $T_3$ and Diet being fixed effects and the effect of Subjects being a random effect. According to the model used, the F-statistics for testing the main effect of $T_3$ is formed as the ratio of the mean square of $T_3$ to the mean square of the $T_3$–subject interaction. The F-statistic for testing the main effect of diet is formed as the ratio of the mean square of diet to the mean square of diet–subject interaction. The F-statistic for testing the diet–$T_3$ interaction is formed as the ratio of the mean square of the diet–$T_3$ interaction to the diet–subject–$T_3$ interaction. The mean squares for each enzyme were calculated using the computer program BMDO8V (9). For a thorough discussion of the analysis of variance for factorial experiments see Steele and Torrie (1960) (10). In addition, the Neuman–Keuls multiple comparison procedure was used to compare means of each diet-hormone combination (11).

*Results.* The results of enzyme assays for normal subjects are shown in Fig. 1. The jejunal glycolytic enzymes PK, FDPA and F1PA showed adaptive increases in activity as the diet was sequentially changed from

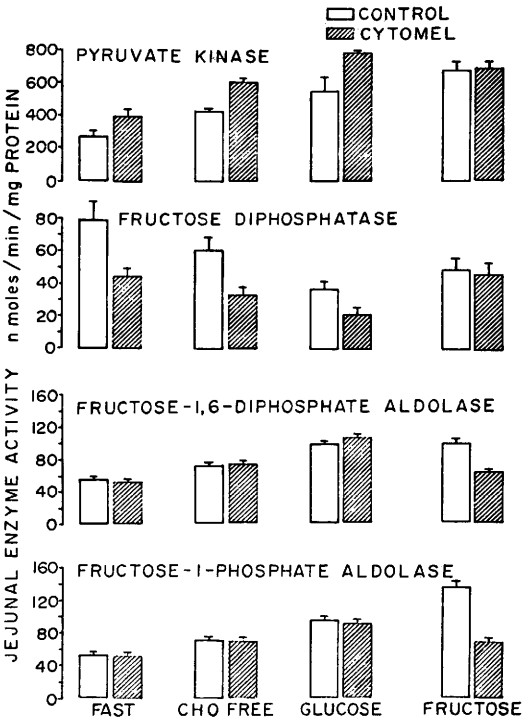


FIG. 1. Jejunal enzyme activities in four normal men expressed as mean ± SEM.

fasting to carbohydrate-free, glucose and fructose. An opposite effect was seen for the gluconeogenic enzyme, FDPase, whose activity was highest with fasting, declined with carbohydrate-free and glucose diet, and was intermediate with the fructose diet. These changes were as expected from previous studies in normal and obese men (12).

The $T_3$ administration caused increased activity of jejunal PK and decreased activity of FDPase during fasting, carbohydrate-free and glucose diets, as compared to these same periods without $T_3$. However, $T_3$ showed no interaction with the dietary effect on these enzymes during fructose feeding. $T_3$ caused no change in enzyme activities for FDPA and F1PA activities except during fructose feeding, where it decreased the activities of both enzymes. These findings are summarized in Table III, which indicates significant general effect of (1) diet upon enzyme activities, (2) $T_3$ upon enzyme activities, and (3) diet–$T_3$ interaction on enzyme activities in the normal subjects.

The enzyme data from the three hypothyroid patients is shown in Table IV and in comparison to the normal subjects reveals frequently decreased activities of PK and increased activities of FDPase during all diet periods. The effect of $T_3$ was variable and restored enzyme activities to normal only occasionally.

*Discussion.* The oral administration of $T_3$ to normal subjects significantly increased the activities of jejunal PK and decreased FDPase during fasting, carbohydrate-free and glucose feeding, but were decreased by $T_3$ during fructose feeding. This indicates that $T_3$ interacts with dietary carbohydrates to affect human jejunal glycolytic and glu-

TABLE III. ANALYSES OF VARIANCE[a] OF ENZYME ACTIVITIES OF FOUR NORMAL SUBJECTS.

| Source of variation | Degrees of freedom | Mean squares[b] | | | |
|---|---|---|---|---|---|
| | | PK | FDPase | FDPA | F1PA |
| $T_3$ | 1 | 135,174.00[c] | 3234.09[d] | 546.15[e] | 2666.33[e] |
| Diet | 3 | 217,910.16[e] | 1411.68[d] | 3087.39[e] | 3870.49[e] |
| Subject | 3 | 21,049.62 | 1143.39 | 358.62 | 444.71 |
| Diet-$T_3$ | 3 | 19,380.55[c] | 377.40[c] | 772.65[e] | 2345.34[e] |
| Subject-$T_3$ | 3 | 6,772.46 | 119.28 | .90 | 3.44 |
| Diet–subject | 9 | 5,504.81 | 39.62 | 5.31 | 6.37 |
| Diet–subject-$T_3$ | 9 | 3,972.51 | 44.49 | 9.52 | 4.07 |

[a] Four analyses of variance were performed, one for each enzyme.

[b] The mean squares are the squares of the enzyme activities expressed as nmoles substrate metabolized/min/mg protein.

[c] $P < 0.05$.

[d] $P < 0.025$.

[e] $P < 0.005$.

TABLE IV. EFFECT OF DIET AND $T_3$ ON JEJUNAL ENZYME ACTIVITIES IN THREE HYPOTHYROID MEN

| Enzyme | Patient | Fast | | CHO free | | Glucose | | Fructose | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | $T_3$ | Control | $T_3$ | Control | $T_3$ | Control | $T_3$ |
| Pyruvate kinase | 1 | 177.6[a] | 149.0 | 167.5 | 154.5 | 145.0 | 212.5 | 101.5 | 84.4 |
| | 2 | — | 559.0 | 389.4 | 585.0 | 340.7 | 419.4 | 305.5 | 728.0 |
| | 3 | 288.8 | — | 346.3 | 388.7 | 366.8 | 164.0 | 387.0 | 289.0 |
| FDPase | 1 | 64.8 | 44.0 | 55.0 | 53.8 | 43.8 | 51.9 | 63.0 | 21.5 |
| | 2 | — | 113.6 | 39.4 | 34.2 | 54.6 | 62.6 | 247.1 | 103.6 |
| | 3 | 92.4 | — | 113.3 | 54.6 | 55.1 | 32.7 | 43.6 | 33.6 |
| FDPA | 1 | 104.3 | 91.4 | 99.3 | 94.7 | 89.2 | 147.3 | 79.5 | 70.2 |
| | 2 | — | 81.3 | 64.2 | 70.2 | 73.2 | 87.2 | 103.2 | 108.2 |
| | 3 | 57.2 | — | 73.0 | 72.5 | 81.4 | 85.0 | 87.6 | 59.2 |

[a] Enzyme activities are expressed as nmoles substrate metabolized/min/mg protein.

coneogenic enzyme activities. The effect on jejunal aldolases (FDPA and F1PA) was mediated through fructose and $T_3$ rather than glucose and $T_3$. $T_3$ alone caused no difference in aldolase enzyme activity except during fructose feeding when the activities of both aldolases were decreased.

The hypothyroid patients demonstrated abnormalities in adaptive responses of these enzymes to both dietary changes and $T_3$ administration. The administration of $T_3$ alone, under the conditions stated, was insufficient to fully correct the abnormalities of jejunal enzyme responses in hypothyroidism.

The explanation of these abnormalities in hypothyroid males awaits further investigation. Perhaps $T_3$ alone, in the absence of thyroxine, was inadequate to restore enzyme activities to normal. Chopra *et al.* (13) have shown that thyroxine ($T_4$) has intrinsic hormonal activity separate from that resulting from its conversion to $T_3$ *in vivo*. If this hypothesis is correct, the administration of both $T_3$ and $T_4$ in physiological quantities should restore enzyme activities to normal. We cannot exclude a defect in cellular metabolism of $T_3$ in these patients, which was not corrected by oral $T_3$ administration. Such a defect could exist at one or more points in the metabolism of $T_3$, such as at the site(s) of cellular binding of $T_3$ (14, 15) or in the processes of transcription and translation (16). Read *et al.* (17) have shown that malabsorption of thyroid hormone does not occur during hypothyroidism.

Numerous enzyme systems have been shown to be both increased and decreased by thyroid hormones (18). Thyroidectomy changes the activities of many enzymes, both mitochondrial and cytoplasmic. The net importance of these enzyme changes in relation to glycolysis and gluconeogenesis remains unclear. We have studied four hypothyroid patients who had reactive hypoglycemia in association with delayed and excessive insulin secretion, all of whom were corrected by $T_3$ therapy. Fasting hypoglycemia could not be demonstrated, suggesting that a severe defect in gluconeogenesis was not present (19). Although the jejunum is not a physiologically significant site of glycolysis and gluconeogenesis in man, it reflects the type of response that may be occurring in the liver as a consequence of $T_3$ administration.

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