

Response of Hepatic Mitochondrial α -Glycerophosphate Dehydrogenase and Malic Enzyme to Constant Infusions of L-Triiodothyronine in Rats Bearing the Walker 256 Carcinoma

Evidence for Divergent Postreceptor Regulation of the Thyroid Hormone Response

Joseph M. Tibaldi, Naima Sahnoun, and Martin I. Surks
Division of Endocrinology and Metabolism, Department
of Medicine, Montefiore Medical Center and The Albert Einstein
College of Medicine, Bronx, New York 10467

Abstract. To characterize the hepatic response to L-triiodothyronine (T_3) in an experimental nonthyroidal disease, we determined the activity of hepatic mitochondrial α -glycerophosphate dehydrogenase (α -GPD) and cytosol malic enzyme (ME) as a function of the saturation of the nuclear T_3 receptor during constant T_3 infusions in rats bearing the Walker 256 carcinoma. Groups of control and tumor-bearing rats were infused by minipumps (Alza Corp., Palo Alto, CA) with vehicle, 1.2 or 4.5 $\mu\text{g } T_3/100$ body wt per day for 3 d. The range for serum T_3 was 47.2 ± 4.1 to 165 ± 17.3 ng/dl for the control rats and 13.2 ± 1.3 to 135 ± 14.3 ng/dl for the tumor-bearing rats. Nuclear T_3 receptor concentration was between 0.41 ± 0.06 and 0.47 ± 0.02 ng/mg DNA in control rats and was decreased in tumor-bearing rats to between 0.23 ± 0.03 and 0.26 ± 0.03 ng/mg DNA. Nuclear T_3 receptor concentrations were not influenced by the T_3 infusions. Specifically bound nuclear T_3 , determined by radioimmunoassay of extracts of isolated nuclei, was decreased nearly 50% in the tumor-bearing rats. However, the calculated percentage saturation of the T_3 nuclear receptor remained similar in control and tumor-bearing rats at each level of T_3 infusion. Dose-response curves for α -GPD and ME were curvilinear and showed an

exponential increase in enzyme activity with progressive receptor saturation. In tumor-bearing rats, the activity curves or calculated appearance rate curves for α -GPD were shifted significantly upward and to the left, indicating greater sensitivity to T_3 , and those of ME were shifted downward and to the right, indicating decreased responsiveness to T_3 . Our findings suggest that cellular factors result in postreceptor amplification of the α -GPD response and diminution of the ME response to T_3 in tumor-bearing rats. Augmentation of the α -GPD response may be a prototype for other hormonal responses that enable the tumor-bearing rat to maintain an apparent euthyroid state in association with decreased serum T_3 .

Introduction

In the human being, nonthyroidal illness results in marked alterations in thyroid hormone concentrations and binding to serum proteins (1–12). Many patients with a variety of nonthyroidal disorders have normal circulating L-thyroxine (T_4)¹ with an increase in free T_4 . This change is associated with a fall in serum L-triiodothyronine (T_3) and free T_3 . The decrease in serum T_3 probably results from a decrease in T_3 production from T_4 under these conditions. Since T_3 is responsible for at least 80% of the biological activity of iodothyronines in human beings (13–15), it is surprising that sick patients do not appear hypothyroid. Such patients are usually judged to be euthyroid upon clinical evaluation, and this impression is supported by the finding of a normal serum thyrotropin (TSH). We have recently reported that many patients who have nonthyroidal diseases (16) or who are of advanced age (17) may have a

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Address reprint requests to Dr. Surks, Montefiore Medical Center.

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1. Abbreviations used in this paper: GPD, glycerophosphate dehydrogenase; ME, malic enzyme; T_3 , L-triiodothyronine; T_4 , L-thyroxine; TSH, thyrotropin.

blunted thyrotropin-releasing hormone-induced TSH response to an experimental decrease in serum T_4 and T_3 . These findings suggest that a normal serum TSH may not be a reliable index of the euthyroid state in some individuals with nonthyroidal illness. Thus, both biological and practical clinical implications of a low serum T_3 in sick patients remain unclear at the present time.

To resolve the paradox of decreased serum T_3 without apparent hypothyroidism in nonthyroidal disease, we have measured the concentration of thyroid hormones, T_3 receptor binding, and biological responses in rats bearing a Walker 256 carcinoma in the thigh. Recent reports suggest that rats with these tumors may be a useful experimental model of nonthyroidal disease of human subjects (18–21). Tumor-bearing rats have decreased circulating total and free T_4 and T_3 . The decrease in T_4 results from the much more rapid rate of T_4 metabolism in the rat ($t_{1/2} = 12$ h) (20) in comparison with human subjects ($t_{1/2} = 6$ d) (22), and from the further acceleration of T_4 metabolism in tumor-bearing rats (20). Similar to sick patients, tumor-bearing rats have a normal TSH concentration (21). Although hepatic nuclear T_3 receptor levels were uniformly decreased in tumor-bearing rats, the activities of two thyroid hormone-responsive hepatic enzymes, mitochondrial α -glycerophosphate dehydrogenase (α -GPD) (L-glycerol-3-phosphate/cytochrome c-oxidoreductase [EC 1.1.99.5]) and cytosol malic enzyme (ME) (L-malate/NADP⁺ oxidoreductase decarboxylating [EC 1.1.1.40]), were variable (19). Basal α -GPD activity remained normal, suggesting that there is normal thyroidal activity under these conditions. In contrast, ME activity was decreased. Since the decrease in ME activity antedated significant changes in concentration of circulating iodothyronines and nuclear T_3 receptors, the decrease in ME activity was considered not to be a primary effect of thyroid hormone. Both enzymes were induced by doses of T_3 sufficient to saturate all nuclear T_3 receptor sites, although the absolute increment in ME activity of tumor-bearing rats was significantly less than in control animals. Pair-feeding experiments suggested that these findings were not due to malnutrition (19).

In our previous studies, we did not measure the concentration of T_3 that was specifically bound to nuclear T_3 receptors. Hence, we were unable to compare the enzyme response of tumor-bearing rats with that of control rats as a function of the fractional occupancy of nuclear receptor sites. Moreover, our results did not distinguish between potential changes in the rate of appearance and degradation of these enzymes. Accordingly, we undertook the present studies to assess the appearance rate of α -GPD and ME as a function of fractional occupancy of the nuclear T_3 receptor. The data to be presented below suggest an augmentation in the appearance rate of α -GPD and a reduction in the appearance rate of ME in tumor-bearing rats in comparison with control animals. Augmentation of α -GPD appearance in tumor-bearing rats may be a prototype of other augmented hormonal responses that enable the rat to

maintain an apparent euthyroid state despite decreased serum and nuclear T_3 .

Methods

Male Sprague-Dawley rats (Charles River Breeding Laboratories Inc., Wilmington, MA) were used in all studies. They were maintained on the Wayne Laboratory Rat Diet (Allied Mills, Inc., Memphis, TN) and were allowed tap water ad lib. Rats usually weighed between 180 and 200 g at the start of each experiment. There was no significant difference between the mean body weight of control and experimental groups.

The dose-response relationship between mitochondrial α -GPD and cytosol ME activities as a function of nuclear T_3 and nuclear T_3 receptor saturation was determined in control and tumor-bearing rats. At the beginning of each experiment (day 0), groups of rats were placed under light ether anesthesia and injected in the right quadriceps with either 0.2 ml of a Walker 256 carcinoma suspension or with an equivalent volume of 0.9% NaCl, as previously described (18). The tumors usually became palpable after 3 d. At that time (day 3), both control and tumor-bearing animals received a minipump (Alzet Osmotic; Alza Corp., Palo Alto, CA) subcutaneous in the intrascapular area. Groups of control and tumor-bearing rats were given pumps containing either vehicle, 0.01 N NaOH, 5% rat serum (23), or vehicle containing enough T_3 (Sigma Chemical Co., St. Louis, MO) to provide a T_3 infusion rate of 1.2 or 4.5 μ g/100 g body wt per d. A tracer amount of [¹²⁵I] T_3 was added to each minipump that contained T_3 . The counting rate of each pump was determined before implantation and after the rats had been killed (see below). The volume of vehicle delivered to each animal was then calculated as the product of the percentage difference in counting rate of individual pumps and the initial volume of each pump. Each dose-response study was carried out twice. Data for most variables were pooled from six animals at each level of T_3 infusion.

Additional groups of four control and four tumor animals were studied to determine the maximum response of α -GPD and ME to T_3 . 3 d after injection of Walker 256 carcinoma suspension or 0.9% NaCl, rats received an intrascapular minipump in order to establish a T_3 infusion rate of 4.5 μ g/100 g body wt per d. On the fourth and fifth days after tumor injection, rats were also injected with 1,000 μ g T_3 i.p. This dose was judged sufficient to effect 100% saturation of hepatic nuclear T_3 receptors (24).

Rats were killed 6 d after injection of Walker 256 carcinoma suspension or 0.9% NaCl and 72 h after implantation of the minipumps. The animals were killed by exsanguination through the abdominal aorta under light ether anesthesia. Tumor size was measured with a caliper and the tumor weight was estimated by the formula developed by Schrek (25). We have previously demonstrated an excellent correlation between estimates of tumor weight by this method and tumor weight determinations by dissection and weighing (19). Plasma was obtained for measurement of the concentrations of T_3 (26) and T_4 (Clinical Assays Kit; Travenol Laboratories, Inc., Cambridge, MA). The liver was quickly removed and placed on ice. Weighed samples were taken for preparation of nuclei, mitochondria, and cytosol. The liver parenchyma and lymph nodes of the porta hepatis were free of tumor by gross examination. We have previously demonstrated the absence of liver parenchymal carcinoma by light microscopy (18).

Nuclear T_3 binding capacity was measured in isolated nuclei by a

method previously described by this laboratory (27). We also determined, in the same samples of nuclei, the concentration of specifically bound nuclear T_3 by a modification of the method of Surks and Oppenheimer (28). Nuclei isolated from 2 g of liver were suspended in 2 ml of 0.32 M sucrose, 3 mM $MgCl_2$, and washed with an equal volume of cold ($5^\circ C$) 0.25 M sucrose, 20 mM Tris-HCl, 1.1 mM $MgCl_2$ (STM-buffer), which contained 1% Triton X-100, pH 7.0, at $25^\circ C$ to remove T_3 that was nonspecifically bound. Triton X-100 was obtained from Packard Instrument Co., Inc., United Technologies (Downers Grove, IL). Nuclei from rats infused with $4.5 \mu g T_3/100 g$ body wt per d were washed twice with 10 ml of STM-0.5% Triton X-100 buffer in order to minimize nonspecific T_3 binding. Nuclei were pelleted by centrifugation at 800 g for 15 min. Nuclei were then washed with 2 ml H_2O at $5^\circ C$ and again centrifuged at 800 g for 15 min. T_3 was extracted from the washed nuclei by three successive treatments with 3 ml of absolute ethanol. Parallel studies that used $[^{125}I]T_3$ indicated that this procedure resulted in extraction of $>98\%$ of the nuclear T_3 . The ethanol extracts were then evaporated to dryness under a stream of N_2 at $37^\circ C$ and the T_3 was taken up in 0.3 ml of 0.4% bovine serum albumin in 0.075 M barbital buffer, pH 8.6. The concentration of T_3 in these solutions was then determined by radioimmunoassay (26). Nuclear and plasma T_3 were measured in the same assay for each experimental group. The intraassay coefficient of variation was 9.1% and the interassay coefficient of variation was 14.6%. Plasma protein binding of T_3 was measured by a charcoal method and free T_3 index was calculated as previously described (3).

Cytosol ME activity was determined by the method of Ochoa (29) as modified by Hsu and Lardy (30), and mitochondrial α -GPD activity was determined by the method of Lee and Lardy (31). DNA was measured by a modification of the Burton procedure (32). Protein concentrations were determined by the method of Lowry et al. (33). Data are expressed as mean \pm SEM and were analyzed statistically by analysis of variance (34).

The rate of appearance of ME and α -GPD activities per milligram of protein was estimated by a modification of the method described by Oppenheimer and Schwartz (35). This method requires determination of the half-life of each enzyme activity. To determine the half-life of cytosol ME and mitochondrial α -GPD activities in control and tumor-bearing rats, groups of rats were injected with either a suspension of Walker 256 carcinoma or 0.9% NaCl, as described above. These groups were equally subdivided and, at the same time, equal numbers of tumor-injected and 0.9% NaCl-injected rats were given either vehicle or 2,000 $\mu g T_3/100 g$ body wt i.p. On the third day, when enzyme induction was judged to be maximal (24, 35), groups ($n = 4$) of control and tumor-bearing rats who had received either T_3 or vehicle were killed for determination of activities of mitochondrial α -GPD and cytosol ME. Similar determinations of enzyme activities were made in comparable groups 6 d after the start of the experiment. This experimental protocol provided both baseline and induced enzyme activities for control and tumor-bearing animals. The data obtained allowed calculation of the half-life of enzyme activity that was induced over baseline values. The appearance rate of each enzyme activity could then be calculated in the basal state as well as in T_3 -infused tumor-bearing and control animals by the method of Oppenheimer and Schwartz (35). For these calculations, 48 h was assumed to be the period of enzyme induction for animals who received continuous T_3 infusions by means of the minipumps.

Since little stimulation of cytosol ME activity occurred in the tumor-bearing rats, the half-life of ME was estimated as follows: groups

of rats were injected intraperitoneally with either vehicle or T_3 (1,500 $\mu g/100 g$ body wt) on day 0 and day 1. Because of the report of Oppenheimer et al. (24), the activity of cytosol ME was judged to be maximal on day 4. At that time, groups of rats injected with T_3 or vehicle were killed for determination of the cytosol ME activity. Walker 256 carcinoma suspension or 0.9% NaCl was then injected into the quadriceps as described above. The ME activity was again determined 5 d after tumor implantation in groups of control and tumor-bearing animals.

Results

Preliminary experiments (not illustrated) suggested that plasma T_3 concentration became relatively constant 16–24 h after the insertion of osmotic minipumps containing T_3 . This finding confirmed the observations of Connors and Hedge (23). To determine whether plasma T_3 remained constant during the next 2 d, plasma T_3 was measured before and 2 and 3 d after T_3 infusion by osmotic minipump was started (Fig. 1). Plasma T_3 remained relatively constant 2 and 3 d after pumps were implanted and no significant difference between control and tumor-bearing rats was noted.

Mean plasma T_3 concentrations of control and tumor-bearing rats are shown as a function of the T_3 infusion rate in Fig. 2. Plasma T_3 of tumor-bearing rats was markedly diminished in vehicle-injected animals but was restored to the normal range (40–80 ng/dl) when tumor-bearing rats were infused with 1.2 $\mu g T_3/100 g$ body wt per d. The greater T_3 infusion rate, 4.5 $\mu g/100 g$ body wt per d, resulted in a further increase in mean plasma T_3 to values greater than those found in euthyroid rats but below those estimated to result in $>95\%$ saturation of nuclear T_3 receptor sites (24). Mean plasma T_3 concentration of tumor-bearing rats (13.2 ± 2.5 ng/dl) was significantly lower than the control group (47.2 ± 4.1 ng/dl) in the vehicle-infused rats ($P < 0.005$). In accordance with previous

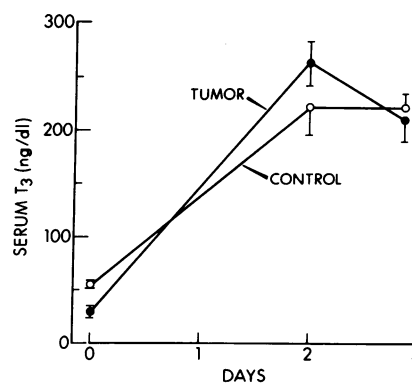


Figure 1. Plasma T_3 in control and Walker 256 carcinoma-bearing rats. Plasma was collected before and 2 and 3 d after minipumps were implanted subcutaneously in the intrascapular space. The minipumps contained enough T_3 to infuse 4.5 $\mu g/100 g$ body wt per d. Results are mean \pm SE for groups of six rats.

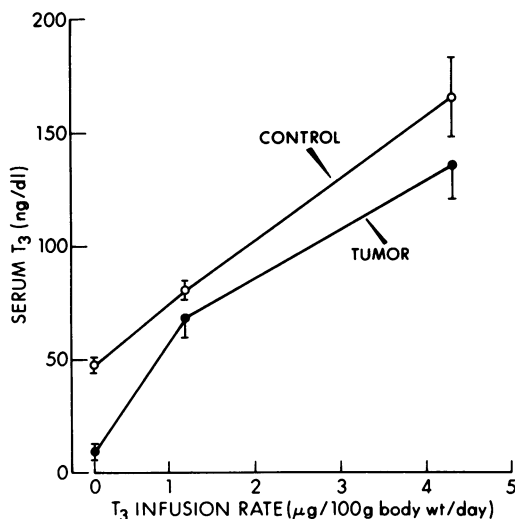


Figure 2. Plasma T_3 in control and Walker 256 carcinoma-bearing rats. Plasma was collected 3 d after minipumps were implanted subcutaneously in the intrascapular region. The minipumps contained either vehicle (0.01 N NaOH, 5% rat serum) or vehicle supplemented with enough T_3 to establish an infusion rate of 1.2 or 4.5 $\mu\text{g}/100\text{ g body wt per d}$. Results are mean \pm SE of values pooled from two separate experiments. ($n = 6$).

observations (18), plasma protein binding of T_3 was decreased in the vehicle-infused tumor-bearing rats. The T_3 binding index of the tumor-bearing rats, 1.68 ± 0.04 , was significantly greater than for control rats, 1.10 ± 0.04 ($P < 0.005$). However, the free T_3 index of the tumor-bearing rats infused with vehicle, 22.4 ± 5.3 , was significantly less than the control group, 51.9 ± 2.3 ($P < 0.01$). Mean plasma T_3 was also decreased in the group infused with the higher T_3 dosage, 4.5 $\mu\text{g}/100\text{ g body wt per d}$

($P < 0.01$). The decrease in mean plasma T_3 concentration noted in tumor-bearing rats at this T_3 infusion rate may result from an increase in the metabolic clearance rate of this iodothyronine. An increase in metabolic clearance rate of T_4 has been reported previously for animals bearing Walker 256 carcinoma (20). The mean serum T_4 concentration of the control group that was infused with vehicle was $3.4 \pm 0.4\text{ }\mu\text{g/dl}$. Serum T_4 levels were $<1\text{ }\mu\text{g/dl}$ in all other animals.

The tumor-bearing rat has been proposed as a model for the syndrome of nonthyroidal illness with decreased serum T_3 (18). Previous pair-feeding experiments suggested that food deprivation was not responsible for altered thyroid hormone economy in tumor-bearing rats (19). Since Oppenheimer and Schwartz (35) have reported that starvation alters the appearance rate of mitochondrial α -GPD and cytosol ME, we reinvestigated the putative relationship between malnutrition, altered thyroid hormone concentrations, and biological responses in tumor-bearing rats by determining the liver weight and concentrations of cytosol protein and DNA (Table I and Fig. 3). Mean body weight of the tumor-bearing rats increased at about the same rate as that of controls during infusion of vehicle or T_3 (Fig. 3). At the end of the experiments, mean body weight of tumor-bearing rats was decreased only 7.5% from their respective control groups at all T_3 infusion rates ($P < 0.05$ – <0.025) (Table I). In contrast, the mean liver weight of tumor-bearing rats was significantly greater than in the respective control groups. The mean percentage increase in liver weight was $23.1 \pm 2.1\%$ for all tumor-bearing rats and this change appeared to be independent of the T_3 infusion rate. The increase in hepatic weight of tumor-bearing rats was associated with a significant increase in mean hepatic DNA content. Mean cytosol protein content was not altered in tumor-bearing rats and was not influenced by the T_3 infusion rate. Since Oppenheimer and Schwartz (35) have reported that a 28–40% decrease

Table I. Effect of T_3 Infusion on Body and Liver Weight and Liver DNA and Protein Content in Control and Walker 256 Carcinoma-bearing Rats*

T_3 infusion	Rats	Body weight	Tumor size	Liver weight	Cytosol protein	DNA
$\mu\text{g}/100\text{ g body wt per d}$		g	% body wt	g/100 g body wt	mg/liver	mg/liver
0	C	247.2 ± 4.0		4.29 ± 0.17	747.1 ± 25.2	27.8 ± 1.9
	T	$233.0 \pm 3.7\ddagger$	5.2	$4.90 \pm 0.15\ddagger$	737.2 ± 33.8	$32.4 \pm 2.5\S$
1.2	C	265.3 ± 9.7		3.73 ± 0.05	776.7 ± 31.8	23.5 ± 0.3
	T	$236.3 \pm 4.4^{\parallel}$	7.8	$4.95 \pm 0.28^{\parallel}$	746.7 ± 61.8	$34.2 \pm 3.5\ddagger$
4.5	C	259.5 ± 5.2		4.15 ± 0.06	779.7 ± 24.7	28.1 ± 1.1
	T	$243.6 \pm 1.6\ddagger$	6.1	$5.14 \pm 0.25^{\parallel}$	817.8 ± 26.7	$37.6 \pm 4.5^{**}$

* Results are mean \pm SE from groups of six rats. Data are pooled from two separate experiments. Statistical analyses were performed using analysis of variance. C, control; T, tumor-bearing rats. Comparisons are T vs. C for each T_3 infusion rate. $\ddagger P < 0.025$. $\S P > 0.05$ (not significant statistically). $^{\parallel} P < 0.05$. $^{\parallel\parallel} P < 0.001$. $^{**} P < 0.005$.

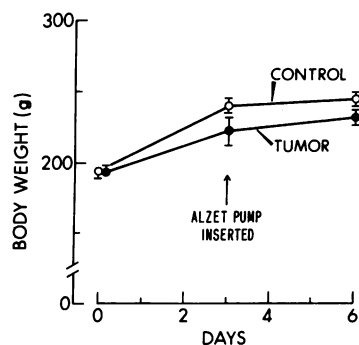


Figure 3. Growth curves of control and Walker 256 carcinoma-bearing rats. Tumor or vehicle was injected at $t = 0$ and Alzet pumps were inserted on day 3. Results are mean \pm SE for groups of six rats.

in body weight, hepatic weight, and content of DNA and protein occurs during starvation, the results in Table I and Fig. 3 suggest that malnutrition was not a significant factor in the present studies.

In order to determine the relationship between hepatic nuclear T_3 receptor occupancy and induction of α -GPD and ME, nuclear T_3 receptor concentration and specifically bound nuclear T_3 were determined in control and tumor-bearing rats at different T_3 infusion rates (Table II). Mean nuclear T_3 receptor concentration of tumor-bearing rats was significantly decreased to 63, 50, and 51% of the values for the respective control groups infused with vehicle, 1.2 and 4.5 $\mu\text{g } T_3/100 \text{ g body wt per d}$. In agreement with our previous study (19), there was no significant difference in mean dissociation constant between control and tumor-bearing animals. Moreover, mean

Table II. Effect of T_3 Infusion on Nuclear T_3 and Nuclear T_3 Receptor Concentration and Percentage Saturation in Control and Walker 256 Carcinoma-Bearing Rats*

T_3 infusion $\mu\text{g}/100 \text{ g}$ body wt per d	Rats	Nuclear T_3 ng/mg DNA	Nuclear T_3 receptor	
			ng/mg DNA	% saturation
0	C	0.21 ± 0.01 (6)	0.41 ± 0.06 (5)	52.6 ± 6.6 (5)
	T	0.10 ± 0.01 (5)‡	0.26 ± 0.03 (5)§	42.1 ± 5.5 (5)¶
1.2	C	0.36 ± 0.03 (6)	0.46 ± 0.02 (6)	79.8 ± 5.3 (6)
	T	0.20 ± 0.02 (6)‡	0.23 ± 0.03 (6)‡	82.1 ± 10.3 (6)¶
4.5	C	0.38 ± 0.07 (3)	0.47 ± 0.02 (6)	87.4 ± 17.6 (3)
	T	0.20 ± 0.01 (3)‡	0.24 ± 0.01 (6)‡	88.3 ± 12.1 (3)¶

*Results are mean \pm SE for data pooled from two separate experiments. Number of samples is in parentheses. C, control; T, tumor-bearing rats. Statistical analyses were performed by analysis of variance. Statistical comparisons listed are T vs. C for each T_3 infusion rate.

‡ $P < 0.001$.

§ $P < 0.005$.

¶ $P > 0.05$ (not significant statistically).

nuclear T_3 receptor concentration of the control or tumor-bearing rats did not change significantly at any of the T_3 infusion rates employed. Thus, nuclear T_3 receptor concentration did not appear to be influenced by the different T_3 infusion rates used in these studies. In the groups infused with vehicle alone, the mean concentration of T_3 bound specifically to the nuclear T_3 receptor was also decreased significantly in tumor-bearing rats ($P < 0.001$), from 0.21 ± 0.01 to 0.10 ± 0.01 ng/mg DNA. Infusion of T_3 raised the mean nuclear T_3 concentration both in control and tumor-bearing rats ($P < 0.005$). However, the mean nuclear T_3 concentration of tumor-bearing rats remained significantly lower than that of the control groups at each of the two T_3 infusion rates. The percentage saturation of the nuclear T_3 receptor was calculated by dividing the nuclear T_3 concentration by the nuclear T_3 receptor concentration. For control and tumor-bearing rats, respectively, mean receptor saturation was 52.6 ± 6.6 and $42.1 \pm 5.5\%$ in vehicle-infused groups, and it increased to $\sim 80\%$ in groups infused with 1.2 $\mu\text{g } T_3/100 \text{ g body wt per d}$, and to $\sim 88\%$ in groups infused with 4.5 $\mu\text{g } T_3/100 \text{ g body wt per d}$. The differences observed in mean percentage receptor saturation between control and tumor-bearing rats at each T_3 infusion rate were not significant statistically.

The dose-response curves for mitochondrial α -GPD activity in control and tumor-bearing rats are presented in Fig. 4. Although the abscissa expresses percentage saturation of the nuclear T_3 receptor, similar dose-response relationships were obtained when the abscissa showed either serum T_3 or T_3 that was specifically bound to the nuclear T_3 receptor. Similarly, although the α -GPD activity is expressed per milligram of mitochondrial protein, similar curves were developed when α -GPD activity was expressed per milligram of DNA or per total liver. The principal finding of this study was that mean α -GPD activity of tumor-bearing rats was significantly greater than that of control rats at all levels of saturation of the nuclear T_3 receptor between 40 and 100%. The differences

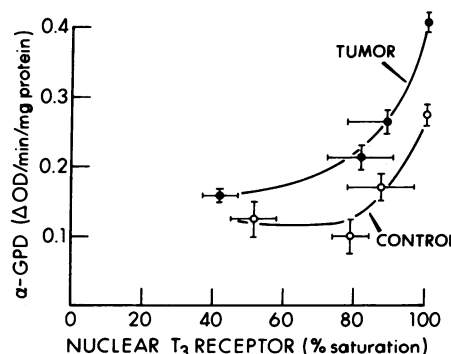


Figure 4. Dose-response of mitochondrial α -GPD in control and Walker 256 carcinoma-bearing rats. Results are mean \pm SE of values pooled from two separate experiments ($n = 6$).

between the mean α -GPD values of the control and tumor-bearing rats that were infused with vehicle or T_3 (1.5 and 4.5 $\mu\text{g}/100$ g body wt per d) were significant statistically ($P < 0.05$, 0.001, and 0.001, respectively). The nonlinear dose-response curves for α -GPD of both control and tumor-bearing rats agree with the observations of Oppenheimer et al. (24, 36). It is also notable that the maximal induction of GPD activity (100% saturation) in tumor-bearing rats was 50% greater than in control groups ($P < 0.001$).

The dose-response curve for cytosol ME activity is shown in Fig. 5. Although the abscissa is presented as percentage saturation of the nuclear T_3 receptor, similar dose-response relationships were obtained when the abscissa expressed serum T_3 . When the abscissa expressed T_3 specifically bound to the nuclear T_3 receptor, differences between the control and tumor-bearing rats were noted only at maximal nuclear T_3 . Moreover, although ME activity is expressed per milligram of cytosol protein, similar curves were developed when ME activity was expressed per milligram DNA or per total liver. In contrast to the dose-response relationships for α -GPD (Fig. 4), a marked depression in ME response to T_3 was observed in tumor-bearing rats in comparison with control animals. As shown in Fig. 5, the dose-response curve for ME activity of control rats was nonlinear and qualitatively similar to the results of Oppenheimer et al. (24, 36). For the control animals, a marked amplification of enzyme activity was noted at high levels of receptor saturation. The ME dose response of tumor-bearing rats appeared qualitatively similar to the controls, but values of enzyme activity at high levels of receptor saturation were considerably lower than the controls. The differences between the mean ME activities of control and tumor-bearing rats were significant statistically, $P < 0.01$ and $P < 0.001$, in the groups infused with 1.5 and 4.5 $\mu\text{g } T_3/100$ g body wt per d, respectively.

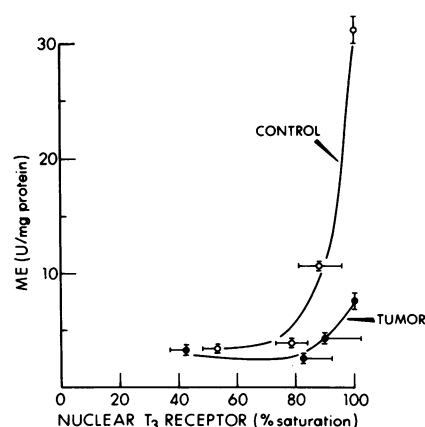


Figure 5. Dose-response of cytosol ME in control and Walker 256 carcinoma-bearing rats. Results are mean \pm SE of values pooled for two separate experiments ($n = 6$).

To investigate further the mechanism underlying the differences in the dose-response curves for ME and α -GPD activities between control and tumor-bearing rats, we estimated the appearance rate of these enzymes by the method of Oppenheimer and Schwartz (35). The half-life for α -GPD activity was 2.1 d for both control and tumor-bearing rats. Thus, the increase in α -GPD activity in tumor-bearing rats (Fig. 4) did not appear secondary to a decrease in the rate of metabolism of this enzyme. The half-life for ME activity was 2.3 d in control rats and 4.5 d for tumor-bearing rats. Since the determination of half-life for ME and α -GPD required pulse injection of T_3 to augment initial enzyme activities (5), these results cannot be assumed to be completely representative of the euthyroid rat. However, the half-life of ME and α -GPD activities by this method has been employed in similar studies previously (35). The half-lives of α -GPD and ME activities were used in the equation derived by Oppenheimer and Schwartz (35) in order to calculate the appearance rate of these enzymes in control and tumor-bearing rats under the present experimental conditions. In this analysis, we assumed a 48-h period of enzyme stimulation by T_3 . The resulting curves for enzyme appearance rate (Fig. 6) suggest that the calculated GPD appearance rate of tumor-bearing rats was $\sim 100\%$ greater than that of control rats in the absence of T_3 infusion. Moreover, the enhanced α -GPD appearance rate was maintained as receptor saturation progressively increased. The curve for α -GPD appearance rate was exponential. In contrast, the appearance rate of ME activity was markedly decreased in tumor-bearing rats. The ME appearance rate of tumor-bearing rats was only 5% of the control rats at all levels of T_3 receptor saturation that were studied. Tumor-bearing rats demonstrated only a slight increase in the appearance rate of ME in response to exogenous T_3 infusion.

Discussion

The present studies provide new insights into the regulation of α -GPD and ME in animals bearing the Walker 256 carcinoma. In our earlier study (19), we measured α -GPD and ME activities only in the basal state and in rats injected with enough T_3 to occupy $>95\%$ of nuclear T_3 receptors. The present study provides a limited characterization of the dose-response curve for both enzymes in control and tumor-bearing rats. In control rats, the dose-response curve for each enzyme appeared nonlinear, with an exponential increase in enzyme activity apparent as receptor sites were progressively saturated. These findings agree with the earlier observations of Oppenheimer et al. (24, 36). A curvilinear dose-response curve for these enzymes was also noted in the tumor-bearing rats. However, the α -GPD curve was shifted upward and to the left, indicating greater α -GPD sensitivity to T_3 , whereas the ME curve was shifted downward and to the right, indicating lower sensitivity of ME to T_3 in tumor-bearing rats. These findings differ somewhat from our previous study in which we

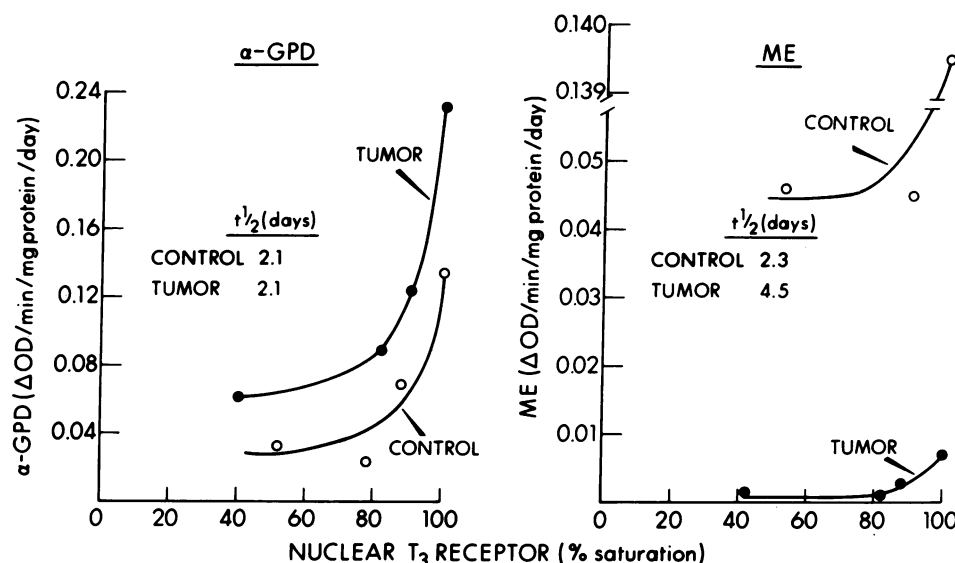


Figure 6. Calculated appearance rate of mitochondrial α -GPD and cytosol ME in control and tumor-bearing rats.

did not show a significant increase in maximal α -GPD response to T_3 in tumor-bearing rats (19). One reason for the difference between the two studies may be the smaller number of animals in each experimental group in our earlier work. When data from the two previously reported experiments are combined (Table 2 in reference 19), the maximal α -GPD response of tumor-bearing rats is significantly increased ($P < 0.025$) in comparison with that of control groups. Compared with vehicle-injected groups, mean percentage increase in maximal α -GPD activity was 48% in the earlier study (19) and 71% in the present experiments.

The possibility that some of the changes observed in tumor-bearing rats are due to starvation has been considered previously (19) and in the present studies. When control rats were paired with tumor-bearing rats, no changes were apparent in any thyroidal parameters that were measured (19). This observation suggested that food deprivation was not a major contributor to the alterations in thyroid hormone economy and biological responses that were observed in tumor-bearing rats. In the present studies, tumor-bearing rats gained weight almost at the same rate as controls and showed an increase in liver weight and DNA content and unchanged cytosol protein content. Since Oppenheimer and Schwartz reported that each of these parameters was significantly decreased in starved rats (35), significant food deprivation probably was not a factor in our studies. Moreover, the changes in the dose-response curves of tumor bearing rats shown in Figs. 4 and 5 were also evident when the enzyme activities were calculated on the basis of the total protein of the respective subcellular fractions, or in terms of total liver protein or DNA. Thus, it seems reasonable to conclude that the present findings resulted from the presence of the tumor and not food deprivation.

An important aspect of the present studies was the simul-

taneous measurement of α -GPD and ME activities and nuclear T_3 and T_3 receptor concentrations. The range of nuclear T_3 receptor concentrations of control rats, 0.41–0.47 ng/mg DNA, agrees with published reports (18, 19, 27, 37), and the 50–63% decrease in nuclear T_3 receptor concentration observed in tumor-bearing rats confirms our previous findings (18, 19). Although published reports indicate either increased (38) or unchanged (39) nuclear T_3 receptor concentrations in liver of hyperthyroid rats, the present data suggest that neither the decreased T_3 nuclear receptor concentration of tumor-bearing rats nor the T_3 receptor concentration of control rats was influenced by a range of plasma T_3 between 10 and 163 ng/dl. In addition, the nuclear T_3 concentration of tumor-bearing rats remained significantly decreased from control at each level of T_3 infusion. The fact that the ME and α -GPD response curves were plotted as a function of T_3 nuclear receptor saturation (Figs. 4 and 5) does not imply a specific biological determinant of receptor saturation. If the α -GPD response to T_3 is plotted as a function of nuclear T_3 , the difference between tumor-bearing and control rats becomes even more marked than illustrated in Fig. 2. In contrast, a similar plot for the ME response to T_3 shows little difference between the groups except at the dosage of T_3 calculated to occupy >95% of T_3 nuclear receptor sites. At this maximal level of nuclear T_3 , the ME response in tumor-bearing rats was significantly smaller than in controls. The ME appearance rate (see below), however, remained significantly decreased in tumor-bearing rats even when expressed in terms of specifically bound nuclear T_3 (see below).

Since the activities of α -GPD and ME observed in these studies are a consequence of the rates of appearance and degradation for each enzyme, we measured the degradation rate and estimated the appearance rate of both enzymes by

the method described by Oppenheimer and Schwartz (35). Assumptions made for the study of enzyme decay rates have been discussed by those investigators (35). The half-life of α -GPD, 2.1 d, and ME, 2.3 d, determined in control rats, agree with values reported by Oppenheimer et al. (24). In tumor-bearing rats, the half-life of α -GPD was the same as control but the half-life of ME was prolonged to 4.5 d. Since Oppenheimer and Schwartz (35) reported a significant increase in the rate of decay of both ME and α -GPD in starved rats, these findings support further the conclusion that nutritional deprivation either of the entire animal or, specifically, of the liver, was not a significant factor in the current studies. Calculations made according to Oppenheimer and Schwartz (35) suggest that the appearance rate of ME was markedly decreased in tumor-bearing rats. Since the decreased ME appearance rate in tumor-bearing rats could be due to the decrease in specifically bound nuclear T_3 , we also estimated the specific appearance rate of ME (appearance rate divided by specifically bound nuclear T_3) according to equation V of Oppenheimer and Schwartz (35). These calculations suggest that the specific appearance rate of ME in tumor-bearing rats is only 10% that of control rats both at nuclear T_3 levels present in the basal state and when nuclear T_3 receptors are maximally saturated. Thus, similar to conclusions from studies of starved rats (35), as yet undefined postreceptor factors appear to inhibit the ME response to T_3 in tumor-bearing rats. In contrast, calculations suggest a twofold increase in the appearance rate of α -GPD in tumor-bearing rats in the basal state. The α -GPD appearance rate is further augmented to fourfold when calculated on the basis of specifically bound nuclear T_3 . These calculations suggest that, in the tumor-bearing rat, the major portion of the increase in α -GPD appearance rate results from postreceptor amplification of the hormone signal in association with a significant decrease in plasma T_3 , nuclear T_3 , and T_3 nuclear receptor concentrations.

The complex nature of the biological response to T_3 in tumor-bearing rats highlights the difficult problem of assaying the thyroidal status of animals with nonthyroidal disease. The results of the present studies clearly indicate that as yet undefined postreceptor factors may modulate the biological response to T_3 in different directions: the α -GPD response was enhanced but the ME response was depressed. The regulation of TSH in tumor-bearing rats appears to be another example of an enhanced response to thyroid hormone in nonthyroidal disease. We have demonstrated normal regulation of TSH secretion in tumor-bearing rats which have decreased concentrations of pituitary nuclear T_3 (21). Our finding of postreceptor regulation of thyroidal response in Walker 256 carcinoma-bearing rats is similar to that reported in starved rats (35) and in rats fed a high carbohydrate diet (40, 41). In the latter, both ME activity (40, 41) and ME messenger RNA activity (41) were increased in comparison with levels in control animals. Thus, dietary factors may under some conditions contribute to the cellular regulation of thyroidal response. Other factors

may include alterations in the concentration of hormones different from those of the thyroid system. Thus, glucagon concentration is increased in starvation (42), and this results in a decrease in concentration of nuclear T_3 receptor sites as well as in the activity of ME (43). Other examples of multi-hormonal regulation of cellular responses include the regulation of α_{2U} globulin messenger RNA in rats by testosterone and glucocorticoids, in addition to thyroid hormones (44, 45) and the synergistic regulation of growth hormone production by glucocorticoid and thyroid hormones in cultured growth hormone-producing pituitary tumor cell lines (46, 47). The possibility exists that products from the tumor itself may have influenced cellular metabolism in the present studies.

In view of the data from the present studies, the finding of a euthyroid clinical state in association with decreased serum T_3 in patients with nonthyroidal disease should not be surprising. In the rat with nonthyroidal disease, we have shown that the α -GPD and ME response to T_3 cannot be predicted a priori from the plasma T_3 or even the nuclear T_3 concentration. Thus, in patients with nonthyroidal disease, enhancement of those responses to T_3 important, for the clinical manifestations of the euthyroid state may account for the normal clinical findings. Since the response of individual biological parameters may be either enhanced or depressed by cellular factors that affect postreceptor mechanisms, probably no single measurement will provide guidance as to the thyroidal state of the entire animal. Indeed, even within individual tissues, postreceptor factors may result in divergent regulation of different T_3 -sensitive biological responses.

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