JCI The Journal of Clinical Investigation

Thyroid Hormone-Carbohydrate Interaction in the Rat: *CORRELATION BETWEEN AGE-RELATED REDUCTIONS IN THE INDUCIBILITY OF HEPATIC MALIC ENZYME BY TRIIODO-l-THYRONINE AND A HIGH CARBOHYDRATE, FAT-FREE DIET*

Mary Ann Forciea, … , F. E. Kaiser, J. H. Oppenheimer

J Clin Invest. 1981;67(6):1739-1747. https://doi.org/10.1172/JCI110212.

Previous studies from this laboratory have demonstrated an age-related decrease in hepatic malic enzyme (ME) levels and in the response of ME to triiodo-l-thyronine (T_3). Moreover, we have recently shown a synergistic interaction of T_3 and a high carbohydrate diet in the induction of this enzyme. Studies were therefore undertaken to assess the response of aging rats to a high carbohydrate diet and to test the effect of such dietary manipulations on the responsiveness of ME to T_3. For this purpose, a new radio-immunoassay for ME was developed that, because of a 10-fold higher sensitivity, was particularly suited to the measurement of the low concentrations of hepatic enzyme in older animals. The level of ME per milligram of DNA fell ~70% between 1 and 6 mo with only minor further changes demonstrated between 6 and 18 mo. In contrast, the level of ME per milligram DNA in brain was slightly increased in the older animals. Although the absolute increment of hepatic ME resulting from seven daily injections of T_3 (15 μg/100 g body wt) fell with age, the ratio of the ME content per milligram DNA to that observed in control animals maintained on a regular chow diet remained relatively constant with an average value of 11.1. The responsivity of hepatic ME to a high carbohydrate, fat-free diet also decreased […]

Thyroid Hormone-Carbohydrate Interaction in the Rat

CORRELATION BETWEEN AGE-RELATED REDUCTIONS IN THE INDUCIBILITY OF HEPATIC MALIC ENZYME BY TRIIODO-L-THYRONINE AND A HIGH CARBOHYDRATE, FAT-FREE DIET

MARY ANN FORCIEA, HAROLD L. SCHWARTZ, HOWARD C. TOWLE, CARY N. MARIASH, F. E. KAISER, and J. H. OPPENHEIMER, *Division of Endocrinology and Metabolism, Department of Medicine, Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455*

ABSTRACT Previous studies from this laboratory have demonstrated an age-related decrease in hepatic malic enzyme (ME) levels and in the response of ME to triiodo-L-thyronine (T_3). Moreover, we have recently shown a synergistic interaction of T_3 and a high carbohydrate diet in the induction of this enzyme. Studies were therefore undertaken to assess the response of aging rats to a high carbohydrate diet and to test the effect of such dietary manipulations on the responsiveness of ME to T_3. For this purpose, a new radioimmunoassay for ME was developed that, because of a 10-fold higher sensitivity, was particularly suited to the measurement of the low concentrations of hepatic enzyme in older animals. The level of ME per milligram of DNA fell ~70% between 1 and 6 mo with only minor further changes demonstrated between 6 and 18 mo. In contrast, the level of ME per milligram DNA in brain was slightly increased in the older animals. Although the absolute increment of hepatic ME resulting from seven daily injections of T_3 (15 μg/100 g body wt) fell with age, the ratio of the ME content per milligram DNA to that observed in control animals maintained on a regular chow diet remained relatively constant with an average value of 11.1. The responsivity of hepatic ME to a high carbohydrate, fat-free diet also decreased with age and could not be attributed exclusively to a reduction in food consumption. The age-related reduction in ME responsivity to dietary stimuli appeared to be due to a reduction in the formation of the specific messenger, (m)RNA for ME as determined in an in vitro translational assay. Our data are consistent with the following hypothesis. There is an age-related decreased hepatic responsivity to a high carbohydrate dietary stimulus. Thyroid hormone administration, as previously postulated by us, interacts with a product or an intermediate of carbohydrate metabolism in a multiplicative fashion. As a consequence, the absolute increment of ME induced by T_3 administration also declines with age.

INTRODUCTION

In a previous study we have shown that in rat liver there appears to be a progressive age-related decrease in the level of two triiodo-L-thyronine (T_3)[1] responsive enzymes, cytosolic malic enzyme (ME), and mitochondrial α-glycerophosphate dehydrogenase, as well as a decrease in the response of the enzymes to a single injection of triiodothyronine (T_3) (1). Since differences in basal enzyme levels and their response to T_3 could not be attributed to alterations in nuclear T_3 occupancy, we concluded that these age-related changes reflected postreceptor modifications of the T_3 nuclear signal. In more recent studies from our laboratory we have also shown what appears to be a synergistic interaction between thyroid hormone and a factor associated with a high carbohydrate, fat-free diet in the induction of ME and other lipogenic enzymes (2). The concept was advanced that the stimulus for the generation of the specific messenger (m)RNA coding for ME represented the product of the signal derived from the T_3 nuclear interaction and that derived from the carbohydrate-induced stimulus. As a corollary of this formulation, it appeared at least theoretically possible that the diminished response of ME to T_3 in the aging rat was related to a primary reduction in the carbohydrate-

Address reprint requests to Dr. J. H. Oppenheimer.
Received for publication 7 November 1980 and in revised form 30 January 1981.

[1] *Abbreviations used in this paper*: ME, malic enzyme; RIA, radioimmunoassay; T_3, triiodothyronine.

generated signal. Accordingly, we undertook a series of experiments to assess the response of the aging animals to a high carbohydrate diet and the influence of concomitant T_3 administration on such dietary manipulation. We specifically examined the relationship between daily food intake and the intrinsic hepatic responsivity to the induction stimulus. In order to increase the sensitivity of our measurements in the aging rat and to be certain that we were determining enzyme mass rather than activity we developed a new radioimmunoassay for this enzyme. Our findings further emphasize the important influence of carbohydrate metabolism on the expression of thyroid hormone effects at a postreceptor level. The results document an age-related diminution in hepatic lipogenic enzyme response to dietary carbohydrate administration, which may be central to the decreased responsivity to T_3.

METHODS

Male Sprague-Dawley rats from 1 to 18 mo of age were purchased from Taconic Farms, Germantown, N. Y. Control rats were maintained on Purina Lab Chow (Ralston Purina Co., St. Louis, Mo.). Groups of rats were treated with daily intraperitoneal injections of 15 μg of T_3 (Sigma Chemical Co., St. Louis, Mo.) per 100 g body wt for 7 d. Dietary induction of ME was carried out by feeding rats a high carbohydrate diet (fat free test diet, ICN Nutritional Biochemicals, Cleveland, Ohio) for 4 d. This diet differs from the normal lab chow mainly in the absence of fat and in the presence of all carbohydrate in the form of a simple sugar, sucrose (60% by weight) (3).

The methods utilized for preparation of purified rat hepatic ME and rabbit anti-ME anti-serum have been previously described (4). Rat hepatic and brain cytosol was prepared by centrifugation of 10% tissue homogenate in 0.32 M sucrose − 3 mM $MgCl_2$ at 158,000 g for 60 min in a Beckman TY65 rotor (Beckman Instruments, Inc., Fullerton, Calif.). Purified ME was iodinated by the method of Bolton and Hunter (5). Standard curves, ranging from 5 to 250 ng were routinely included in all assays. All samples were diluted in phosphosaline buffer (0.01 M sodium phosphate − 0.15 M sodium chloride − 0.1% sodium azide) containing 1% bovine serum albumin. Sample volume used for standards or cytosol, was 400 μl to which were added 100 μl of the anti-ME antiserum (diluted 1/5,000 in phosphosaline buffer containing 3% normal rabbit serum), and 100 μl of phosphosaline buffer − 1% bovine serum albumin containing ~10,000 cpm of ^{125}I-labeled ME. This mixture was incubated for 24 h at 4°C. At the end of this period 100 μl of a 1:1 dilution of goat anti-rabbit serum antiserum (Antibodies, Inc., Davis, Calif.) in phosphosaline buffer − 1% bovine serum albumin were added and the mixture incubated for a further 24 h. At that time samples were centrifuged and decanted. The resulting pellets were counted in a Packard Autogamma (Packard Instruments, Inc., Downers Grove, Ill.). Calculations were performed by use of a computer program kindly supplied by Dr. David Rodbard (National Institutes of Health) for best fit of log-dose response data (6).

ME activity was measured by the method of Hsu and Lardy (7). 1 U of activity was defined as that amount of enzyme required to reduce 1 nmol of $NADP^+$ in 1 min. Specific activity of the purified ME was 39,600 U/mg.

Quantitation of the binding capacity of the specific T_3-nuclear receptors in rat liver was carried out as previously described (8). Nuclear content of specifically bound T_3 was calculated from the product of the nuclear/plasma [^{125}I]T_3 ratio and the plasma concentration of T_3 (9). Plasma T_3 concentration was measured by the immunoassay method of Surks et al. (10). The activity levels of the mRNA for ME were quantitated using a previously described in vivo translational assay in an endonuclease-treated reticulocyte lysate system (4).

Average daily food intake was measured during a period of 4 d. Younger animals were housed four per cage. Older rats (6 to 27 mo) were grouped two per cage. Measurements were made for at least four groups at each age.

For the determination of the rate of plasma disappearance of T_3, rats at 2 mo and 2 y of age were injected intravenously with a tracer dose (5 ng/100 g body wt) of [^{125}I]T_3. Serial blood samples were taken from the cut tail. Aliquots of plasma were treated with TCA to remove accumulating [^{125}I]-iodide (11).

DNA was measured as described by Giles and Myers (12) and RNA according to Munro and Fleck (13). Protein was measured by the method of Lowry et al. (14).

RESULTS

Radioimmunoassay for ME. A representative standard displacement curve obtained by the logit-log transformation of values and a best fit computer generated curve is illustrated in Fig. 1. The intraassay coefficient of variation was 10% whereas interassay coefficient of variation averaged 15.5%. Fig. 1 also illustrates that the shape of the standard displacement curve produced by serial dilution of brain cytosol is indistinguishable from that obtained with hepatic cytosol, thus justifying the use of the antiserum in the

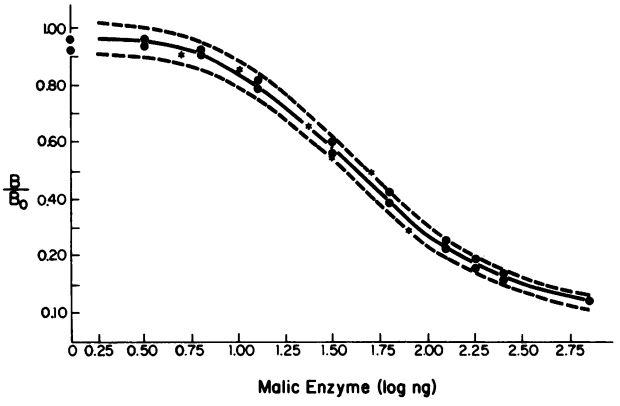

FIGURE 1 RIA of ME. ●, represent duplicate samples of increasing concentrations of purified rat hepatic ME prepared as previously described (4). A best-fit curve was computed using the logit-log linearization program of Dr. D. Rodbard (National Institutes of Health), Bethesda, Md. (7). The data are then plotted in conventional fashion. – – –, 95% confidence limits of the curve. Serial dilutions of rat brain cytosol (*) were simultaneously assayed as described in Methods. The parallelism of these points to those of the standard curve indicates identity of the immunologic determinants of ME from liver and brain: B/B_0 = ratio of total counts per minute bound at any concentration to the cpm bound at the lowest concentration used.

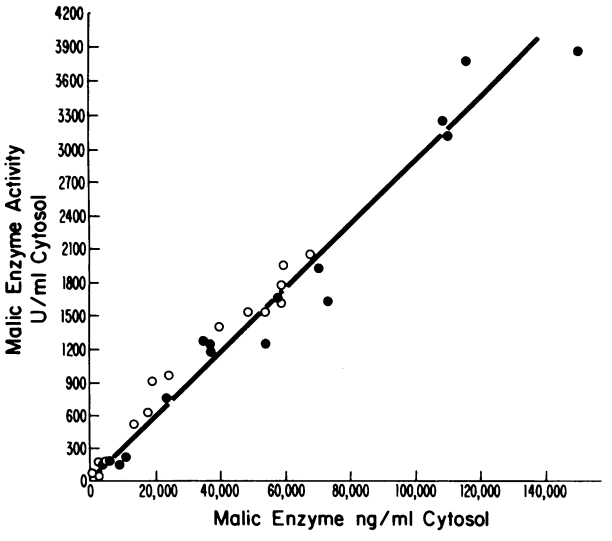

FIGURE 2 Comparison of the mass of hepatic ME measured by RIA with the ME activity measured by conventional spectrophotometric assay (8). Hepatic cytosol from animals on regular diet, high carbohydrate diet, and under treatment with T_3 was analyzed. ●, 1 mo rat; ○, 6 mo rat.

quantitation of brain ME. The correspondence between concentrations of ME mass measured by radioimmunoassay (RIA) and ME activity determinations performed by a standard spectrophotometric assay is illustrated in Fig. 2. The large range in ME concentrations was obtained by the intraperitoneal injection of T_3 (200 μg/100 g body wt) into 1- and 6-mo-old animals for a variable number of days. The correlation coefficient between the standard spectrophotometric assay and the RIA was 0.98, confirming earlier inferences that the changes in ME activity reflected changes in enzyme mass (1). We estimated from the specific activity of purified preparations that the limit of resolution of the spectrophotometric method was 235 ng/ml cytosol, whereas the limit for the RIA is only 25 ng/ml cytosol. The enhanced sensitivity was especially helpful in our studies of the ME activity in older rats since the basal concentration of this enzyme in such animals was exceedingly low.

Nuclear binding parameters as a function of age. Table I lists nuclear T_3 binding parameters as well as some general biochemical characteristics of liver in relationship to age. In confirmation of our previous report, the concentration of plasma T_3 was higher in animals at 1 mo of age (0.67±0.04 ng/ml) than in the older groups in which it ranged from 0.38±0.05 to 0.48±0.07 ng/ml. The nuclear T_3 content determined from the product of the isotopic nuclear/plasma [^{125}I]T_3 ratio and the plasma T_3 RIA, remained remarkably constant

TABLE I
Hepatic Parameters in the Euthyroid Rat as a Function of Age

	Age			
	mo			
	1.5	6	12	18
Body wt, *g*	143±29	435±71	615±46	586±44
Plasma T_3, *ng/ml*	0.67±0.04	0.38±0.05	0.48±0.07	0.44±0.09
$\frac{\text{N/P}}{\text{mg DNA}}$	0.389±0.117	0.530±0.180	0.533±0.083	0.516±0.089
Nuclear T_3, *ng/mg DNA*	0.26±0.08	0.20±0.07	0.26±0.04	0.23±0.05
Receptor B_{max}, *ng T_3/mg DNA*	0.39±0.10	0.49±0.19	0.57±0.17	0.45±0.07
$k_a \times 1$ nM	0.87±0.18	0.90±0.15	1.10±0.13	1.05±0.21
Liver wt, *g*	5.8±1.1	13.7±2.3	16.4±0.9	16.6±1.7
$\frac{\text{Liver}}{\text{Body}} \times 100$	4.0±0.3	3.2±0.2	2.7±0.2	2.8±0.4
DNA, *mg/g liver*	3.39±0.52	2.93±0.38	2.94±0.31	3.28±0.25
RNA/DNA	1.68±0.4	1.72±0.42	1.46±0.66	1.39±0.58
Cytosol protein, *mg/g liver*	85.0±7.8	86.4±10.0	91.4±3.9	91.8±4.0
n	4	8	8	8

All measurements were carried out as described in Methods. Values represent the mean and SD for the number (*n*) of animals indicated. (N/P)/mg DNA = nuclear:plasma ^{125}I-T_3 concentration ratio; B_{max}, maximal binding capacity.

TABLE II

Effect of Age, Diet, and Treatment on ME Content

		Chow diet					High carbohydrate diet				
		Weight		ME			Body wt.		ME		
Age	Status	Begin	Final	U/mg DNA	Δ ME	R	Begin	Final	U/mg DNA	Δ ME	R
mo											
1.0	Basal	74 ±8	115 ±17	669 ±183			71 ±6	108 ±7	6,232 ±1,343		
					5,615 ±1,583	9.4				2,819 ±687	1.5
	+T_3	71 ±7	107 ±8	6,284 ±1,583			70 ±6	101 ±12	9,051 ±687		
1.5	Basal	113 ±5	115 ±10	482 ±42			100 ±8	136 ±15	4,432 ±909		
					3,383 ±1,048	8.0				2,627 ±1,391	1.6
	+T_3	103 ±15	139 ±19	3,866 ±1,048			106 ±9	125 ±11	7,059 ±1,391		
6.0	Basal	439 ±54	448 ±53	215 ±41			418 ±38	425 ±40	2,427 ±496		
					2,964 ±113	14.8				1,384 ±511	1.6
	+T_3	399 ±22	361 ±21	3,179 ±113			393 ±15	349 ±10	3,811 ±511		
12.0	Basal	571 ±63	578 ±70	262 ±130			614 ±25	595 ±32	782 ±494		
					3,095 ±1,655	12.9				2,165 ±1,169	3.8
	+T_3	583 ±51	513 ±62	3,375 ±1,655			604 ±26	520 ±20	2,947 ±1,169		
18.0	Basal	608 ±67	610 ±71	251 ±140			560 ±74	546 ±76	1,053 ±491		
					2,326 ±905	10.3				1,642 ±1,010	2.6
	+T_3	597 ±44	519 ±44	2,577 ±905			627 ±27	546 ±64	2,695 ±1,009		

Age-related response of hepatic malic enzyme to T_3 and a high carbohydrate diet. T_3 (15 μg/100 g body wt) was administered by daily intraperitoneal injection for 7 d. High carbohydrate diet was fed to the rats for 4 d. When the diet was combined with T_3 it was given during the last 4 of the 7 d of hormone treatment. R = ratio of ME in T_3-treated rats to the untreated control animals. ΔME = T_3-induced increment in ME above basal levels.

over the age span examined. The binding capacity determined from whole nuclear assays also showed singularly little variation. The fractional occupancy of T_3 (=nuclear T_3 content/binding capacity) averaged ~50%. No significant age-dependent alterations in the nuclear T_3 affinity constant[2] were observed in the whole nuclear assay. Neither were there significant changes in the DNA per gram liver, the total cellular RNA/DNA ratios, or the cytoplasmic protein content per gram liver. Liver weight as a percentage of total body weight fell significantly from 1.5 to 6 mo (4.0 − 2.8%, $P < 0.001$). Total body weight continued to increase markedly over the first year of life, after which the rate of increase slowed.

ME and protein levels in liver and brain as a function of age. Fig. 3 illustrates the results of measurements of the basal cytosol ME content of liver and brain in animals of different ages. In agreement with our previous report, the major fall in hepatic ME content occurred between 1 and 6 mo with only a slight decrease thereafter. In contrast, brain cytosolic ME content exhibits a modest rise with age. No consistent alteration in cytosolic protein content per milligram DNA was observed in either tissue.

[2] Note that the association constant for whole nuclear T_3 binding cited in our previous studies (1) is approximately five times greater than the comparable value cited in this report (Table I). In large part, this is due to an inadvertent error in the calculation of our previous data. The results should have averaged ~0.5 nM. Thus, our current results are actually approximately twice those in the earlier study, a discrepancy that is probably within the interassay variation of the method. Of particular pertinence was that in neither study was there a significant age-related change and the constancy of the in vitro assay was supported by the estimated nuclear T_3 content based on in vivo isotopic measurements.

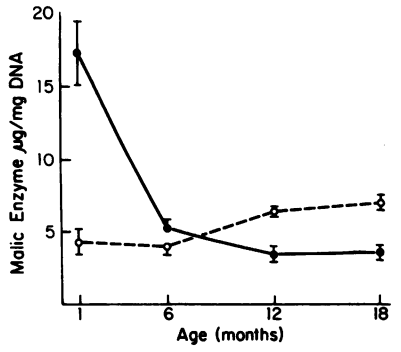

FIGURE 3 ME per milligram DNA as a function of age in liver and brain. Liver ME concentration falls, whereas brain ME concentration rises slightly. ⊕, mean±SE; ●, liver; ○, brain.

Effect of thyroid hormone and high carbohydrate diet on ME activity. As indicated in Table II, 7 d of T_3 administration (15 μg/100 g body wt) resulted in a striking increase in the level of ME in all age groups. In confirmation of our previous report, however, the absolute increment achieved as a result of T_3 administration also diminished with age, the largest decrease occurring between 1 and 6 mo. This pattern resembled the fall in basal enzyme levels. These changes could not be attributed to age-related alterations in nuclear binding, as noted above. It also appeared unlikely that these changes were due to alterations in the metabolism of T_3 as a function of age, since the nuclear sites are nearly saturated with the level of T_3 used. Nevertheless, additional experiments (Fig. 4) showed no appreciable difference in the disappearance rate of T_3 in old as compared to young rats. Of interest was the finding that the response of ME to the high carbohydrate, fat-free diet also diminished with age. As is apparent from Table II a similar age-related decrease in response was observed when animals were simultaneously subjected to both stimuli, daily T_3 administration (15 μg/100 g body wt) for 7 d and a high carbohydrate diet during the last 4 d. The level in ME achieved in 1-mo-old animals was in excess of that achieved with either T_3 or carbohydrate alone but not in excess of the sum of both. In the 12- and 18-mo-old animals the high degree of variability in the results may have prevented the demonstration of greater effectiveness of combined stimulation.

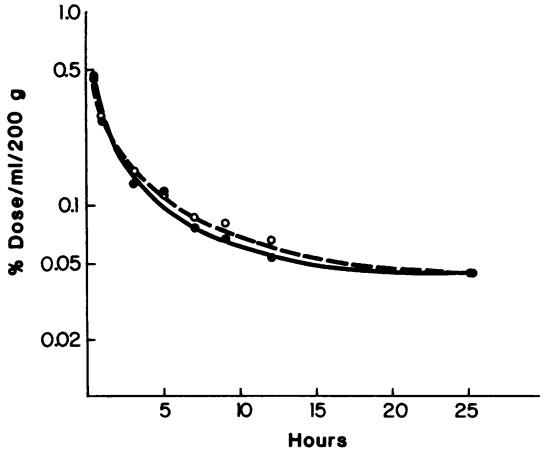

FIGURE 4 Plasma disappearance of [^{125}I]T_3. Data were corrected to an ideal weight of 200 g. Each point is the average of a group of three animals. No significant age-related differences in the disappearance curves are apparent. ●, 2 mo; ○, 2 yr.

Some insight into the mechanistic basis of these interactions can be derived from a consideration of the proportional changes in enzyme levels induced by the two stimuli rather than by the absolute increment achieved. Of interest was the finding in every age group that the administration of T_3 led to approximately the same proportional increase in ME levels, averaging 11-fold over the base line (Table II). This was similar to the degree of steady-state induction, which we had previously reported as a consequence of T_3 administration with the nuclear sites fully saturated and the animals maintained on a regular chow diet (15). We have designated this an "amplified" response. Under physiological conditions the nuclear sites are one-half occupied. Thus, the rate of response would simply double if the relationship between nuclear occupancy and response were linear. We have also shown that when animals are maintained on a high carbohydrate diet, the response to T_3 changes from a highly amplified to a nearly linear response (2). The present results confirm this finding and indicate that on the average there is only a 2.2-fold increase when animals at every age group are maintained on a high carbohydrate diet (Table II).

In agreement with recent studies from this laboratory (4), the level of mRNA for ME in 1-mo-old rats was markedly increased after 4 d of the high carbohydrate diet. In contrast, little change could be detected in 1-yr-old rats. In both age groups, base-line levels of mRNA in uninduced animals were undetectable. Thus, the diminished response in ME to carbohydrate administration in the older rats appears to be reflected at the pretranslational level. These observations are clearly limited to the liver since brain ME levels neither fall with age nor do they respond to T_3 administration (200 μg for 4 d) or a high carbohydrate diet for 4 d either at 1 or 12 mo (data not shown).

Relationship of hepatic ME level to food intake. The diminished basal level of ME in the aging rat and the decrease in responsivity to carbohydrate could be a reflection of decreased food intake, intrinsic diminution in responsivity of the liver, or both. In an attempt to resolve this issue measurements were made of the daily food intake in the 1-, 12-, and 18-mo-old animals studied in the experiments in Table II. Results of these measurements have been incorporated into Table III. It

TABLE III
Total Hepatic ME Content and Food Consumption

Status	Age	Daily food consumption		Liver	Total ME
		Per 100 g	Total		
				g	*Units/liver × 10^{-3}*
Euthyroid chow	1.0	17.2	15.6	4.9±0.8	11.8±3.5
	1.5	12.9	21.8	6.6±0.5	11.5±1.6
	6.0	9.5	30.8	13.8±1.9	10.2±2.5
	12.0	5.2	30.1	16.9±2.5	10.6±2.9
	18.0	4.7	28.1	17.1±3.7	12.0±4.3
Hyperthyroid chow	1.0	13.8	12.3	4.3±0.6	118.9±31.3
	1.5	—	—	4.9±0.5	82.9±29.2
	6.0	—	—	9.8±0.6	145.0±43.0
	12.0	3.9	23.1	13.5±4.1	142.0±16.9
	18.0	3.8	21.6	12.2±2.0	120.9±26.3
Euthyroid Hi CHO	1.0	16.2	13.1	5.8±0.9	117.7±31.7
	1.5	—	—	6.7±1.0	96.7±31.1
	6.0	—	—	14.8±2.0	101.4±27.9
	12.0	4.5	25.1	15.6±2.5	35.9±27.0
	18.0	2.5	14.5	14.2±2.3	45.6±21.7
Hyperthyroid Hi CHO	1.0	17.8	15.4	4.2±0.5	180.6±32.1
	1.5	—	—	5.1±0.7	171.3±46.9
	6.0	—	—	9.3±0.5	175.4±16.8
	12.0	2.5	14.5	12.3±0.9	159.3±45.7
	18.0	2.5	16.0	12.1±1.2	143.6±51.8

Food consumption and total hepatic ME content in animals used in the experiment summarized in Table II. Hi CHO, high carbohydrate diet.

is apparent that there was a major decrease in food consumption per 100 g body wt in the older animals. The weight of food consumed by 12- and 18-mo old animals was approximately one-quarter of that consumed by 1-mo-old animals. Curiously, the daily food intake per 100 g body wt did not increase in the hyperthyroid compared to the euthyroid groups.

Another unanticipated finding illustrated in Table III was that despite the age-related fall in ME per milligram DNA the total hepatic content of ME was remarkably constant. It therefore appeared possible to relate at least in part, the diminished hepatic ME pool per 100 g body wt to a diminished food intake. Nevertheless, the calculations summarized in Table IV show that the livers of the older animals also exhibit a diminished accumulation of total ME per gram of food consumed. These findings suggest that both diminished food intake per 100 g body wt and a decrease in hepatocellular responsivity are responsible for the decline in ME levels per milligram DNA.

DISCUSSION

The present findings confirm previous results from our laboratory that show that the basal level of hepatic ME and the response of this enzyme to T_3 administration decline with age. In our previous report (1) the oldest animal studied was 6 mo of age, whereas the range in the present series of studies was extended to 12 and 18 mo. Nevertheless, our current study showed that 12- and 18-mo-old animals exhibit only a modest further decline in basal enzyme level and response to T_3 administration. Also in confirmation of

TABLE IV
Calculated Hepatic Responsivity to Dietary Induction

Age	Regular chow		Hi CHO diet	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
mo				
1	757 (100)	774 (100)	8,080 (100)	5,267 (100)
1.5	529 (50)			
6	330 (44)			
12	353 (47)		1,005 (12)	
18	427 (56)	301 (39)	2,313 (29)	1,235 (23)
27		310 (40)		835 (10)

Responsivity is defined for regular diet as total hepatic enzyme mass per gram of chow consumed; for high carbohydrate (CHO) diet, as the increase in ME hepatic content per gram CHO diet consumed. Numbers in parentheses represent percentage of values at 1 mo. Primary data for experiment 1 in Table II; primary data for experiment 2 are not shown. Hi CHO, high carbohydrate; fat-free diet.

our previous results was a failure to detect significant changes in nuclear T_3 content as determined from the product of the isotopic nuclear:plasma [^{125}I]T_3 ratio and the concentration of circulating plasma T_3. Moreover, no significant age-related changes were observed in the nuclear binding capacity as measured by in vitro saturation techniques, nor were there significant changes in T_3 metabolism with aging. As a consequence, it is apparent that age-related changes both in basal enzyme level and that induced by T_3 cannot be related to alterations in receptor content or affinity or to the metabolism of T_3, thus justifying our previous conclusion that thyroid hormone expression in the aging rat is a reflection of postreceptor modifications. Our use of a new RIA procedure has also permitted us to quantitate relatively low levels of ME and has allowed us to confirm our previous conclusions that age-related differences in ME activity are due to alterations in enzyme mass and not to changes in enzyme activity.

Perhaps the most important results from the present series of studies was the striking correlation between the age-related decline in basal ME levels and the diminution in ME response both to T_3 and to carbohydrate administration. Previous studies from a number of laboratories have indicated that ME, together with other lipogenic enzymes, is induced by a high carbohydrate low-fat diet (16, 17). More recent experiments from our laboratory have established that there is a synergistic relationship between T_3 and carbohydrates in the induction of ME and other lipogenic enzymes (2). We postulated a multiplicative process between the signal created by the T_3 nuclear interaction and that arising from the high carbohydrate diet. As a consequence, thyroidectomy results in a sharp curtailment in the generation of ME by carbohydrate. Conversely, in starvation the response of ME to T_3 is markedly inhibited (18). The structural basis for this interaction is unknown and the nature of the signal that is elicted by the high carbohydrate diet has not been defined. Since fructose can interact with T_3 in animals rendered diabetic with streptozotocin to induce ME, the carbohydrate-derived signal does not appear to be insulin itself (19). Insulin, however, may play a permissive role in allowing normal intracellular metabolism of glucose, a process that is bypassed by fructose in the diabetic liver. Recent studies with isolated hepatocytes by Mariash et al. in our laboratory (20) have shown that glucose by itself is effective in the induction of ME enzyme in primary hepatocyte cultures. Thus, the carbohydrate-generated signal is either a glycolytic intermediate or a product of intracellular glucose metabolism. Other studies from our laboratory have shown that the induction of ME both by T_3 and carbohydrate can be attributed exclusively to increased formation of the specific mRNA coding for ME (4). Therefore, the high carbohydrate and T_3 nuclear signals appear to interact at a nuclear level.

In the context of these considerations we were interested to find an age-related decrease in hepatic response to a high carbohydrate diet that appeared to parallel both the decrease in the basal enzyme contents per milligram DNA and the response to T_3 administration. All three parameters exhibited the largest percentage fall between 1 and 6 mo. Despite the age-related decrease in the mass of ME induced by T_3, the relative or "fold-increase" in all age groups remained relatively constant (Table II). This is precisely what one would anticipate if as previously postulated T_3 serves to "multiply" the carbohydrate-induced signal.

In animals maintained on a high carbohydrate diet, T_3 administration resulted in a much smaller (two to three)-fold increase. This again is in agreement with our previous results, which showed a much lesser degree of amplification by T_3 maintained on a high carbohydrate diet (2). We have speculated that the high degree of amplification observed on the Chow diet is due to the stimulation of carbohydrate metabolism by T_3 leading to an augmented rate of generation of the putative carbohydrate-related factor. We should now like to propose that the combined administration of T_3 and a high carbohydrate, fat-free diet results in the generation of a large abundance of the proposed intermediate or product directly responsible for ME induction, far in excess of what is required to drive the induction process at maximal rates. Our working hypothesis, illustrated in Fig. 5, would serve to explain the finding (Table III) that the combined administration of T_3 and diet increases the total hepatic ME mass to nearly the same level in all age groups studied. Under any circumstance, the current data strongly support our previous proposal that T_3 acts as a multiplier of the carbohydrate dietary factor.

The concept that the hepatic ME response to T_3 is linked to carbohydrate metabolism is strengthened by the observation that the level of brain ME enzyme does not fall with age and fails to respond either to T_3 or to carbohydrate administration. Although our data do not exclude the possibility that ME in brain and liver are distinctive isoenzymes, the hepatic and brain enzymes were not distinguishable immunologically with the antiserum used in the present studies.

The mechanism leading to the demonstrated age-related reduction in ME content per milligram hepatic DNA appears to have two components, a reduction in daily food intake per 100 g body wt and per total liver mass, and a decrease in hepatocellular responsivity to the administration of carbohydrate. The age-related decrease in hepatic responsivity to carbohydrate administration is apparent from the ratio of the induced hepatic ME mass and the daily weight of food consumed. The reduced hepatic responsivity may well be due to an impaired intracellular metabolism of glucose.

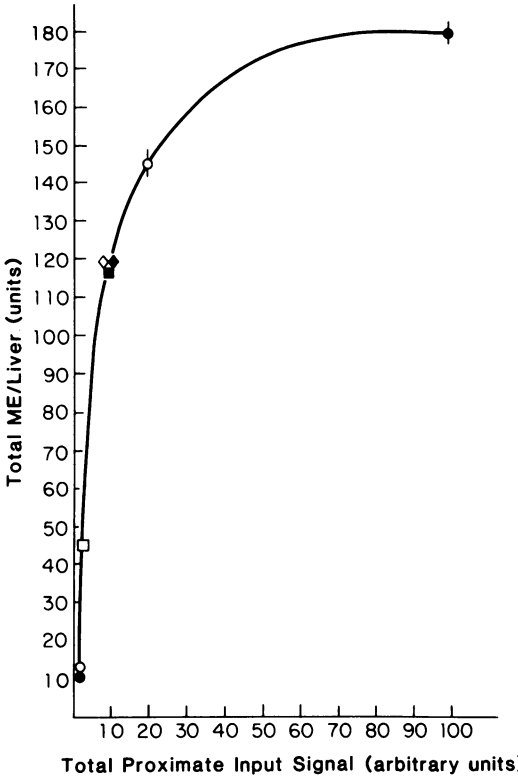

FIGURE 5 Hypothetical model to explain age-related changes in ME response to a high carbohydrate diet and to T_3 administration. We assume that the ME response is governed by an input signal determined both by the amount of carbohydrate delivered to the liver and the thyroidal status of the animal. The input signal is expressed in arbitrary units, and the ME response system is postulated to be saturable. The postulated relationship between steady-state response and the input is represented by the function indicated. The input signal for liver ME on a regular diet for young (●) and old animals (○) is assigned arbitrarily a value of 1, since the total ME per liver in 1 mo and 18 mo animals is approximately the same (Table III). Administration of carbohydrates to the young animals (■) results in an ~10-fold increase, yielding a postulated signal strength of 10, whereas the input response of the older animal (□) is postulated to lead to a signal strength of only 2. (Responsivity of old animals to carbohydrate is ~20%, Table IV.) T_3 is postulated to cause a 10-fold increase in signal strength (Table II) yielding a value of 10 for young (◆) and old (◇) animals. Administration of T_3 plus the high carbohydrate diet to old animals (ϕ) yields an input value of 20 (=2 × 10) and combined stimuli in young animals (◆) a value of 100 (=10 × 10).

Further studies are required to establish whether there is any relationship between this phenomenon and the documented impairment of glucose tolerance in the aging rat (21). The impaired response in the level of specific mRNA sequences coding for ME in the carbohydrate-stimulated aging rat indicates that the basis of the defect resides at the pretranslational level.

Lastly, previous experiments have also shown that the level of the mitochondrial enzyme α-glycerophosphate dehydrogenase falls with age as does its response to T_3 (1). Unfortunately, little is known about the biological function of this enzyme. Nevertheless, by analogy with the present results it is at least theoretically conceivable that factors as yet unidentified are primarily responsible for inducing α-glycerophosphate dehydrogenase and that such inducers decline with age. One could thus hypothesize that T_3 serves to multiply such a signal. Several examples of a coordinate interrelationship between T_3 and other hormones have recently drawn considerable attention. Thus, the induction of the exportable hepatic protein α_{2u}-globulin in the rat requires not only T_3 but dihydrotestosterone and cortisol (22). There also appears to be a synergistic interaction between cortisol and T_3 in the induction of mRNA for growth hormone in rat pituitary tumor cells (23, 24). These examples prompted the speculation that T_3 may function in general by amplifying primary signals involved in genetic regulation. Recent studies have shown that receptors for T_3 are bound to a 5.8S linker fragment in a portion of chromatin that is highly accessible to nuclease digestion and thus presumably to endogenous polymerase II action (25). There also appear to be no gross differences in the susceptibility of chromatin from hypothyroid animals to nuclease digestion. These findings support the concept that T_3 does not primarily increase the accessibility of genes but in some undefined fashion T_3 in concert with other hormones and factors accelerates the rate of transcription or processing of specific mRNA sequences.

ACKNOWLEDGMENTS

We should like to acknowledge the expert technical support of Mr. Robert Gunville, Ms. Ana Martinez, and Ms. Carolyn McSwigan, as well as the editorial-secretarial support of Ms. Nancy Rutledge and Ms. Colleen Jahnel.

This work was supported by National Institutes of Health grants AM019812 and AM26919, Special Service Research Awards AM05880 and AG05125, Clinical Investigator Award AM00800, and Training Grant AM07203.

REFERENCES

1. Schwartz, H. L., M. A. Forciea, C. N. Mariash, and J. H. Oppenheimer. 1979. Age-related reduction in response of hepatic enzymes to 3,5,3'-triiodothyronine administration. *Endocrinology.* **105:** 41–46.
2. Mariash, C. N., F. E. Kaiser, H. L. Schwartz, H. C. Towle, and J. H. Oppenheimer. 1980. Synergism of thyroid hormone and high carbohydrate diet in the induction of lipogenic enzymes in the rat. *J. Clin. Invest.* **65:** 1126–1134.
3. Wooley, J. G., and W. H. Sebrell. 1945. Niacin (nicotinic acid) an essential growth factors for rabbits fed a purified diet. *J. Nutr.* **29:** 191–199.
4. Towle, H. C., C. N. Mariash, and J. H. Oppenheimer. 1980. Changes in the hepatic levels of messenger ribonucleic acid for malic enzyme during induction by thyroid hormone or diet. *Biochemistry.* **19:** 579–585.
5. Bolton, A. E., and W. M. Hunter. 1973. The labeling of proteins to high specific radioactivities by conjugation

to an I-containing alkylating agent. *Biochem. J.* **133:** 529–539.
6. Rodbard, D. 1974. Apparent positive cooperative effects in cyclic AMP and corticosterone production by isolated adrenal cells in response to ACTH analogues. *Endocrinology.* **94:** 1427–1437.
7. Hsu, R. Y., and H. A. Lardy. 1969. Malic enzyme. *Methods Enzymol.* **13:** 230–235.
8. Schuster, L. D., H. L. Schwartz, and J. H. Oppenheimer. 1979. Nuclear receptors for 3,5,3′-triiodothyronine in human liver and kidney. Characterization, quantitation and similarities to rat receptors. *J. Clin. Endocrinol. Metab.* **48:** 627–632.
9. Oppenheimer, J. H., H. L. Schwartz, and M. I. Surks. 1974. Tissue differences in the concentration of triiodothyronine nuclear binding sites in the rat; liver, kidney, pituitary, heart, brain, spleen and testis. *Endocrinology.* **95:** 897–903.
10. Surks, M. I., A. R. Schadlow, and J. H. Oppenheimer. 1972. A new radioimmunoassay for plasma L-triiodothyronine. Measurements in thyroid disease and in patients maintained on hormonal replacement. *J. Clin. Invest.* **51:** 3104–3113.
11. Oppenheimer, J. H., H. L. Schwartz, H. C. Shapiro, G. Bernstein, and M. I. Surks. 1970. Differences in primary cellular factors influencing the metabolism and distribution of 3,5,3′-L-triiodothyronine and L-thyroxine. *J. Clin. Invest.* **49:** 1016–1024.
12. Giles, K. W., and A. Meyers. 1965. An improved diphenylamine method for estimation of deoxyribonucleic acid. *Nature (Lond.).* **206:** 93.
13. Munro, H. N., and A. Fleck. 1966. The determination of nucleic acids. *Methods Biochem. Anal.* **14:** 113–176.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
15. Oppenheimer, J. H., P. Coulombe, H. L. Schwartz, and N. W. Gutfeld. 1978. Nonlinear (amplified) relationship between nuclear occupancy by triidothyronine and the appearance rate of hepatic α-glycerophosphate dehydrogenase and malic enzyme in the rat. *J. Clin. Invest.* **61:** 987–997.
16. Tepperman, H. M., and J. Tepperman. 1964. Patterns of dietary and hormonal induction of certain NADP-linked liver enzymes. *Am. J. Physiol.* **206:** 357–361.
17. Tarentino, A. L., D. A. Richert, and W. W. Westerfield. 1966. The concurrent induction of hepatic α-glycerophosphate dehydrogenase and malate dehydrogenase by thyroid hormone. *Biochem. Biophys. Acta.* **124:** 295–309.
18. Dillmann, W. H., H. L. Schwartz, and J. H. Oppenheimer. 1978. Selective alterations in hepatic enzyme response after reduction of nuclear triiodothyronine receptor sites by partial hepatectomy and starvation. *Biochem. Biophys. Res. Commun.* **80:** 259–266.
19. Kaiser, F. E., C. N. Mariash, H. L. Schwartz, and J. H. Oppenheimer. 1980. Inhibition of malic enzyme induction by triiodothyronine in the diabetic rat: reversal by fructose feeding. *Metab. Clin. Exp.* **29:** 767–772.
20. Mariash, C. N., C. McSwigan, H. L. Schwartz, and J. H. Oppenheimer. 1981. *Clin. Res.* **29:** 414A (Abstr.).
21. Bracho-Romero, E., and G. M. Reaven. 1977. Effect of age and weight on plasma glucose and insulin responses in the rat. *J. Am. Geriatr. Soc.* **25:** 299–302.
22. Roy, A. K., and D. J. Dowbenko. 1977. Role of growth hormone in the multi-hormonal regulation of messenger RNA for alpha$_{2U}$-globulin in the liver of hypophysectomized rats. *Biochemistry.* **16:** 3918–3922.
23. Samuels, H. H., Z. D. Horwitz, F. Stanley, J. Casanova, and L. E. Shapiro. Thyroid hormone controls glucocorticoid action in cultured GH cells. *Nature (Lond.).* **268:** 254–257.
24. Martial, J. A., J. D. Baxter, H. M. Goodman, and P. H. Seeburg. 1977. Regulation of growth hormone messenger RNA by thyroid and glucocorticoid hormones. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 1816–1820.
25. Jump, D. B., and J. H. Oppenheimer. 1980. Thyroid hormone receptor-containing fragment release from chromatin by deoxyribonuclease I and micrococcal nuclease. *Science (Wash. D. C.).* **209:** 811–813.