

Synergism of Thyroid Hormone and High Carbohydrate Diet in the Induction of Lipogenic Enzymes in the Rat

MECHANISMS AND IMPLICATIONS

CARY N. MARIASH, FRAN E. KAISER, HAROLD L. SCHWARTZ, HOWARD C. TOWLE,
and JACK H. OPPENHEIMER, *Division of Endocrinology and Metabolism,*
Department of Medicine, University of Minnesota, Minneapolis,
Minnesota 55455

ABSTRACT We have investigated the relationship between the administration of triiodothyronine (T_3) and a high carbohydrate (CHO) fat-free diet in the induction of lipogenic enzymes in two rat tissues, liver, and fat. Male thyroidectomized rats were treated with graded daily doses of T_3 and either supplemented with a high CHO diet or left on a regular diet. Enzymes studied included malic enzyme (ME), fatty acid synthetase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. In the liver, all four lipogenic enzymes showed a synergistic response between T_3 administration and high CHO feeding. In fat, ME also responded synergistically. The interaction was reflected in an increased sensitivity to T_3 . The dose of T_3 required to achieve 50% maximal response was reduced three- to seven-fold by the high CHO diet. This phenomenon could not be attributed to a dietary-induced alteration either in T_3 metabolism or in number or affinity of the T_3 -nuclear receptors. Moreover, studies of the relative rate of synthesis of ME suggested a simultaneous time of onset in the induction of ME, within 2 h after the application of either T_3 or CHO. Thus, it is unlikely that either stimulus is secondary to the other. Since parallel experiments from this laboratory (Towle, Mariash, and Oppenheimer. 1980. Changes in hepatic levels of messenger ribonucleic acid for malic enzyme during induction by thyroid hormone or diet. *Biochemistry*. 19: 579-585.) show that ME induction both by CHO and T_3 is mediated by an increase in specific messenger RNA for ME, the interaction of T_3 and the dietary factor occurs at a pretranslational level.

Dr. Mariash is a recipient of National Institutes of Health Research Fellowship 1-F32-AM05880. Address reprint requests to Dr. Mariash.

Received for publication 24 July 1979 and in revised form 16 January 1980.

INTRODUCTION

Hepatic lipogenic enzymes respond in a coordinated fashion to multiple physiological stimuli (1-3). For instance, administration of a high carbohydrate (CHO)¹ fat-free diet results in an increase in the activity of these enzymes as a result of *de novo* protein synthesis (3, 4). In three instances increased induction has been shown to correlate with the level of polysomal messenger RNA (mRNA) (5-8). An increase in activity and mass of lipogenic enzymes also follows administration of thyroid hormone (9-12). Moreover, previous studies from our laboratory have shown that the rate of lipogenic enzyme induction is related to nuclear occupancy in a nonlinear and highly amplified fashion (13-15). Thus, in the euthyroid state when approximately one half of the nuclear sites are occupied, the rate of ME induction is less than one-tenth of that which characterizes the response when the sites are fully occupied by triiodothyronine (T_3).

The ability of these two apparently unrelated stimuli to induce the formation of the same lipogenic enzymes appears intriguing and raised fundamental questions with respect to potential interrelationships of the stimulating mechanisms. Accordingly, we undertook a systematic study to analyze the effect of T_3 on the induction of lipogenic enzymes in animals maintained on a normal chow or a high CHO diet. Four lipogenic enzymes were studied: malic enzyme (ME), fatty acid synthetase (FAS), and the two hexose monophosphate shunt enzymes, glucose-6-phosphate dehydrogenase (G6PD), and 6-phosphogluconate dehydrogenase

¹Abbreviations used in this paper: CHO, carbohydrate; D_{50} , half maximally effective dose of triiodothyronine; FAS, fatty acid synthetase; G6PD, glucose-6-phosphate dehydrogenase; ME, malic enzyme; mRNA, messenger RNA; 6PGD, 6-phosphogluconate dehydrogenase; T_3 , triiodothyronine.

(6PGD). The effect of a high CHO diet on the metabolism and distribution of T_3 and specific T_3 nuclear receptor binding were assessed. Additional information was obtained from the time-course of ME induction by T_3 or high CHO diet from serial determinations of the relative rate of synthesis of this enzyme. Our results indicate a synergistic interaction between the T_3 nuclear complex and an as yet unidentified factor generated in response to the high CHO diet. Our findings, furthermore, emphasize that metabolic as well as hormonal determinants can influence the expression of thyroid hormone action at a postreceptor level.

METHODS

Sprague-Dawley male rats (150–200 g) were purchased from Taconic Farms, Germantown, N. Y., and were provided with water ad lib. except where noted. Rats were rendered hypothyroid by surgical thyroidectomies performed by the supplier and the administration of 100 μ Ci of 131 I 1 wk after the institution of a low iodine diet. Hypothyroid animals were used only after cessation of weight gain for a minimum of 2 successive wk. T_3 (Sigma Chemical Co., St. Louis, Mo.) was dissolved in a minimum of 0.1 N NaOH and made up to 2 mg/ml with distilled water. Dilutions of T_3 stock solutions were made with 2% human albumin in saline. Animals were fed either a regular Purina diet (23% protein; 55% complex CHO; 22% fat; Ralston Purina Co., St. Louis, Mo.) or a high CHO (fat-free, test diet; ICN Nutritional Biochemicals, Cleveland, Ohio; 20% casein; 60% sucrose; 20% nonabsorbable filler) which had been conventionally used in the study of lipogenic enzyme induction. Although the latter is generally referred to as a high CHO diet it is apparent that the percentage of total diet as CHO does not differ greatly in both diets. The high CHO diet was administered for 4 d because studies by others (1, 16, 17) as well as preliminary experiments in our laboratory indicated that this period was sufficient to attain equilibrium values. In those experiments in which the time of onset was determined for high CHO, 5 ml of 60% sucrose, 20% casein (wt/vol) was administered by gavage.

Tissue was prepared and ME was assayed as previously described (14, 18). G6PD and 6PGD were assayed by a modification of the technique of Bottomley et al. (19). The initial rate of reduction of NADP was followed spectrophotometrically at 30°C on a Beckman model 25 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) at 340 nm. Each 1-ml assay contained 120 μ mol Tris-HCl pH 8.0, 10 μ mol $MgCl_2$, 0.9 μ mol NADP, 0.6 μ mol 6-phosphogluconate for the measurement of 6PGD activity or 0.6 μ mol 6-phosphogluconate plus 2 μ mol of glucose-6-phosphate for the determination of combined hexose monophosphate shunt enzyme. 200–400 μ g protein of the 100,000 g supernate was used for enzyme determination. FAS was assayed by a modification of the technique of Nepokroeff et al. (20). Immediately after centrifugation, the 100,000 g supernate was diluted into an equal volume of 1 M potassium phosphate buffer, pH 7.0, 10 mM dithiothreitol. 150–300 μ g protein of this solution was used for enzyme assay. The assay contained in a final volume of 1.0 ml; 200 μ mol potassium phosphate buffer, pH 7.0; 0.33 μ mol acetyl-CoA; 0.1 μ mol malonyl-CoA; 0.1 μ mol NADPH; 1 μ mol EDTA; 1 μ mol 2-mercaptoethanol. The enzyme was preincubated for 15 min at 37°C and the initial rate of reaction was followed spectrophotometrically at 340 nm.

Enzyme activity is reported as units per milligram cytosol protein. One unit is defined as that amount of enzyme needed

to catalyze the oxidation or reduction of 1 nmol of NADP per min, using an extinction coefficient for NADPH of $E_{340}^{mM} = 6.3$. All assays were linear with respect to protein concentration in the ranges used. Protein was determined by the method of Lowry et al. with bovine serum albumin as the standard (21).

ME was purified from hyperthyroid rats fed a high CHO diet by a modification of the methods of Yeung and Carrico (22). A 100,000 g supernatant fraction was prepared from a 20% (wt/vol) liver homogenate. A 60–80% ammonium sulfate fraction was then adsorbed onto a N^6 -(6-aminoethyl)-adenosine 2',5'-diphosphate (P-L Biochemicals, Inc. Milwaukee, Wis.) affinity column. ME was eluted with 0.5 mM NADP⁺, concentrated by ultrafiltration, and chromatographed on a Bio-Gel A-0.5m column (Bio-Rad Laboratories, Richmond, Calif.). ME was stored at 4°C where it was stable for several months. Electrophoresis in sodium dodecyl sulfate-polyacrylamide gel revealed a single protein peak. Final specific activity was 40,600 U/mg.

Antisera to purified ME was prepared by subcutaneous injection of 500 μ g enzyme in complete Freund's adjuvant into New Zealand white rabbits three times at 1-wk intervals. 1 wk after the last injection, animals were bled for antisera. Double immunodiffusion revealed only one precipitation band of identity between purified ME and total rat liver homogenate.

The relative rate of hepatic ME synthesis was determined by intraperitoneal administration of 0.5 mCi [3 H]leucine (ICN Nutritional Biochemicals; 40–50 mCi/mmol) 45 min before killing. Livers were homogenized in 2–3 vol 0.32 M sucrose, 3 mM $MgCl_2$ and centrifuged at 100,000 g for 60 min. The supernate (cytosol) was brought to 10 mM Tris-HCl, pH 7.6, 1% Triton X-100. Incorporation of [3 H]leucine into total cytosolic protein was determined by precipitation with 20% (wt/vol) TCA. The precipitate was washed three times with 20% TCA and dissolved in 0.3 ml of 88% formic acid. Incorporation of [3 H]leucine into malic enzyme was determined by adding enough antisera to 1.7 ml of cytosol to precipitate all enzyme activity. Purified unlabeled malic enzyme was added to bring each sample up to 6,500 U. After 2 h of incubation at 30°C, the sample was layered over a cushion of 1 M sucrose, 10 mM Tris-HCl, pH 7.6, 1% Triton X-100 and centrifuged at 10,000 g for 10 min. Pellets were washed three times with 0.9% (wt/vol) sodium chloride and dissolved in 0.3 ml of 88% formic acid. Both the TCA and immunoprecipitates were counted in 10 ml of Aquasol (New England Nuclear, Boston, Mass.). Immunoprecipitate backgrounds were determined by reprecipitating the original sample with an equal amount of antisera and 6,500 U of unlabeled purified malic enzyme. (23). The relative rate of synthesis (RRS) was calculated from: percent RRS = (counts per minute in ME immunoprecipitate/counts per minute in TCA precipitate) \times 100.

Isolation of liver nuclei and determination of the nuclear T_3 receptor content and affinity were carried out as previously described (24). Similarly, the ratio of isotopically labeled T_3 in nuclei and plasma was determined according to methods previously described (25). The concentration of radioimmunoassayable T_3 was determined by the methods of Surks et al. (26). In preliminary experiments the concentration of T_3 in serum and livers of hypothyroid animals was determined by the procedures outlined by Surks and Oppenheimer (27).

Comparisons between groups were analyzed by the Student's *t* test with two tails. Log dose-response curves were fitted to the Rodbard equation (28) by a computer program kindly provided by Dr. David Rodbard (National Institutes of Health). This program fits dose-response data to the four parameter logistic function proposed by Dr. Rodbard and supplies confidence intervals for each of the four parameters. Square deviations were pooled where appropriate; otherwise the SEM of groups were calculated from more precise formula (29).

RESULTS

In preliminary experiments we noted that the induction of lipogenic enzymes by the high CHO diet was less marked in hypothyroid than in euthyroid animals (Fig. 1). Our initial findings prompted us to examine in detail the effect of high CHO diet over the entire range of thyroidal status, from severe hypothyroidism to extreme hyperthyroidism. Accordingly, hypothyroid animals were treated with daily intraperitoneal T_3 for 7 d. Half the animals received a high CHO diet during the last 4 d of the study whereas the remaining half were continued on a regular chow diet. The results of these studies are summarized in Figs. 2 and 3 and a compendium of the parameters derived from an analysis of the results is given in Table I. Inspection of Fig. 2 reveals that for both diets the level of enzyme rises as the dose of T_3 is increased until a plateau level is achieved. However, the maximal level of enzyme attained in the hyperthyroid state was greater in the CHO-treated animals than in animals on the regular diet. For three of the enzymes, ME, FAS, and 6PGD, the extent of excursion of the enzyme from the hypothyroid to the hyperthyroid level was similar on both diets. G6PD constitutes a striking exception since the excursion was 109 U/mg on the high CHO diet and only 22 U/mg on the regular diet. In fact, the activity of G6PD on a regular diet with maximal levels of administered T_3 was less than the level in the hypothyroid animals on the high CHO diet.

Of particular interest was that the half-maximally effective dose of T_3 (D_{50}) was markedly decreased by administration of a high CHO diet. Thus, the ratio of the

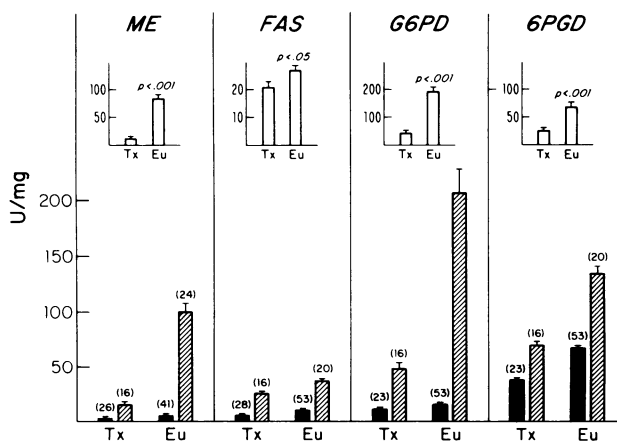


FIGURE 1 Influence of high CHO feeding on lipogenic enzyme induction in either thyroidectomized (Tx) or euthyroid (Eu) rats. The bars represent the mean \pm SEM for each group, n is in parenthesis. Animals were given the high CHO diet for 4 d before killing (hatched bar). The solid bar represents animals on a regular diet. The insert for each enzyme compares the increment induced by carbohydrate in Tx vs. Eu. The ordinate represents the enzyme specific activity.

D_{50} on the CHO diet to the D_{50} on a regular diet varied from 0.03 for FAS to 0.39 for ME. As a necessary consequence of the reduction in D_{50} by CHO feeding, the degree of amplification of the T_3 response was markedly reduced. As indicated in Fig. 2 and Table I the shift of the dose-response curve by the CHO diet leads to a virtual abolition of amplification. For ME the amplification factor on a regular diet ($f = 13.1$) is reduced to 2.5, which represents an almost linear relationship between nuclear occupancy and enzyme induction. A comparable reduction in f values characterizes the other enzymes studied.

The reduction in D_{50} by the high CHO diet can be shown to be a reflection of the cooperative interaction between diet and T_3 in the induction of lipogenic enzymes. The degree of cooperativity can be quantitated from the ratio of the instantaneous slopes of the dose-response curves to T_3 on the different diets (Appendix). Any ratio greater than one signifies a synergistic interaction. Values and ratios for the instantaneous slope at $D = 0.03 \mu\text{g}$ are given in Table I. Since all the ratios were clearly above unity, these findings emphasize the cooperative interaction between T_3 and CHO feeding in the induction of lipogenic enzymes.

The linear plot of the variables shown in Fig. 3 underscores the divergence in the initial slope on the two diets and thus, the cooperative relationship between T_3 and CHO feeding. Moreover, inspection of the plot suggests that extrapolation of both curves appears to reach a common negative intercept on the abscissa, $-125 \text{ ng } T_3/\text{d}$, $\sim 1/3$ the normal replacement value. If true, this is compatible with the hypothesis that the induction by CHO feeding in thyroidectomized animals is contingent on the presence of small residual quantities of thyroid hormone. To test this hypothesis we extracted T_3 from the liver and plasma of two thyroidectomized animals that had undergone complete arrest of growth. The material was concentrated and measured by radioimmunoassay in two dilutions. The livers contained 1.4 and 1.5 ng T_3/g and two simultaneously extracted euthyroid rat livers contained 4.9 and 3.7 ng T_3/g . The corresponding plasma T_3 in the pooled thyroidectomized plasma was 0.23 ng T_3/ml , whereas in the euthyroid animal the concentration was 0.6 ng T_3/ml . These results indicate that the thyroidectomized rats used in our studies may have as much as one-third the normal circulating tissue and plasma concentration of T_3 .

Before considering the possible mechanisms responsible for the interaction between T_3 and CHO feeding, studies were undertaken to ascertain whether the dietary induction of lipogenic enzyme activity was due to an increase in appearance rate or a decrease in fractional disappearance of the enzyme. For this purpose we measured the fractional rate of decrease in enzyme activity following the substitution of a regular diet to rats that had been on a high CHO diet the

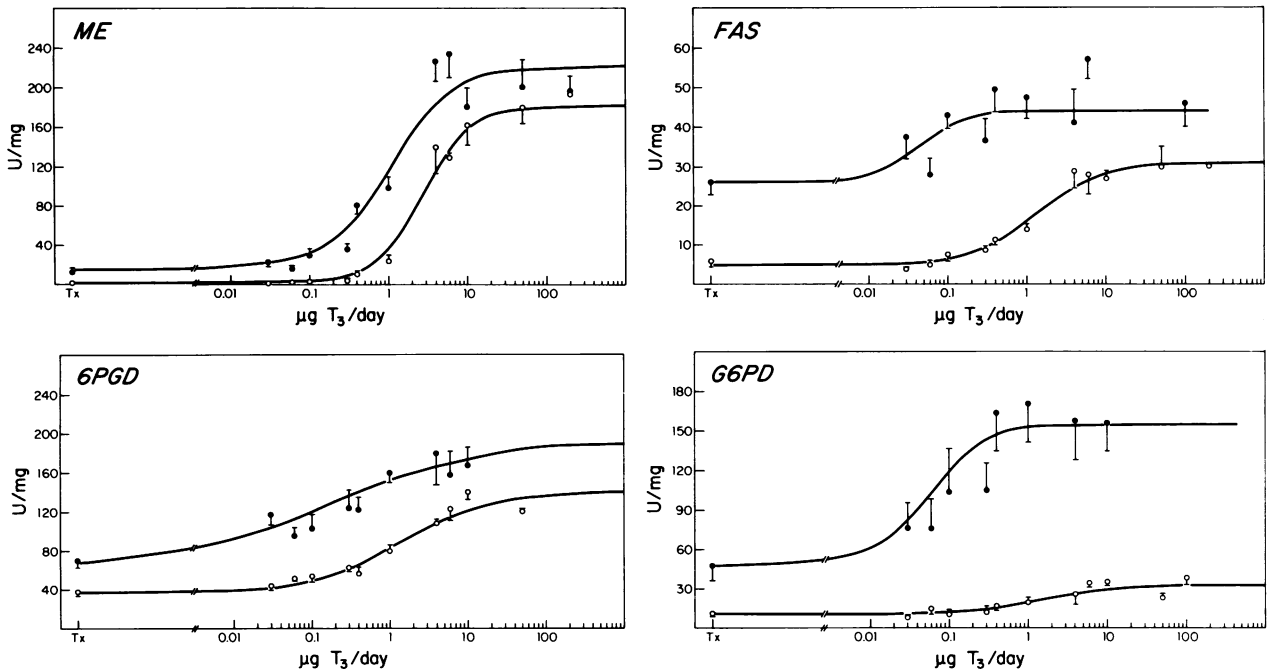


FIGURE 2 Complete dose-response relationship of lipogenic enzymes to T_3 on regular and high CHO diets. Thyroidectomized rats were given graded doses of T_3 for 7 d and either given a high carbohydrate diet (closed circles) or left on a regular diet (open circles) for the last 4 d. Each point represents the mean \pm SEM of at least four animals (except only one rat at 200 μg on a regular diet for ME and FAS). The line drawn is the computer generated best fit curve for the data.

previous 4 d. A semilogarithmic plot of the enzyme level as a function of time indicated an apparent half-life of decline of 3.8 d for ME, 3.8 d for 6PGD, 2.6 d for G6PD, and 0.9 d for FAS. These values are similar to

previously observed values after discontinuation of T_3 treatment (15). This would suggest that for each enzyme the same limiting factor, probably the half-life of the enzyme itself, determines its fractional disappearance rate after withdrawal of either stimulus.

We next examined the potential mechanisms which might account for the observed interaction between dietary and hormonal stimuli. First, we considered the possibility that the high CHO diet might potentiate the effect of thyroid hormones either by decreasing the metabolic clearance rate of T_3 or by increasing the number or affinity of the T_3 nuclear receptor sites. Measurements of the T_3 clearance rate of isotopically injected T_3 in euthyroid animals using noncompartmental methods (30) (Table II), however, showed only a 12% reduction by CHO feeding. This change is of uncertain significance and under any circumstance could not account for the observed dietary-induced events. Direct measurements of the binding capacity and affinity of isolated nuclei both in hypothyroid and euthyroid animals failed to reveal significant differences attributable to diet (Table II). Moreover, *in vivo* measurements of the nuclear/plasma ratio of T_3 showed no substantial differences. Thus, there is no evidence favoring an increase in overall strength of nuclear binding as a consequence of CHO feeding.

Next, we considered the possibility that the induction

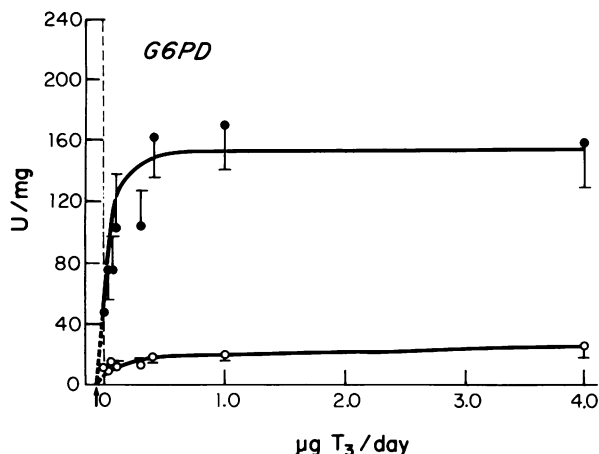


FIGURE 3 Low dose-response relationships of G6PD to T_3 administration on regular and high CHO diets. Data from Fig. 2 are plotted on linear coordinates up to a dose of 4 μg T_3 /d/100 g body wt. Symbols and curves are as in Fig. 2. Extrapolation to the abscissa (arrow) intercepts at a common value of ~ 125 ng T_3 /d. Similar steep initial curves characterize plots of the other enzymes.

TABLE I
Compendium of Dose-Response Parameters

Enzyme	Diet	E_{max}	f	b	D_{50}	Slope at $D = 0.03 \mu g^*$
					μg	
ME	Regular	180.4	13.1	1.47	2.8	11.2
	High CHO	209.3	2.5	1.07	1.1	151.4
	High CHO/Regular					13.5
FAS	Regular	26.5	6.0	1.02	1.4	17.2
	High CHO	17.8	1.7	1.51	0.043	173.9
	High CHO/Regular					10.1
G6PD	Regular	22.0	4.2	0.85	1.4	22.2
	High CHO	108.7	0.7	1.19	0.057	881.1
	High CHO/Regular					39.7
6PGD	Regular	102.1	3.5	0.77	1.44	122.1
	High CHO	122.6	1.8	0.47	0.18	464.3
	High CHO/Regular					3.8

Abbreviations used in this table: b, Hill coefficient (see Appendix); E_{max} , maximum response above a hypothyroid baseline; f, amplification factor (see Text) = [Hyperthyroid - Tx/Euthyroid - Tx].

* Calculated from Eq. 2 (Appendix).

of lipogenic enzymes was mediated exclusively by the high CHO dietary factor and that the effects of T_3 could be explained simply by an enhanced generation of this factor. Since the maximal effects of thyroid hormone-mediated events are characterized by a delay of many hours after the injection of hormone, one might then anticipate that the lag time for ME induction after the administration of a maximal intravenous dose of T_3

would be substantially longer than the lag time after a pulse gavage of the high CHO diet. To evaluate the time of onset of ME induction by T_3 or CHO feeding the relative rates of synthesis of ME were determined 45 min after the administration of [3H]leucine. The results of these studies are illustrated in Fig. 4 and clearly show that the increased rate of synthesis of ME after both stimuli begins at ~ 2 h after administration

TABLE II
Effect of Diet on T_3 Metabolism and T_3 Nuclear Binding

	High CHO diet	Regular diet
MCR	14.7 ml/h/100 g	16.8 ml/h/100 g
Half-life	6.2 h	7.6 h
$\left[\frac{\text{Nuclear}}{\text{Plasma}} \right]^*$	0.387 \pm 0.009	0.361 \pm 0.023
Maximum nuclear T_3 binding Eu	0.32 \pm 0.03 ng T_3 /mg DNA	0.31 \pm 0.02 ng T_3 /mg DNA
Equilibrium association constant Eu	6.2 \pm 0.1 $\times 10^8$ M $^{-1}$	7.7 \pm 0.5 $\times 10^8$ M $^{-1}$
Maximum nuclear T_3 binding Tx	0.56 \pm 0.02 ng T_3 /mg DNA	0.61 \pm 0.02 ng T_3 /mg DNA
Equilibrium association constant Tx	1.4 \pm 0.1 $\times 10^9$ M $^{-1}$	1.2 \pm 0.1 $\times 10^9$ M $^{-1}$

Abbreviations used in this table: Eu, euthyroid rats, $n = 4$; MCR, metabolic clearance rate of T_3 determined from average plasma values in six rats on each diet; Reg, regular diet; Tx, thyroidectomized rats, $n = 5$.

* Nuclear/plasma ratios of T_3 30 min after intravenous injection. $n = 4$ for each diet at both T_3 tracer and T_3 load injections. Values are mean \pm SEM.

DISCUSSION

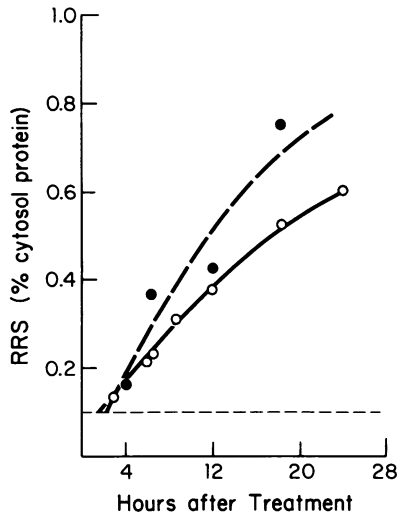


FIGURE 4 Relative rate of synthesis (RRS) of ME after either T_3 or high CHO administration. Rats were given T_3 ($200 \mu\text{g}/100 \text{g body wt i.v.}$) or gavaged with high CHO diet at $t = 0$ and given $0.5 \text{ mCi } [^3\text{H}]\text{leu i.p.}$ 45 min before killing. The counts per minute in immunoprecipitable ME are compared to the cpm in TCA-precipitable cytosol as a function of time after the stimulus. Open circles are T_3 -induced rats, closed circles are CHO induced rats. Each point is the mean of two separate rats. The range at each point is no more than $\pm 15\%$. The horizontal dashed line represents the RRS of untreated base-line rats ($\text{RRS} = 0.10\% \pm 0.006$, $n = 6$).

of both stimuli. These results, in conjunction with the lack of effect of high CHO diet on T_3 metabolism and receptor binding provide no support for the hypothesis that either the CHO diet or T_3 is in any sense "primary" with respect to the other. Additional studies indicated that 24 h after the institution of the high CHO diet the relative rate of synthesis was shown to be eightfold increased above baseline values, proportional to the increase in the steady-state ME activity. The augmented synthesis rate could thus completely account for the increase in ME levels.

To determine whether or not synergism also characterizes the interaction of CHO and T_3 in other tissues, studies were carried out with epididymal fat. The level of lipogenic enzymes was determined in hypothyroid, euthyroid and hyperthyroid animals (Table III). Of the four lipogenic enzymes studied only the response of ME was synergistic to T_3 and CHO feeding. Thus, the increment in ME activity produced by CHO feeding in hypothyroid animals was only 10 U/mg, whereas in euthyroid animals the increase was 140 U/mg. Failure to demonstrate a cooperative interaction with the other lipogenic enzymes may have been due to the large experimental error encountered. A limited number of studies were performed with heart, kidney, and brain. No consistent evidence of induction either by CHO feeding or T_3 administration was noted.

A number of recent reports have suggested the potential importance of the interaction of T_3 with other hormones in producing specific biologic effects. Thus, two groups have studied the interrelationship of T_3 , growth hormone and steroids in the induction of $\alpha_2\text{-u-globulin}$, an hepatic protein of unknown function found in male rats (31, 32). This interaction appears to occur predominately at a pretranslational level. Similarly, T_3 and cortisol interact synergistically in the induction of the specific mRNA for growth hormone in GH₁ cells, a cell line derived from a rat pituitary tumor (33). Results of the present series of experiments emphasize the physiologic relevance of the interaction of T_3 with other stimuli. Thus, we have shown the induction of lipogenic enzymes by the high CHO diet is closely related to the thyroidal status of the animal and conversely, the effect of T_3 in stimulating lipogenic enzymes is strongly influenced by dietary factors.

The degree of interaction of T_3 with the high CHO diet varied with each of the lipogenic enzymes examined. The strongest degree of interaction occurred with G6PD. As illustrated in Fig. 2 for G6PD, a high CHO diet increases the sensitivity of T_3 induction 40-fold over that on a regular diet. The minimal T_3 induction on normal chow raises the interesting possibility that other thyroid-dependent activities may not be apparent until the appropriate physiological or hormonal stimuli are applied.

Our findings also touch on the fundamental question as to whether thyroid hormone constitutes an absolute or only a relative requirement for the induction of lipogenic enzymes. Inspection of Fig. 3 raises the possibility that the carbohydrate induction observed in hypothyroid animals may be attributed to residual tissue T_3 . Measurement of the T_3 concentration in liver and plasma in animals subjected to combined thyroidectomy and ^{131}I treatment showed that the pool size of T_3 was approximately one-third that observed in normal animals. Further studies are required to determine whether the residual T_3 in these animals is due to incomplete thyroidectomy, extrathyroidal T_3 formation or an unsuspected dietary source of hormone. Regardless of the eventual resolution of this problem, the steep initial slope and early plateau noted in Fig. 3 points to the potential importance of "permissive" effects of T_3 , which can easily be overlooked if animals are not made sufficiently hypothyroid.

Our studies have also clarified several aspects governing the interaction of T_3 and the high CHO diet. Our findings effectively exclude the possibilities that CHO feeding either decreases the metabolic clearance rate of T_3 or enhances T_3 -nuclear binding (Table II). Furthermore, we have shown that T_3 does not act exclusively by altering carbohydrate metabolism since the onset of

TABLE III
Lipogenic Enzymes in Epididymal Fat*

	ME	FAS	C6PD	6PGD
Tx	10.2±7.0 (8)	7.4±1.6	52.6±4.8	19.7±4.7
Tx + CHO	20.3±4.4 (8)	59.9±5.5	87.6±13.0	53.8±7.5
ΔTx†	10.1±8.5	52.5±5.7	35.0±13.9	34.1±8.9
Eu	51.8±19.0 (12)	24.2±6.7	90.5±7.2	51.5±7.2
Eu + CHO	192.4±41.2 (8)	74.7±10.7	103.5±10.4	74.8±17.4
ΔEu	140.6±45.4	50.5±12.6	13.0±12.6	23.3±18.8
Hyper	87.4±26.7	35.8±11.2	79.5±8.3	54.6±6.8
Hyper + CHO	334±47.5	86.2±6.7	140.2±30.2	99.7±10.5
ΔHyper	246.6±54.5	50.4±13.1	60.7±31.3	45.1±12.5

Abbreviations used in this table: +CHO, high CHO diet given 4 d before killing; Eu, euthyroid; hyper, 15 μg T₃/100 g BW intraperitoneal for 7 d; Tx, thyroidectomized.

* Values are mean±SEM, n = 4 except where indicated in parenthesis.

† Δ, carbohydrate induced increase in activity. Statistics compare ΔEu and Δhyper to the ΔTx value. Differences are not significant except ΔEu for ME (P < 0.025) and ΔHyper for ME (P < 0.005).

induction by T₃ or CHO feeding is simultaneous (Fig. 4). Thus, it appears reasonable to conclude that there is a common point of interaction of T₃ and the high CHO factor and that neither stimulus is primary to the other.

Recent studies in our laboratory have indicated that the induction of ME either by CHO or by T₃, individually or in combination, is proportional to the level of mRNA for ME as measured by a translational reticulocyte lysate assay (6). Since there is general agreement that T₃ induces protein synthesis at a nuclear receptor level (13) we can infer that the signal created by the T₃ receptor complex interacts with the signal generated by the high carbohydrate diet at a pretranslational level.

The nature of the carbohydrate-induced signal has not been defined. Previous studies have shown that in diabetic rats neither CHO nor T₃ can effectively induce lipogenic enzymes (34). Nevertheless, the block created by insulin lack can be overcome by fructose which enters the liver via fructokinase (35). Furthermore, in other experiments from our laboratory² synergism between T₃ and fructose has also been demonstrated in diabetic rats. Thus, it appears that the most proximal signal for CHO induction of ME is not insulin itself but probably an intermediate of CHO metabolism or a signal generated by such an intermediate.

We have previously shown in animals maintained on a regular diet that the rate of induction of ME is not related in a linear fashion to the nuclear occupancy

by T₃ but that the relationship which exists is strongly amplified. When the T₃-nuclear sites are fully saturated, the rate of induction is 14-fold greater than when the sites are half saturated. The results of the current experiments indicate that when the animals are fed a high CHO diet the relationship between nuclear occupancy and response becomes almost linear in nature. The following and admittedly speculative explanation can be advanced. It is possible that in addition to the primary effects of T₃ on lipogenic enzyme induction discussed above, continued administration of T₃ eventually results in increased turnover of CHO (36–38) leading to an enhanced formation of the putative intermediate responsible for the induction of lipogenic enzymes. On a regular diet with increased occupancy of the T₃-nuclear sites and an increased rate of generation of the intermediate the interaction of the two systems would result in an exponential increase in the rate of induction of lipogenic enzymes. On the other hand, with the high CHO diet one might expect a maximal formation of the putative intermediate. This would result in a simple linear increase in the rate of induction of ME as the T₃-nuclear sites become progressively occupied (see Appendix for a formal exposition). Alternatively, it is conceivable that the hyperthyroid state serves to reduce the concentration of an inhibitor of lipogenesis. A high CHO diet could also result in a decrease in such an inhibitor.

The interaction of thyroid hormones and CHO metabolism has received considerable attention in recent years. The conversion of T₄ to T₃ is inhibited (39) and the induction of ME is blocked with starvation (40). It is therefore of interest to ask whether the decrease in lipogenic enzyme induction by T₃ in starvation is the

² Kaiser, F. E., N. Mariash, H. L. Schwartz, and J. H. Oppenheimer. Inhibition of malic enzyme induction by triiodothyronine in the diabetic rat. Reversal by fructose feeding. *Metab. Clin. Exp.* In press.

mirror image of the enhanced induction by T_3 observed in the present set of experiments with a high CHO diet. Alterations in T_3 levels, nuclear T_3 receptor concentration and postreceptor factors may have an important regulatory function with respect to lipogenic enzyme induction. The cessation of lipogenesis in starvation and its resumption with CHO availability clearly represent an important adaptive biological process. The interaction of T_3 with a high CHO diet may also be a generally useful physiological model for the study of the interaction of T_3 with other biological processes.

APPENDIX

Derivation of the slope of the dose-response curve. The four parameter logistic function proposed by Rodbard (28) to describe dose-response data can be rearranged to:

$$E = \frac{E_{\max} \cdot D^b}{(D_{50})^b + D^b}, \quad (1)$$

where E is the incremental enzyme response, E_{\max} is the maximal incremental enzyme response, D is the dose administered, D_{50} is the dose which gives 50% of the maximum response, and b is the Hill coefficient.

Differentiating E with respect to D yields:

$$\frac{dE}{dD} = \frac{b \cdot E_{\max} \cdot D^{b-1}}{D_{50}^b + 2D^b + D^{2b}/D_{50}^b}, \quad (2)$$

from which the instantaneous slope of the dose-response curve can be obtained for any dose. Substitution of the computer generated values of the dose-response data into Eq. 2 allows one to easily determine whether any dietary manipulation produces a synergistic relationship with T_3 for any dose of T_3 .

Mechanism of T_3 amplification. The model proposed in the Discussion holds that the generation of ME mRNA is proportional to the product of the concentrations of the T_3 nuclear receptor complex (T_3N) and the postulated carbohydrate-induced factor (ϕ):

$$\text{mRNA} = \alpha(T_3N)(\phi), \quad (3)$$

where α is a proportionality constant. This model further implies that when the animal is on a regular diet, T_3 directly influences one or more sequential steps in the generation of ϕ . If each step is linearly related to (T_3N), then

$$\phi = \beta(T_3N)^n, \quad (4)$$

where β is another proportionality constant and n is the number of steps influenced by T_3 . Thus,

$$\text{mRNA} = \alpha\beta(T_3N)^{n+1} \quad (5)$$

Since $(T_3N) = 0.5$ in the euthyroid animal (24), and if $n = 3$, then it follows that the amplification factor ($16 = (1)^4(0.5)^4$) would approximate previous values on a regular diet (14). On the other hand, if the high CHO diet produces maximal quantities of ϕ independent of (T_3N), then Eq. 3 would predict that the ME response would be linearly related to (T_3N) with an amplification factor of ~ 2 . Differences in ϕ and n for each

of the lipogenic enzymes could account for variable degrees of amplification.

ACKNOWLEDGMENTS

We thank Dr. David Rodbard of the National Institutes of Health for supplying us with his computer programs for the analysis of the dose-response relationship, Dr. Christopher Bingham of the Department of Applied Statistics, University of Minnesota for help in the statistical analysis of our data, and Mr. George Runger of the same department for computer programming and very useful discussions. The technical assistance of Robert Gunville and Ana Martinez, as well as the secretarial assistance of Betty Sokolosky and Nancy Rutledge are gratefully acknowledged.

This work was supported by National Institutes of Health grants AM-19812 and National Service Research Training grant AM-07203.

REFERENCES

- Gibson, D. M., R. T. Lyons, D. F. Scott, and Y. Muto. 1972. Synthesis and degradation of the lipogenic enzymes of rat liver. *Adv. Enzyme Regul.* **10**: 187-204.
- Garcia, D. R., and D. Holten. 1975. Inhibition of rat liver glucose-6-phosphate dehydrogenase synthesis by glucagon. *J. Biol. Chem.* **250**: 3960-3965.
- Volpe, J. J., and P. R. Vagelos. 1976. Mechanisms and regulation of biosynthesis of saturated fatty acids. *Physiol. Rev.* **56**: 339-417.
- Winberry, L., and D. Holten. 1977. Rat liver glucose-6-P dehydrogenase: Dietary regulation of the rate of synthesis. *J. Biol. Chem.* **252**: 7796-7801.
- Hutchison, J. S., and D. Holten. 1978. Quantitation of messenger RNA levels for rat liver 6-phosphogluconate dehydrogenase. *J. Biol. Chem.* **253**: 52-57.
- Towle, H. C., C. N. Mariash, and J. H. Oppenheimer. 1980. Changes in the hepatic messenger ribonucleic acid for malic enzyme during induction by thyroid hormone or diet. *Biochemistry.* **19**: 579-585.
- Nepokroeff, C. M., and J. W. Porter. 1978. Translation and characterization of the fatty acid synthetase messenger RNA. *J. Biol. Chem.* **253**: 2279-2283.
- Flick, P. K., J. Chen, A. W. Alberts, and P. R. Vagelos. 1978. Translation of rat liver fatty acid synthetase mRNA in a cell-free system derived from wheat germ. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 730-734.
- Diamant, S., E. Gorin, and E. Shafir. 1972. Enzyme activities related to fatty-acid synthesis in liver and adipose tissue of rats treated with triiodothyronine. *Eur. J. Biochem.* **26**: 553-559.
- Kumar, S., D. K. Das, A. E. Dorfman, and N. Asato. 1977. Stimulation of the synthesis of hepatic fatty acid synthesizing enzymes of hypophysectomized rats by 3,5,3'-L-triiodothyronine. *Arch. Biochem. Biophys.* **178**: 507-516.
- Volpe, J. J., and J. C. Marasa. 1975. Hormonal regulation of fatty acid synthetase, acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. *Biochim. Biophys. Acta.* **380**: 454-472.
- Roncari, D. A. K., and V. K. Murthy. 1975. Effects of thyroid hormones on enzymes involved in fatty acid and glycerolipid synthesis: Evidence for tissue specificity. *J. Biol. Chem.* **250**: 4134-4138.
- Oppenheimer, J. H. 1979. Thyroid hormone action at the cellular level. *Science (Wash. D. C.)*. **203**: 971-979.
- Oppenheimer, J. H., P. Coulombe, H. L. Schwartz, and N. W. Gutfeld. 1979. Nonlinear (amplified) relationship

- between nuclear occupancy and triiodothyronine and the appearance rate of hepatic α -glycerophosphate dehydrogenase and malic enzyme in the rat. *J. Clin. Invest.* **61**: 987-997.
15. Mariash, C. N., F. E. Kaiser, and J. H. Oppenheimer. 1980. Comparison of the response characteristics of four lipogenic enzymes to triiodothyronine administration: evidence for variable degrees of amplification of the nuclear-T₃ signal. *Endocrinology*. **106**: 22-27.
 16. Rudack, D., E. M. Chisholm, and D. Holten. 1971. Rat liver glucose 6-phosphate dehydrogenase: Regulation by carbohydrate diet and insulin. *J. Biol. Chem.* **246**: 1249-1254.
 17. Rudack, D., E. M. Gozukara, E. M. Chisholm, and D. Holten. 1971. The effect of dietary carbohydrate and fat on the synthesis of rat liver 6-phospho-gluconate dehydrogenase. *Biochim. Biophys. Acta.* **252**: 305-313.
 18. Oppenheimer, J. H., E. Silva, H. L. Schwartz, and M. I. Surks. 1977. Stimulation of hepatic mitochondrial α -glycerophosphate dehydrogenase and malic enzyme by L-triiodothyronine: characteristics of the response with specific nuclear thyroid hormone binding sites fully saturated. *J. Clin. Invest.* **59**: 517-527.
 19. Bottomley, R. H., H. C. Pitot, V. R. Potter, and H. P. Morris. 1963. Metabolic adaptations in rat hepatomas v. reciprocal relationship between threonine dehydrase and glucose-6-phosphate dehydrogenase. *Cancer Res.* **23**: 400-409.
 20. Nepokroeff, C. M., M. R. Lakshmanan, and J. W. Porter. 1975. Fatty acid synthase from rat liver. *Methods Enzymol.* **35**: 37-44.
 21. 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**: 256-275.
 22. Yeung, K. K., and R. J. Carrico. 1976. Purification of malic enzyme by affinity chromatography on immobilized N⁶-(6-Aminoethyl)-Adenosine 2',5'-biphosphate. *Anal. Biochem.* **74**: 369-375.
 23. Schimke, R. T., E. W. Sweeney, and C. M. Berlin. 1965. The roles of synthesis and degradation in the control of rat liver tryptophan pyrrolase. *J. Biol. Chem.* **24**: 322-331.
 24. Koerner, D., M. I. Surks, and J. H. Oppenheimer. 1974. *In vitro* demonstration of specific triiodothyronine binding sites in rat liver nuclei. *J. Clin. Endocrinol. Metab.* **38**: 706-709.
 25. 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**: 987-993.
 26. Surks, M. I., A. R. Schadow, 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.
 27. Surks, M. I., and J. H. Oppenheimer. 1977. Concentration of L-thyroxine and L-triiodothyronine specifically bound to nuclear receptors in rat liver and kidney: quantitative evidence favoring a major role of T₃ in thyroid hormone action. *J. Clin. Invest.* **60**: 555-562.
 28. 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.
 29. Diem, K., editor. 1962. Documenta Geigy Scientific Tables. Geigy Pharmaceuticals, Div., Ciba-Geigy Corp., Ardsley, N. Y. Sixth edition. 172.
 30. Oppenheimer, J. H., H. L. Schwartz, and M. I. Surks. 1975. Determination of common parameters of iodothyronine metabolism and distribution in man by noncompartmental analysis. *J. Clin. Endocrinol. Metab.* **41**: 319-324.
 31. Kurtz, D. T., and P. Feigelson. 1978. Multihormonal control of the messenger RNA for the hepatic protein α_{20} globulin. In *Biochemical Actions of Hormones*. G. Litwack, editor. Academic Press, Inc., New York. **5**: 433-455.
 32. Roy, A. K., and D. J. Donbenenko. 1977. Role of growth hormone in the multihormonal regulation of messenger RNA for α_{20} globulin in the liver of hypophysectomized rats. *Biochemistry*. **16**: 3918-3922.
 33. Shapior, L. E., H. H. Samuels, and B. M. Yaffe. 1978. Thyroid and glucocorticoid hormones synergistically control growth hormone mRNA in cultured GH₁ cells. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 45-49.
 34. Ruegamer, W. E., G. H. Newman, D. A. Richert, and W. W. Westerfield. 1965. Specificity of the α -glycerophosphate dehydrogenase and malic enzyme response to thyroid hormone. *Endocrinology*. **77**: 707-715.
 35. Volpe, J. J., and P. R. Vagelos. 1974. Regulation of mammalian fatty-acid synthetase. The roles of carbohydrate and insulin. *Proc. Natl. Acad. Sci. U. S. A.* **71**: 889-893.
 36. Glock, C. E., P. McLean, and J. K. Whitehead. 1956. Pathways of glucose catabolism in rat liver in alloxan diabetes and hyperthyroidism. *Biochem. J.* **63**: 520-524.
 37. Althausen, T. L. 1940. The disturbance of carbohydrate metabolism in hyperthyroidism. *JAMA (J. Am. Med. Assoc.)*. **115**: 101-104.
 38. Elrick, H., C. J. Hlad, and Y. Arai. 1961. Influence of thyroid function on carbohydrate metabolism and a new method for assessing response to insulin. *J. Clin. Endocrinol. Metab.* **21**: 387-400.
 39. Vagenakis, A. G., A. Burger, G. I. Portnay, M. Rudolph, J. T. O'Brian, F. Azizi, R. A. Arky, P. Nicol, S. H. Ingbar, and L. E. Braverman. 1975. Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting. *J. Clin. Endocrinol. Metab.* **41**, 191-194.
 40. 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.