Dietary-induced Alterations in Thyroid Hormone Metabolism during Overnutrition

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ABSTRACT Diet-induced alterations in thyroid hormone concentrations have been found in studies of long-term (7 mo) overfeeding in man (the Vermont Study). In these studies of weight gain in normal weight volunteers, increased calories were required to maintain weight after gain over and above that predicted from their increased size. This was associated with increased concentrations of triiodothyronine (T_3). No change in the caloric requirement to maintain weight or concentrations of T_3 was found after long-term (3 mo) fat overfeeding.

In studies of short-term overfeeding (3 wk) the serum concentrations of T_3 and its metabolic clearance were increased, resulting in a marked increase in the production rate of T_3 irrespective of the composition of the diet overfed (carbohydrate 29.6±2.1 to 54.0±3.3, fat 28.2±3.7 to 49.1±3.4, and protein 31.2±2.1 to 53.2±3.7 μg/d per 70 kg). Thyroxine production was unaltered by overfeeding (93.7±6.5 vs. 89.2±4.9 μg/d per 70 kg). It is still speculative whether these dietary-induced alterations in thyroid hormone metabolism are responsible for the simultaneously increased expenditure of energy in these subjects and therefore might represent an important physiological adaptation in times of caloric affluence.

During the weight-maintenance phases of the long-term overfeeding studies, concentrations of T_3 were increased when carbohydrate was isocalorically substituted for fat in the diet. In short-term studies the peripheral concentrations of T_3 and reverse T_3 found during fasting were mimicked in direction, if not in degree, with equal or hypocaloric diets restricted in carbohydrate were fed.

It is apparent from these studies that the caloric content as well as the composition of the diet, specifically, the carbohydrate content, can be important factors in regulating the peripheral metabolism of thyroid hormones.

Portions of these studies were presented at the XIth Acta Endocrinology Congress, Lausanne, Switzerland, 19–23 June 1977 (*Acta Endocrinol.* **85**: 84), the Annual Meeting of the American Federation for Clinical Research, Atlantic City, N. J., 2 May 1976 (*Clin. Res.* **24**: 271A), and the Annual Meeting of the American Diabetes Association, New York, 15–17 June 1975 (*Diabetes.* **24**: 406).

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INTRODUCTION

An unexpected finding in the Vermont studies of experimental obesity in man (1) was that lean volunteers who gained weight by overeating required more calories to maintain this added weight relative to their increased size than they required before gaining weight. These volunteers gained 25% above their ideal body weight by overeating over a period of 7 mo and required 50% more calories to maintain this new heavier weight than they required at their usual lean weights. More recently, in collaboration with Goldman (2), we have found that short-term overfeeding is associated with increased thermogenesis (energy utilization). Whether there is an adaptive increase in thermogenesis after overnutrition is an old and controversial subject which we have recently reviewed (3, 4). Any number of physiological and biochemical mechanisms have been evoked to explain this phenomenon. Because it is well recognized that thyroid hormones directly increase thermogenesis,

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it is possible that a diet-induced alteration in thyroid hormone metabolism could explain the increased thermogenesis resulting from overnutrition.

We report here the changes in the serum concentrations and metabolism of thyroxine (T_4),[1] 3,5,3′-triiodothyronine (T_3), and 3,3′,5′-triiodothyronine (reverse T_3, rT_3) during long- and short-term overfeeding and after diets of altered composition. We find that serum concentrations of T_3 increase and rT_3 decrease during overfeeding and that this is associated with an accelerated metabolic clearance and production of T_3 without changes in the serum concentration or metabolism of T_4. We have also discovered that the composition of the diet, specifically the carbohydrate content when intake of calories is equal to or below that required to maintain weight, is an important factor in these changes in thyroid hormone metabolism. In view of these results and of parallel studies of thermogenesis (5, 6), it is possible that changes in the peripheral metabolism of T_4 may comprise one of the mechanisms responsible for the increased thermogenesis resulting from overnutrition.

METHODS

Subjects. These studies were performed with the help of 34 normal weight and 13 moderately overweight volunteers. All subjects were between 20 and 29 yr of age and gave no history of recent illness or family history of an endocrinopathy. All volunteers gave their informed consent for these studies. Before the study, they had normal physical examinations, routine blood and urine examinations, chest x rays, electrocardiograms, serum electrolytes, hepatic and renal function tests, lipid and thyroid hormone concentration examinations.

These studies are a part of the larger Vermont Study of Experimental Obesity in Man (1). With the exception of the mixed-diet, long-term overfeeding study, all studies were performed while the subjects lived and ate their meals and supplements under supervision on the General Clinical Research Center of the University of Vermont. The mixed-diet, long-term overfeeding study was performed with volunteers from the Vermont State Prison, Windsor, Vermont. These subjects received their meals each day in an area organized within the prison hospital for this study. In addition to three large, regular meals, dietary supplements were given at the time of an evening television period. The subjects were monitored during all meals by our staff, and the food eaten at each meal was recorded. During the remainder of the day, the subjects performed their regular work assignments and were supervised by the prison guards. Their physical activity was uniform with respect to general activities such as walking or climbing stairs, but the activity required by their jobs within the prison varied. At night they slept in their cells. In all studies, weight maintenance was first established for 3 wk by the use of frozen meals (Swanson Foods, Campbell Soup Co., Camden, N. J.) drawn from single production lots. The dietary composition was provided by the manufacturer, and identical meals were taken during the initial and experimental periods. These were variously supplemented with weighed quantities of standard foods to achieve maintenance of weight or to alter the proportion of carbohydrate and fat in the diet. The calories supplied above maintenance during the overfeeding phases were supplied as part of the regular meals and as between-meal feedings. Demographic data, including age, sex, physical characteristics, duration and type of overfeeding, calories eaten and their composition, body composition, adipose cell size and number for the two long-term overfeeding studies and the short-term overfeeding studies are detailed in tables registered with the National Auxiliary Publications Service.[2]

Long-term overfeeding studies. The long-term study of mixed-diet overfeeding included four subjects studied during periods of weight maintenance before and after 7 mo of overeating an average of 2,000 kcal/d of a mixed diet. This study was designed to dissociate where possible the effect of change in the ratio of carbohydrate and fat in the diet from the effect of gain in weight. For this reason, the subjects underwent two 4-wk-long periods of weight maintenance of the same protein content, one containing 400 and the other 1,200 kcal/m² carbohydrate, before and after their gain in weight.

The long-term study of fat overfeeding included four subjects studied before and after overeating fat for 3 mo. The excess fat in these diets averaged 895 kcal/d consisting of margarine, corn oil, a corn oil colloidal suspension, and fat-enriched soups and cookies. The ratio of saturated to unsaturated fatty acids in these diets was ≅1:2.5.

Short-term overfeeding studies. 17 subjects underwent short-term overfeeding. Studies were performed before and then repeated 3 wk after having taken 1,375 kcal/d excess carbohydrate (six subjects), 1,340 kcal/d excess fat (five subjects), or 965 kcal/d excess protein (six subjects). The subjects who received excess carbohydrate and fat were randomized into two groups. The six subjects overfed protein were studied in a single group. The excess carbohydrate was supplied as Dextrimaltose (Mead-Johnson & Co., Bristol-Meyers Co., Evansville, Ind.), and the additions of fat were the same as for the long-term fat-overfed group. The excess protein was provided as lean meat supplemented with casein (Caseinate, Mead-Johnson & Co.).

Fasting study. Seven moderately overweight subjects fasted for 7 d, except for water and electrolytes, and were then allowed to refeed using a balanced diet.

No carbohydrate study. Three normal weight subjects were studied after weight had been maintained for 1 wk on a balanced liquid diet containing 20% milk protein (Casec, Mead-Johnson & Co.), 40% carbohydrate (Dextrimaltose), and 40% liquid corn oil, and again after the equal-caloric replacement of all carbohydrate in the diet with fat (carbohydrate-free phase) for a week, and then again after taking the original formula diet for a week. In this study, in contrast to the situation in the overfeeding studies, total calories and weights were maintained and therefore the composition of the diet served as the variable.

Low carbohydrate study. Six normal weight subjects were

[1] *Abbreviations used in this paper*: CHO, carbohydrate; FAT, fat; PRO, protein; rT_3, reverse T_3; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TRH, thyroid releasing hormone; TSH, thyroid stimulating hormone.

[2] An Appendix has been deposited with the National Auxiliary Publications Service (NAPS) as NAPS document 03516. This information may be ordered from ASIS/NAPS, Microfiche Publications, P. O. Box 3513, Grand Central Station, New York 10017. Remit in advance, in U. S. funds, $3.00 for microfiche copy, or for photocopy, $5.00 up to 20 pages plus 25¢ for additional pages. Outside the U. S. and Canada add postage of $3.00 for photocopy and $1.00 for microfiche. Checks should be made payable to Microfiche Publications.

studied while their weight was maintained for 1 wk on a balanced diet of regular food containing 40% CHO (carbohydrate), 40% FAT (fat), and 20% PRO (protein), and then again after the equal-caloric replacement of FAT for CHO so that the diet contained 10% CHO, 70% FAT, and 20% PRO for 1 wk, and then again after reinstitution of the initial balanced diet for 1 wk. In this study, weights were maintained and therefore the effects of restricted CHO with increased FAT served as the variable.

Protein-supplemented modified fast study. Six moderately overweight subjects were studied during an initial 2-wk baseline period while they received a weight-maintaining diet consisting of 45% CHO, 40% FAT, and 15% PRO. This was followed by a 6-wk period during which the subjects received a protein-supplemented modified fast including 1.2 g/kg ideal weight per d of lean meat, fish, or fowl. This was supplemented by 25 meq/d of potassium bicarbonate and citrate and 200 mg of calcium as carbonate, plus vitamins and iron.

Measurement of thyroid hormones. All blood samples were drawn when the subjects were supine. To exclude interassay variation, samples from each individual or from each group were measured in the same assay. Serum determinations of T_3 and rT_3 were performed in duplicate or triplicate by radioimmunoassay: T_3 by a modification of the method of Burger et al. (7) and rT_3 by the method of Nicod et al. (8). The intra- and interassay variations were respectively, 3 and 8% for T_3, and 7 and 9% for rT_3. T_4 was determined using a kit from Antibodies, Incorporated, (Davis, Calif.) and T_3 resin binding with the Abbott Trisorb M^{125} Kit (Abbott Laboratories, North Chicago, Ill.), except in studies involving fasting and protein-supplemented fasting, in which T_4 was determined by radioimmunoassay using a polyethylene glycol separation procedure (9).

Thyroid-stimulating hormone (TSH) concentrations were estimated before and after intravenous injection of synthetic thyroid-releasing hormone (THR) by the method of Diamond et al. (10). Human TSH standard (MRC 68/38) was obtained from the Medical Research Council, Holly Hill, London, and human TSH for labeling and the anti-human TSH serum were supplied by the National Pituitary Agency and the National Institute for Arthritis, Metabolism, and Digestive Diseases. In this method, high molecular weight material and iodide are excluded from labeled TSH by gel filtration. The limit of sensitivity of this method is 1 μU/ml.

Kinetic studies. $[^{125}I]T_3$ and $[^{131}I]T_4$ were obtained from Industrial Nuclear Co., Inc., St. Louis, Mo. or from Abbott Laboratories. The two labeled hormones were diluted with human serum albumin and then passed through 0.2-μm filters into sterile containers and used without further purification. Samples ($\cong$40 μCi each) were taken into syringes and injected into an anticubital vein. Venous samples were taken from the opposite arm beginning at 10 min from the injection and at 2, 4, 6, 8, 10, 12, 16, and 24 h, and then three times daily over the next 3 d and twice daily thereafter. Each dose was calculated by either weight or volume of injected isotope. Thyroid uptake of metabolically liberated iodide was minimized by oral administration of one drop of Lugol's iodine solution (10% KI and 5% I_2) twice a day during the first 4 d of the study. Serum $[^{125}I]T_3$ and $[^{131}I]T_4$ were separated from nonthyronine-labeled materials by the method of Nicoloff (11), using 23 $\times$ 0.8-cm Dowex 1-2X anion exchange resin columns supplied by Curtis Nuclear Corp. (Los Angeles, Calif.) and elution with acetic acid. $[^{125}I]T_3$ and $[^{131}I]T_4$ standards were prepared in pooled human serum to approximate the same level of activity as that of the test samples and processed in the same manner. All samples from a group were processed in a single experiment to reduce interassay variability. ^{125}I and ^{131}I activities were estimated in a dual-channel autogamma spectrometer. The counts in serum were expressed as the percentage of the dose per liter and plotted on semilogarithmic graph paper.

Calculation. Metabolic clearance rate and body distribution volume were calculated using the noncompartmental approaches used by Openheimer (12, 13). The results obtained over the first 3 d were used to calculate the metabolic clearance rate of T_3 and from all points to calculate the kinetics of T_4. Production rate was calculated as the product of the metabolic clearance rate and the serum concentration (micrograms per liter) of the hormone before the injection of radioactive hormone.

Statistical analysis. The subjects served as their own controls throughout these studies and the data were analyzed by Students paired t test. The results of these kinetic studies represent single determinations done before and at the end of the experimental periods. The thyroid hormone concentrations, except as noted, are the means of samples drawn 3 or 4 d before and at the end of the overfeeding periods. Values given for the concentrations of hormones are the means and SEM.

RESULTS

Long-term overfeeding. Substantial weight was gained by all subjects during the two long-term overfeeding studies. The volunteers overfed the mixed diet for an average excess of 2,000 kcal/d[3] for 7 mo gained 11.2±2.6 kg, whereas the volunteers given fat in excess for an average of 895 kcal/d for 3 mo gained 14.0±0.6 kg. One of the major differences between these two groups was that the group overfed a mixed diet required more calories (2,625 kcal/m^2 per d) to maintain their weights after gaining than did the group overfed fat (1,840 kcal/m^2 per d). There was no difference between the groups in the calories required to maintain initial lean weights (1,870 vs. 1,705 kcal/m^2 per d).

Serum concentrations of T_3 (Table I) were higher after overfeeding and gain in weight in the group overfed a mixed diet when the low carbohydrate periods were compared (136±10 vs. 152±6 ng/dl, $P < 0.05$). T_3 concentrations were similarly increased in this group before overeating when they were shifted from a low to a high carbohydrate diet (136±10 vs. 151±10 ng/dl, $P < 0.01$). No further increase in T_3 concentrations resulted after weight was gained when the subjects took the high carbohydrate diet. Serum concentrations of T_4 were unchanged by overeating and gain in weight or change in dietary composition. Serum concentrations of rT_3 tended to be lower when the subjects ingested the high carbohydrate diets, but were only significantly lower after the subjects had gained weight. There were no changes in the thyroid hormone concentrations or T_3 resin binding in the subjects overfed fat (Table II).

[3] The number of excess calories eaten daily varied widely in the subjects overfed the mixed diet which may partially explain the apparent greater rate of gain in weight by the subjects overfed fat who took a relatively constant daily increment in excess calories. For more details on the course of these studies see Sims et al. (1).

TABLE I

Serum Concentrations of T_3, rT_3, and T_4 at End of Weight Maintenance Periods before and after Long-term, Mixed-diet Overfeeding

Subjects	400 kcal/m²/d CHO (4 wk)			1,200 kcal/m²/d CHO (4 wk)		
	T_3	rT_3	T_4	T_3	rT_3	T_4
	ng/100 ml		*μg/100 ml*	*ng/100 ml*		*μg/100 ml*
Before weight gain (1,800 kcal/m²/d)						
P.T.	113	49.9	7.6	132	50.6	6.7
P.W.	145	51.7	6.8	165	49.6	6.5
M.R.	125	40.9	5.1	136	28.8	4.9
Z.B.	160	61.2	10.2	170	51.9	9.2
Mean	136	50.9	7.4	151	45.2	6.8
SEM	10	4.2	1.1	10	5.5	0.9
P				<0.01	NS	NS
After weight gain (2,700 kcal/m²/d)						
P.T.	147	67.3	6.7	162	54.3	6.1
P.W.	159	62.0	7.3	152	55.8	5.2
M.R.	135	39.5	4.3	139	33.1	3.9
Z.B.	168	50.4	9.0	162	39.8	7.7
Mean	152	54.8	6.8	154	45.8	5.7
SEM	6	6.2	1.0	6	5.6	0.8
P (high vs. low carbohydrate)				NS	<0.01	NS
P (before vs. after gain)	<0.05	NS	NS	NS	NS	NS

Short-term overfeeding. The subjects in these studies (n = 17) experienced a weight gain from 70.5±2 to 74.6±2 kg over the 3-wk period. Serum concentrations of T_3 (Table III) increased whether the volunteers were overfed with CHO (25%), PRO (17%), or FAT (29%). Serum concentrations of rT_3 decreased when CHO (15%) or PRO (23%) were overfed, although no overall change in rT_3 concentrations occurred when fat was overfed. Although there was some variability, these changes in serum concentrations of T_3 and rT_3 were detectable within 2 or 3 d of overfeeding and reached a new relatively stable concentration after 1 wk of overfeeding. To be certain the changes in hormone concentrations were the result of changes in diet and not a compounding effect of the small amount of iodide given to protect the thyroid gland from irradiation during the turnover studies, five volunteers were given comparable amounts of iodide and the same protocol followed. No detectable change was found in the thyroid concentration after the administration of this small amount of iodide.

TABLE II

Serum Concentrations of T_3, rT_3, T_4, and T_3 Resin Binding before (B) and after (A) Long-term Fat Overfeeding

Subject	T_3		rT_3		T_4		T_3 resin binding	
	B	A	B	A	B	A	B	A
	ng/100 ml		*ng/100 ml*		*μg/100 ml*		%	
W.M.	132	138	37.5	33.6	6.7	7.5	35.6	35.3
I.R.	145	144	36.8	39.5	8.4	8.9	30.9	32.9
B.S.	151	159	31.2	28.6	8.1	7.7	28.7	28.6
S.T.	145	128	51.3	28.1	7.5	6.6	33.1	37.0
Mean	143	142	39.2	32.5	7.7	7.7	32.1	33.5
SEM	4	7	4.3	2.7	0.4	0.5	1.5	1.8
P	NS		NS		NS		NS	

TABLE III

Serum Concentrations of T_3, rT_3, T_4, and T_3 Resin Binding before (B) and after (A) Short-term Overfeeding

Subject	T_3		rT_3		T_4		T_3 resin binding	
	B	A	B	A	B	A	B	A
	ng/100 ml		*ng/100 ml*		*μg/100 ml*		%	
Carbohydrate overfed								
R.B.	103	141	33.7	28.0	5.7	5.5	33.5	33.0
J.M.	144	171	30.3	23.5	7.8	5.8	24.4	31.3
J.E.	139	180	34.7	35.0	6.7	8.6	29.7	29.7
H.B.	112	132	27.3	21.3	8.6	7.7	35.9	36.5
J.W.	135	164	30.7	25.3	11.4	9.2	29.2	27.4
L.W.	127	166	25.0	22.0	9.4	7.2	30.2	29.9
Mean	127	159	30.3	25.9	8.3	7.3	30.5	31.3
SEM	7	8	1.5	2.1	0.8	0.6	1.6	1.3
P	<0.0005		<0.005		NS		NS	
Fat overfed								
D.U.	137	202	44.7	41.0	8.0	8.0	26.1	27.9
D.B.	175	210	38.3	39.0	8.3	8.7	27.5	27.6
D.S.	112	132	29.7	30.0	6.7	7.0	29.2	28.2
B.M.	108	127	30.0	27.3	10.8	9.8	36.4	37.0
R.S.	101	144	32.0	34.3	8.1	8.8	35.4	37.5
Mean	127	163	34.9	34.3	8.4	8.5	30.9	31.6
SEM	14	18	2.9	2.6	0.7	0.5	2.1	2.3
P	<0.01		NS		NS		NS	
Protein overfed								
M.B.	106	142	27.3	21.3	5.9	5.8	33.0	34.0
D.E.	126	146	25.0	19.7	7.5	6.6	30.0	30.0
B.C.	154	174	37.0	26.3	—	—	—	—
P.H.	161	193	32.0	24.0	8.2	6.9	31.0	31.0
A.H.	115	137	23.7	22.0	5.6	7.4	35.0	34.0
D.K.	129	133	29.3	20.7	7.4	6.6	31.0	29.0
Mean	132	154	29.1	22.3	6.9	6.7	32.0	31.6
SEM	9	8	2.0	1.0	0.5	0.3	0.9	1.0
P	<0.0025		<0.0025		NS		NS	
All subjects								
Mean (n = 17)	129	158	31.2	27.1	7.9	7.5	31.1	31.5
SEM	5	6	1.3	1.6	0.4	0.3	0.9	0.9
P	<0.0005		<0.0005		NS		NS	
Controls (not overfed)								
Mean (n = 5)	118	122	25.9	26.9	5.6	5.5	—	—
SEM	18	18	3.3	4.6	0.5	0.5	—	—
P	NS		NS		NS			

Kinetics of T_3. T_3 and T_4 turnover studies were performed simultaneously before and during the 2nd wk of overfeeding after the serum concentration of T_3 had reached new stable concentrations. The metabolic clearance rate of T_3 (Table IV) increased in all 17 subjects whether overfed CHO (43%), FAT (32%), or PRO (33%), and there was no difference among the groups. The mean increase for all the subjects was 36% from 23.7±1.4 to 32.4±1.7 liters/d per 70 kg ($P < 0.0005$). Because the serum concentrations of T_3 increased as well in all subjects (129±5 to 158±6 ng/dl), the increase in production rate of T_3 was even greater than the metabolic clearance rate. The production rate for T_3 increased in all 17 subjects whether overfed CHO (82%), FAT (74%), or PRO (71%). The mean increase for all groups was 76% from 29.8±1.5 to 52.3±1.9 μg/d per 70 kg ($P < 0.0005$). When calculated as micrograms per day per square meters, production rates of T_3 increased from 16.0±0.8 to 28.4±1.3 ($P < 0.0005$). There was also an increase in the volume of distribution of T_3 in all

TABLE IV
Kinetics of T_3 Peripheral Metabolism before (B) and after (A) Overnutrition

Subject	Distribution volume		Fractional removal rate		Metabolic clearance rate		Serum concentration		Production rate	
	B	A	B	A	B	A	B	A	B	A
	liters/70 kg		*%/d*		*liters/d/70 kg*		*ng/100 ml*		*μg/d/70 kg*	
Carbohydrate overfed										
R.B.	24.8	36.1	100.9	90.5	25.1	32.7	98	150	24.6	49.1
J.M.	18.8	28.9	92.5	88.7	17.4	25.6	140	167	24.4	42.8
J.E.	21.5	34.9	89.4	81.9	19.2	28.6	136	178	26.1	50.9
H.B.	30.6	43.3	92.3	98.2	28.3	42.5	116	138	32.8	58.7
J.W.	29.4	35.8	83.1	100.8	24.5	36.1	140	157	34.3	56.7
L.W.	35.9	44.3	77.4	86.6	27.8	38.4	128	172	35.6	66.0
Mean	26.8	37.2	89.3	91.1	23.7	34.0	126	160	29.6	54.0
SEM	2.6	2.3	3.3	2.9	1.8	2.6	7	6	2.1	3.3
P	<0.0005		NS		<0.0005		<0.0025		<0.0005	
Fat overfed										
D.U.	20.3	29.1	82.4	76.1	16.6	22.1	128	225	21.2	49.7
D.B.	19.4	28.5	86.8	84.1	16.8	24.0	156	197	26.2	47.3
D.S.	28.1	28.3	65.1	68.2	18.3	26.2	125	144	22.9	37.7
B.M.	32.9	42.0	106.4	110.4	34.9	46.4	120	125	41.9	58.0
R.S.	42.7	43.4	77.3	89.8	33.0	39.0	88	135	29.0	52.7
Mean	28.7	36.3	83.6	85.7	23.9	31.5	123	165	28.2	49.1
SEM	4.3	3.2	6.8	7.2	4.1	4.7	11	20	3.7	3.4
P	<0.01		NS		<0.0025		<0.025		<0.0005	
Protein overfed										
M.B.	35.9	35.4	72.1	94.1	25.9	33.3	110	150	28.5	50.0
D.E.	31.7	42.0	65.1	78.9	20.6	33.2	130	156	26.8	51.8
B.C.	29.8	29.4	63.2	79.6	18.8	23.4	152	225	28.6	52.7
P.H.	46.3	48.6	59.2	68.0	27.4	33.1	156	214	42.7	70.8
A.H.	28.7	31.8	80.5	91.9	23.1	29.2	120	154	27.7	45.0
D.K.	35.4	43.5	72.4	84.0	25.6	36.5	128	134	32.8	48.9
Mean	34.6	38.5	68.8	82.8	23.6	31.5	133	172	31.2	53.2
SEM	2.6	3.0	3.2	3.9	1.4	1.9	7	15	2.1	3.7
P	<0.05		<0.0005		<0.0005		<0.005		<0.0005	
All subjects										
Mean (n = 17)	30.1	36.7	80.4	86.6	23.7	32.4	128	166	29.8	52.3
SEM	1.9	1.6	3.3	2.7	1.4	1.7	5	8	1.5	1.9
P	<0.0005		<0.01		<0.0005		<0.0005		<0.0005	

subjects. Overfeeding CHO increased the volume of distribution 39%, FAT 26%, and PRO 11%. The mean increase for all groups was 22% from 30.1±1.9 to 36.7 ±1.6 liters/70 kg ($P < 0.0005$). The fractional turnover rate increased in 12 of the 17 subjects with no change or a small decrease in the other 6. For all the groups, the fractional turnover rate increased 8% from 80.4 ±3.3 to 86.6±2.7%/d ($P < 0.01$).

Kinetics of T_4 (Table V). T_4 peripheral turnover studies were performed in 12 subjects. These included three subjects overfed carbohydrate, three overfed fat, and six overfed protein. No change was found in the kinetic parameters of T_4 metabolism in any of these subjects. The overall distribution volumes (11.9±0.5 vs. 11.1±0.5 liters/70 kg), fractional turnover rates (11.3±0.6 vs. 11.4±0.4%/d), and metabolic clearance rates (1.33±0.08 vs. 1.27±0.06 liters/d per 70 kg) were unchanged. Because T_4 concentrations did not change after overfeeding (7.1±0.3 vs. 7.0±0.3 μg/100 ml), there was no change in the T_4 production rates (93.7±6.5 vs. 89.2±4.9 μg/d per 70 kg).

TRH stimulation. TRH stimulation tests (Figs. 1 and 2) were performed before and after overfeeding carbohydrate and fat. The basal serum concentrations of TSH were normal in all subjects and overfeeding produced no change in these basal concentrations. Mean basal concentrations for all subjects was 2.8±0.5 μU/ml before overfeeding and 2.8±0.8 μU/ml after

TABLE V

Kinetics of T_4 Peripheral Metabolism before (B) and after (A) Overnutrition

Subject	Distribution volume		Fractional removal rate		Metabolic clearance rate		Serum concentration		Production rate	
	B	A	B	A	B	A	B	A	B	A
	liters/70 kg		*%/d*		*liters/d*		*μg/100 ml*		*μg/d/70 kg*	
Carbohydrate overfed										
R.B.	12.2	12.0	10.1	11.2	1.35	1.35	5.7	5.5	77.0	7.43
J.M.	9.5	9.7	11.3	10.1	1.07	0.99	7.8	5.8	83.5	57.4
J.E.	10.7	9.5	12.1	13.6	1.29	1.29	6.7	8.6	86.4	110.9
Mean	10.8	10.4	11.5	11.6	1.24	1.21	6.7	6.6	82.3	80.9
SEM	0.8	0.8	0.3	1.0	0.09	0.11	0.6	0.9	2.8	15.8
P	NS		NS		NS		NS		NS	
Fat overfed										
D.U.	10.3	8.4	10.4	11.2	1.01	0.94	8.0	8.0	80.8	75.2
D.B.	9.6	10.3	13.7	10.8	1.32	1.12	8.3	8.7	109.6	97.4
D.S.	10.8	9.4	11.4	13.0	1.23	1.22	6.7	7.0	82.4	85.4
Mean	10.2	9.4	11.8	11.7	1.19	1.09	7.7	7.9	90.9	86.0
SEM	0.4	0.6	1.0	0.7	0.09	0.08	0.5	0.5	9.3	6.4
P	NS		NS		NS		NS		NS	
Protein overfed										
M.B.	14.4	13.3	12.8	12.1	1.85	1.61	5.9	5.8	109.2	93.4
D.E.	15.0	13.1	5.9	9.2	0.89	1.23	7.5	6.6	66.8	81.2
B.C.	10.7	10.8	11.5	9.5	1.23	1.04	—	—	—	—
P.H.	13.4	12.6	12.8	11.1	1.70	1.40	8.2	6.9	139.4	96.6
A.H.	13.0	11.7	11.3	12.7	1.46	1.48	5.6	7.4	81.8	109.5
D.K.	13.2	12.3	11.7	12.3	1.54	1.51	7.4	6.6	114.0	99.7
Mean	13.3	12.3	11.0	11.5	1.45	1.38	6.9	6.7	102.2	96.1
SEM	0.6	0.4	1.0	0.6	0.14	0.09	0.5	0.3	1.3	4.6
P	NS		NS		NS		NS		NS	
All subjects										
Mean (n = 17)	11.9	11.1	11.3	11.4	1.33	1.27	7.1	7.0	93.7	89.2
SEM	0.5	0.5	0.6	0.4	0.08	0.06	0.3	0.3	6.5	4.9
P	NS		NS		NS		NS		NS	

overfeeding. The highest TSH concentration reached after TRH administration was similar in all subjects. The peak concentration of TSH reached after TRH (23.2±2.6 vs. 19.5±2.3 μU/ml, $P < 0.1$) and peak Δ increase in TSH (20.3±2.4 vs. 16.8±1.8 μU/ml, $P < 0.1$) tended to be lower after overfeeding. However, significantly lower values were found after overfeeding only at 120 and 180 min. As expected, the basal concentration of T_3 was higher in each subject after overfeeding carbohydrate or fat. However, no differences were found after overfeeding in the response of T_3 to TRH when expressed as the percentage change above base line (Fig. 2, inset).

Eucaloric diets. When fat was equal-calorically substituted for carbohydrate in a weight maintaining diet for 1 wk (Fig. 3B), the serum concentrations of T_3 fell from 172±9 to 116±9 ng/dl ($P < 0.005$), and then returned toward their initial concentrations (157±9 ng/dl, $P < 0.01$) when carbohydrate was restored to the diet. Serum concentrations of rT_3 responded in the opposite direction to those of T_3. Beginning at 56±1 ng/dl, the rT_3 rose to 73±9 ng/dl ($P < 0.01$) and then fell with addition of carbohydrate back to the diet to 43±8 ng/dl ($P < 0.01$). The concentrations of T_4 and T_3 resin binding were unaffected by this change in the composition of the diet. Similar alterations in the concentrations of T_3 and rT_3 were produced when fat was equally but not completely substituted for carbohydrate in the diet for 7 d (Fig. 3D). Beginning at 130 ±8 ng/dl, T_3 fell to 105±9 ng/dl ($P < 0.01$) and then rose to 127±7 ng/dl ($P < 0.01$) when these subjects were returned to the diet containing 40% CHO. CHO accounted for only 10% of the total calories necessary to maintain weight during the experimental phase of this study.

Hypocaloric diets. Fasting over the same period

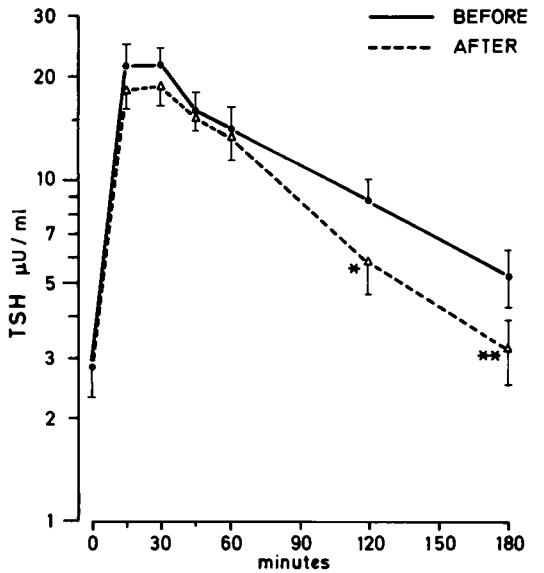

FIGURE 1 Comparison of the serum TSH response to TRH (500 μg i.v.) before and during carbohydrate and fat overfeeding for 3 wk. Each point represents the mean±SEM ($n = 6$). Statistical significance is reached only at 120 ($P < 0.025$) and 180 ($P < 0.005$) min after TRH injection.

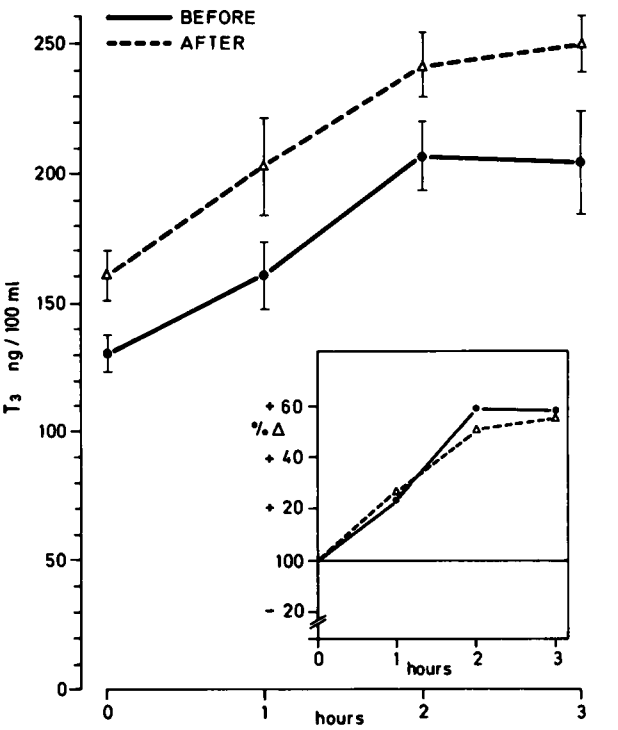

FIGURE 2 Comparison of the serum T_3 response to TRH (500 μg i.v.) before and during carbohydrate and fat overfeeding for 3 wk. Each point represents the mean±SEM ($n = 6$). The inset represents the percent change in T_3 from control values. The increased base-line concentration ($P < 0.01$) after overfeeding persisted throughout the response. However, the percent change in T_3 from base line is unchanged by overfeeding.

as the above carbohydrate-restricted studies resulted in similar changes in the serum concentrations of T_3 and rT_3 (Fig. 3A). The initial concentration of T_3 in these subjects was 155±7 ng/dl, fell to 87±7 ng/dl ($P < 0.0005$) during the 7-d fast, and then rose to 146 ±9 ng/dl with refeeding ($P < 0.001$). Initial rT_3 concentrations were 25±2 ng/dl, rose with fasting to 57±2 ng/dl ($P < 0.0005$), and then fell again to 24±2 ng/dl ($P < 0.001$) with refeeding. Slower but similar changes in the concentrations of T_3 and rT_3 to those of fasting occurred with administration of a protein-supplemented modified fast for 1 wk (Fig. 3C). During the 1st wk of the diet, T_3 concentrations fell from 166±8 to 109±4 ng/dl ($P < 0.0005$) and rT_3 concentrations rose from 31±2 to 53±5 ng/dl ($P < 0.0005$). As the modified fast was continued, T_3 concentrations continued to fall and at 6 wk were equivalent to those found after 1 wk of fasting (88±5 ng/dl, $P < 0.05$). rT_3 concentrations, however, returned toward their initial values as the fast was continued (39±2 ng/dl, $P < 0.05$). Again, T_4 concentrations were unaffected by these dietary manipulations after 1 wk, but were slightly lower ($P < 0.05$) after 6 wk of the diet.

DISCUSSION

We can conclude from these studies that overfeeding increases the serum concentration of T_3 and accelerates its metabolic clearance and production without altering the serum concentration, metabolic clearance, or production of T_4. Our estimates of the peripheral metabolism of T_4 and T_3 in these lean, young, healthy subjects while they were eating a weight-maintaining balanced diet agree with estimates reported by others (11, 14–20) in normal euthyroid subjects. The uniformity of these results is best explained by the careful attention paid to the antecedent diets, because it is known that starvation (21) and overnutrition induce alterations in the peripheral metabolism of thyroid hormones. Overnutrition in these subjects induced a marked and similar increase in the production of T_3 regardless of the component of the diet overfed (CHO 82%, FAT 74%, and PRO 71%). It is known that most of the T_3 found in the serum of euthyroid man is derived from the peripheral monodeiodination of T_4 rather than by direct secretion from the thyroid gland (17, 22–25). Similar studies have not been performed in hypothyroid or euthyroid subjects given thyroid hormone adequate to inhibit endogenous secretion of T_3, but because the serum concentrations and peripheral kinetics of T_4 were unaltered, it is unlikely the altered production of T_3 reflects a selective increased secretion of T_3 from the thyroid. It is more likely that overnutrition is associated with an acceleration of the peripheral

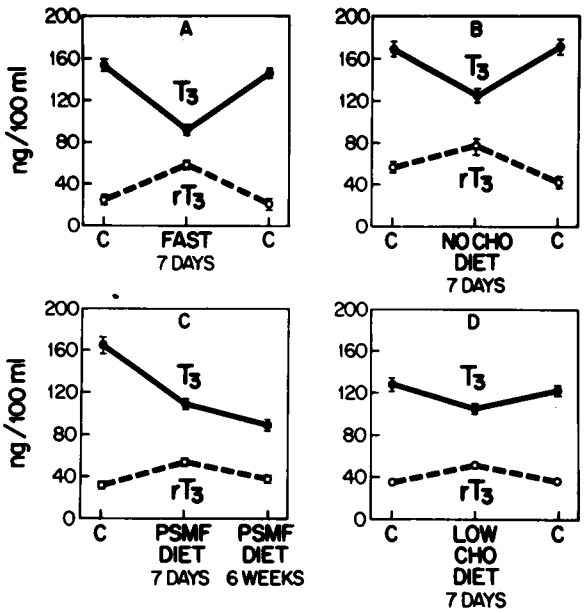

FIGURE 3 Comparison of the changes in the serum concentrations of T_3 and rT_3 produced by fasting (A) ($n = 7$), carbohydrate-free equal-caloric weight maintaining diets (B) ($n = 3$), protein-supplemented modified fasts (PSMF) (C) ($n = 6$), and low carbohydrate (10%) equal-caloric weight maintaining diets (D) ($n = 6$). Each experimental point represents the mean±SEM and is statistically different from control days at $P < 0.01$ level or greater, except the rT_3 values in D that represent the determination in a single individual.

conversion of T_4 to T_3. Serum concentrations of rT_3 decreased in these studies. However, we are unable to say whether the peripheral metabolism of rT_3 is also affected by overnutrition for the reason that studies of the kinetics of rT_3 were not performed.

As noted earlier, our interest in performing these studies came from observations made in studies of long-term overfeeding in which volunteers overfed a mixed diet developed higher serum concentrations of T_3 and required a greater relative number of calories to maintain their heavier weights than volunteers overfed a diet high in fat who had no change in their T_3 concentrations. At first glance the studies of short-term overfeeding do not appear to explain this discrepancy, because the concentrations, metabolic clearance, and production of T_3 increased whether the subjects overate carbohydrates or fat. In this regard, it is important to note that the measurements of thyroid hormones in the two long-term overfeeding studies were performed after relatively long (4 wk) periods at stable weight and not while the subjects were gaining weight, as was the case in the short-term overfeeding studies. It is also important to consider the difference in requirements between the two groups. The number of calories needed to maintain weight before gaining was similar in the two groups. However, after weight was gained the group taking a mixed diet required more calories to maintain weight than the fat-overfed group. Although some of this difference in requirement might be a result of differences between the groups in their institutional setting, length of study, amount of exercise, shifts in body composition, coffee intake or smoking habits, the magnitude of this difference is large enough so that another source of energy expenditure must have occurred in the group overfed the mixed diet. The group overfed a mixed diet was therefore "overeating" in order to maintain their new weights, whereas the fat-overfed subjects were able to maintain their new weights by eating approximately the same relative number of calories they had required before gaining weight. It seems clear therefore that the level of caloric intake is important in regulating the serum concentrations and the peripheral metabolism of thyroid hormone. This observation is supported in a reciprocal manner by the observation that starvation reduces the serum concentration of T_3 and the peripheral conversion of T_4 to T_3 (26).

A second important observation that is not obvious when examining the results of the short-term overfeeding studies is the importance of the carbohydrate content of the diet in determining the serum concentrations of the thyroid hormones. This was first suspected when the results of the long-term, mixed-diet overfed group was studied when taking either a low (400 kcal/m² per d) or a high (1,200 kcal/m² per d) level of carbohydrate in the diet. An increase in T_3 occurred before weight was gained when a weight-maintaining low carbohydrate diet was replaced by an equal-caloric high-carbohydrate diet. However, no further increase in T_3 concentrations resulted when the high carbohydrate diet was given after weight was gained and maintenance established on the increased intake. This suggested a finite phenomenon that could be induced either by increasing the carbohydrate content without increasing the total number of calories in the diet.

The four short-term studies served to clarify the importance of the carbohydrate content of the diet in producing these changes. Changes in the concentrations of T_3 and rT_3 that occur during starvation were mimicked when carbohydrate was eliminated or restricted in diets adjusted to maintain weight by the isocaloric replacement of carbohydrate with fat. Changes similar to those found during complete starvation also occurred in the concentrations of T_3 and rT_3 after 1 wk of a protein-supplemented modified fast containing almost no carbohydrate. T_3 concentrations continued to fall, and were at their lowest concentrations after 6 wk of the protein-supplemented fast. Interestingly, in this study, as has been reported

after prolonged starvation (27), rT_3 concentrations returned toward normal as the fast was continued. It is apparent from these results that the composition of the diet, as well as the caloric content of the diet, can play an important role in determining the concentrations of T_3 and rT_3 in the serum. This conclusion then raises the question not only of the relationship between altered thyroid hormone metabolism and energy utilization where energy intake is above or below maintenance, but also the relationship between thyroid hormone metabolism and energy intake where energy intake is kept the same and only the composition of the diet changed.

There is substantial evidence in man and from in vivo (28–33) and in vitro (34–40) animal studies to support these observations. Others have confirmed our initial report in studies in man that the level of carbohydrate in the diet is an important determinant of the concentrations of T_3 and rT_3. Spaulding et al. (41) found that 800 kcal hypocaloric diets containing no carbohydrate mimicked the fall in T_3 found during starvation. Several investigators have reported a return toward normal of the fasting-induced low T_3 and high rT_3 concentrations when carbohydrate or a mixed diet was refed (42–44), and the absence of such an effect when fat was refed (45). Further support for these observations is found in the studies by Schonborn et al (46), Burman et al. (44), and Davidson and Chopra (47). The latter study confirms our initial report that carbohydrate sources of calories are important modulators of serum concentrations of T_3 in man, and concludes as we do that the influence of total calories is as pronounced as that of carbohydrate when a permissive amount of carbohydrate is present in the diet. If, as we believe, these alterations in thyroid hormone metabolism represent important adaptations of the body to the fed and fasted states, then this is an attractive conclusion because in diets near or below maintenance, the level of carbohydrate, through effects on other hormones and substrates, is the major signal to the body of the fed or the fasted state.

The physiological significance of these diet-induced alterations in thyroid hormone metabolism is presently unknown. We have measured increased thermogenesis (energy utilization) in these subjects (5, 6), and in others overfed carbohydrate (2). It is tempting to speculate that the increased clearance and production of T_3 might be responsible for this increase in thermogenesis. Energy utilization is decreased during starvation, raising the possibility that the decreased production of T_3 in this condition might be responsible. This interpretation is not simple, for the reason that it is now recognized that caloric restriction lowers not only T_3 concentrations but also the putative nuclear T_3-receptor capacity (48–50), suggesting the possibility that the capacity to bind T_3 to its receptor is a function of the nutritional state of the organism. In starvation it seems unlikely that the fall in oxygen consumption is entirely the result of the lowered T_3 concentrations. This is supported by our discovery that the fall in oxygen consumption during starvation occurs in hyperthyroid as well as hypothyroid rats given T_3 (51). During overfeeding, however, an increase in the concentration and production of T_3 in conjunction with an unaltered or even an increase in T_3-receptor capacity, as suggested by the replenishment of T_3-receptor capacity on refeeding, could support a role for T_3 in the increased energy utilization after overnutrition.

Although a strong case for the inhibition of the pituitary secretion of TSH by overfeeding cannot be made, the rise following TRH stimulation was less sustained. It is debated whether in starvation circulating levels of TSH or the response of TSH to TRH is normal or low (27, 42, 45, 52, 53). Sensitization of the pituitary to the inhibition of thyrotropin by T_3 has been suggested by some investigators (54) to explain the normal circulating levels of TSH and response to TRH found during starvation. One mechanism that could explain such altered sensitivity of the pituitary to circulating concentrations of T_3 during starvation and overnutrition is the findings of Silva et al. (55). They found that a greater amount of T_3 bound to receptors in the pituitary is generated locally from T_4 than in the peripheral tissues such as the liver and kidney. There is the possibility therefore that the expression of T_3 activity may vary with the tissues involved. If this is so, then the effect of over- and undernutrition on the peripheral metabolism of T_4 and the resetting of the hypothalamic pituitary thyroid axis might be important mechanisms by which increased amounts of T_3 are made available to the body during times of caloric affluence and decreased amounts during times of caloric deprivation. Thyroid hormones have well recognized effects on the two costliest homeostatic processes of the body, protein synthesis and degradation, and the maintenance of intracellular sodium concentrations by the sodium pump. Diet-induced alterations in thyroid hormone metabolism could, through the regulation of the rate of these processes and therefore the expenditure of energy, represent an important physiological adaptation to feast and famine.

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