0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 3
Printed in U.S.A.

Metabolic Effects of Triiodothyronine Replacement during Fasting in Obese Subjects*

LAURI O. BYERLEY† AND DAVID HEBER

Clinical Research Center, Harbor-University of California-Los Angeles Medical Center, Torrance, California 90509-2910; the Division of Clinical Nutrition, Department of Medicine, University of California School of Medicine, Los Angeles, California 90024

ABSTRACT

The adaptation to fasting reduces muscle protein breakdown by switching from a carbohydrate to fat fuel economy in normal man. With the discovery of T_3 and the observation that its formation from T_4 was reduced significantly during starvation, it was proposed that T_3 mediated many of these changes. To examine this possibility, otherwise healthy, obese subjects were fasted for 10 days and supplemented with T_3 the last 3 days of the fast to bring circulating T_3 levels within normal prefasting (weight maintenance) levels. The effects of the same dose of T_3 for 3 days were tested during the last 3 days of a 10-day weight maintenance diet for comparison. Both metabolic rate and CO_2 production decreased as expected with fasting and did not increase after T_3 supplementation. Hepatic glucose appearance rates fell with fasting and increased significantly during T_3 supplementation, but not to prefasting levels. Urinary urea nitrogen excretion decreased significantly with fasting and decreased further with T_3 supplementation. Lysine appearance did not change during fasting or T_3 supplementation, but leucine appearance decreased with T_3 supplementation during fasting. These observations suggest that the fall in serum T_3 during fasting may not mediate the observed decreases in protein breakdown that occur during fasting and prolonged starvation, but may instead initiate the fall in hepatic glucose appearance. (*J Clin Endocrinol Metab* **81:** 968–976, 1996)

DURING STARVATION, glycogen stores are rapidly depleted to provide glucose for energy. After 24 h of fasting, glucose is derived entirely from gluconeogenesis, requiring breakdown of protein stores to provide glucogenic amino acids to the liver. However, the body gradually adapts, minimizing the rate of muscle catabolism, decreasing basal energy requirements (1), and switching to fat as the predominate fuel. It has been hypothesized that this adaptation, termed protein sparing, is mediated by hormones and metabolites, particularly thyroid hormone.

Altered peripheral metabolism of thyroid hormone occurs commonly in fasting and in patients with many acute and chronic illnesses. It has been proposed that these changes may be a normal adaptation to fasting and illness, serving to reduce energy expenditure and perhaps to help conserve body protein stores (2). As fasting in healthy man results in changes in thyroid hormone metabolism very similar to those observed in nonthyroidal illness, this is a useful experimental model for determining the role of thyroid hormones in sparing protein stores.

T_4 is secreted by the thyroid gland and converted in peripheral tissues to T_3 by a specific monodeiodinase enzyme. T_3 is thought to be more metabolically active than T_4 because it has a 10-fold greater affinity for the thyroid hormone receptor in target tissues (2), T_3 is more rapidly turned over, and a smaller percentage is bound to circulating serum protein. During starvation, serum total and free T_3 (FT_3) concentrations decrease, with little change in serum total and free T_4 (FT_4) concentrations and increased serum concentrations of rT_3, an inactive metabolite of T_4 (3, 4). Also, the enzyme responsible for deiodinating T_4 to T_3 decreases on a hypocaloric diet (5–7).

A number of previous studies have addressed the hypothesis that reductions in thyroid hormone levels during fasting may mediate protein sparing. Several studies (8) have administered supraphysiological doses of T_3 (120–150 μg T_3/day) and concluded that T_3 plays a role in modulating protein, fat, and glucose metabolism during starvation. However, supraphysiological doses increase serum T_3 levels above the normal physiological range, which may interfere with the normal physiological functioning of thyroid hormone.

Other studies have administered T_3 to fasted individuals at the onset of the fast. Gardner *et al.* (9) fasted normal volunteers for 80 h with or without T_3 supplementation (5 μg every 3 h) and measured serum T_3, rT_3, T_4, and TSH as well as urinary urea nitrogen. When the usual decrease in T_3 was prevented by the administration of synthetic T_3, urinary urea nitrogen excretion increased by 9.1%. Vignati *et al.* (10) reported that administration of T_3 during a 20-day fast resulted in restoration of serum T_3 to postabsorptive levels and increased urinary urea nitrogen and ammonia nitrogen excretion. Increased release of glutamine from forearm muscle could account for most of the excess urinary nitrogen losses; a 21% increase in urinary 3-methylhistidine excretion indicated increased muscle proteolysis. These data suggest that the fall in serum T_3 concen-

Received November 15, 1995. Revision received April 21, 1995. Rerevision received August 8, 1995. Accepted September 28, 1995.

Address all correspondence and requests for reprints to: Dr. David Heber, Division of Clinical Nutrition, Department of Medicine, University of California School of Medicine, A1-57 UCLA Rehab, 1000 Veteran Los Angeles, California 90024.

* This work was supported by NIH General Clinical Research Center Grant M01-RR-00425.

† Current address: Division of Nutrition, University of Texas, GEA 115, Austin, Texas 78712-1097.

tration in fasting is a protective mechanism, limiting muscle protein catabolism.

Most recently, Nair *et al.* (11) supplemented five obese individuals with 20 μg T_3 every 8 h for 1 week before fasting and for 1 week during fasting after 7 days of fasting without T_3 administration. Before this 3-week period, a supraphysiological dose of T_3 was administered to better control the T_3 response during the study period. The results from these five obese subjects were compared to those from three obese subjects who followed the same protocol but did not receive T_3. During the fast, administration of T_3 increased serum T_3 to a level similar to prefast levels; however, these levels were 2 times greater than the serum T_3 levels of the controls at any time during the 3-week study. Leucine kinetics did not change with T_3 administration during the fast, suggesting that T_3 does not mediate protein sparing after adaptation to caloric restriction.

In the present study, fasting obese subjects were supplemented with T_3 after 7 days of fasting to return serum T_3 levels to normal prefasting levels to investigate the short term role of T_3 in mediating the changes in protein turnover, glucose metabolism, and basal metabolic rate observed in fasting. In preliminary studies, we determined that supplemental T_3 could only be administered for a short period of time during fasting to avoid the hypothalamic-pituitary adjustments to exogenous hormone supplementation

Materials and Methods

Subjects

Obese [defined as weight >120% of ideal body weight (IBW) (12)] adult euthyroid volunteers without evidence of any major organ system disease except obesity, as determined by routine physical exam and medical history, were admitted for 21 days to the Clinical Research Center at Harbor-University of California-Los Angeles Medical Center. All subjects gave informed written consent to participate in this study. The protocol and procedures were approved by the human subjects committee of the Research and Education Institute at Harbor-University of California-Los Angeles Medical Center.

Twenty-nine subjects (10 men and 19 women), ranging in age from 19–58 yr (mean ± SEM, 33 ± 2) participated in this study. Their average height and weight were 169.9 ± 1.6 cm and 114.2 ± 6.9 kg, respectively; that weight was 170 ± 8% (67.5 ± 1.3 kg) of the IBW. Body mass index (BMI) ranged from 26–70 kg/m^2 (mean ± SEM 38.9 ± 1.8 kg/m^2).

Protocol

The study was 21 days in length and consisted of a 10-day period in which each subject was fed to maintain his/her weight [weight maintenance (WM)], followed by an 11-day fasting period [fasted (F)]. During the last 3 days of both periods, synthetic T_3 (Cytomel, Smith, Kline, and French, Philadelphia, PA; 5 μg) was administered orally every 4 h to return serum T_3 levels at normal prefasting WM levels. In a preliminary study, we found that 5 μg Cytomel every 4 h led to decreased TSH levels after 3 days. Thus, we purposely chose 3 days to observe changes in protein and glucose metabolism and short enough to allow us to return T_3 levels to approximately normal physiological prefasting levels without affecting endogenous hormone secretion. To distinguish each period, the following abbreviations were used: WM, WM + T_3, F, and F + T_3.

Weight on a calibrated scale was measured daily. Starting on day 5, thyroid function (T_3, FT_3, T_4, FT_4, and rT_3) was measured daily. Leucine (n = 18) or lysine (n = 7) kinetics, serum amino acids (n = 27), and glucose appearance (n = 29) were quantitated simultaneously at the end of each period (days 7, 11, 17, and 21). Starting on day 4, urine was collected every 24 h for urea nitrogen and creatinine determination. An aliquot from the 24-h urine collections on days 6, 7, 10, 11, 16, 17, 20, and 21 was analyzed for urinary 3-methylhistidine content. The values corresponding to each period were averaged. Basal metabolic rate was determined on the following days for each period and averaged: days 3, 4, and 7 for WM; days 9, 10, and 11 for the WM + T_3; days 17 and 18 for F; and day 21 for F + T_3.

Diet

All food was prepared daily by the Clinical Research Center dietary staff. Detailed records of the amount of food and beverage consumed were kept to monitor dietary compliance. It was usual for most of the subjects to consume all food presented to them.

The weight maintenance diet consisted of a creatinine-free liquid formula containing 25 Cal/kg actual wt and 1.2 g protein/kg IBW. On the average, the diet contained 11788 ± 732 kilojoules/day, 80.1 ± 1.8 g protein/day, 122 ± 16 g fat/day, and 363 ± 24 g carbohydrate/day. Each participant was allowed *ab libitum* water and ice only.

During the fasting period, each subject was offered and allowed *ab libitum* water, ice, decaffeinated coffee, diet soda (except diet cola), and caffeine-free herbal tea. Such items as regular coffee, sugarless gum, and sugarless mints were not allowed. Potassium chloride supplements were given as necessary to maintain normal serum potassium levels. A multivitamin tablet was given daily.

Methods

Basal metabolic rate was determined using the closed circuit Douglas bag method. Briefly, a face mask was placed over the subject's face. Expired air was collected for 5 min into a Douglas bag from which air samples were withdrawn into oil-sealed 50-mL glass syringes and analyzed for O_2 and CO_2 contents using a Perkin-Elmer mass spectrometer (MGA 1100, Norwalk, CT). The volume of air expired was determined using a Collins gas meter (Instrumentation Associates, New York, NY) calibrated with a Tissot spirometer. Basal metabolic rate was calculated from O_2 consumption and CO_2 production by the Weir equation, as previously described (13).

All biochemical measurements were made in the Clinical Research Center Core Laboratory. Urinary 3-methylhistidine was measured on a Beckman Amino Acid Analyzer (model 120C), as described by Young *et al.* (14). Urinary creatinine (15) and urea nitrogen (16) were measured by colorimetric procedure.

Serum T_3, T_4, and rT_3 were measured by standard RIA (17–19). Serum FT_3 and FT_4 were measured by equilibrium dialysis using 0.1 mL serum, 1.0 mL dialysis cells (BelArts Products, Pittsburgh, PA), and repurified radioiodinated T_3 and T_4 (New England Nuclear Corp., Boston, MA).

Hepatic glucose appearance was determined using a primed continuous infusion of [6-^{3}H]glucose after an overnight fast (20). Starting between 0700–0800 h, a loading bolus of 25 μCi was given at time zero followed by a 4-h continuous infusion of 14.4 μCi [6-^{3}H]glucose/h. Blood was drawn 0, 180, 200, 220, and 240 min after the start of the infusion, immediately iced, then spun to separate the plasma, which was frozen for analysis at a later time. The plasma glucose concentration was determined by the hexokinase method (21) adapted to an ABA 100 automated analyzer (North Chicago, IL). The amount of labeled glucose in the plasma was counted on a Hewlett-Packard liquid scintillation counter (Hewlett-Packard, Palo Alto, CA) after deproteinization with 30% sulfosalicylic acid and removal of water by evaporation (20). Counts per min were corrected for counting efficiency to determine disintegrations per min. The appearance rate (R_a) was calculated from the formula (22): $R_a = (q \times SA_i)/SA_s$, where R_a is the glucose appearance rate in milligrams per min, q is the infusion rate in milliliters per min, SA_i is the specific activity of the infusate in disintegrations per min/mL, and SA_s is the specific activity of plasma glucose at equilibrium in disintegrations per min/mg. The data were normalized to body weight and expressed as the glucose appearance rate in milligrams per kg/min.

Either whole body lysine or leucine flux was determined simultaneously with glucose appearance using a primed continuous infusion of either L-[U-^{14}C]lysine (23) or L-[1-^{14}C]leucine (20), as previously described by our group. Regardless of which amino acid isotope was infused, a priming dose of 2.55 μCi was administered at time zero, followed by a 4-h continuous infusion of the respective isotope (1.6 μCi/h). Blood was drawn at 0, 180, 200, 220, and 240 min; the plasma was separated and frozen for later analysis.

The plasma amino acid concentration was determined after plasma protein precipitation using 10% sulfosalicylic acid (1:0.1, vol plasma/vol sulfosalicylic acid) using a Beckman 119 CL automated amino acid analyzer (Beckman Instruments, Fullerton, CA). The [^{14}C]lysine concentration was determined in the same samples by measuring total acid-soluble radioactivity using a Beckman β-scintillation counter. Using the stream splitter on the amino acid analyzer, the plasma leucine concentration was determined from one tenth of the plasma sample, whereas nine tenths of the sample were collected for specific activity determination. Disintegrations per min were increased by 10% to account for the loss of radioactivity used for leucine peak determination.

Lysine or leucine appearance is equal to lysine or leucine flux (Q) because the subjects were fasted. Appearance was calculated from the formula $Q = (q \times SA_i)/SA_p$, where Q is appearance in micromoles of lysine or leucine per h, q is the infusion rate in milliliters per min, SA_i is the specific activity of infusate in disintegrations per min/mL, and SA_p is the plasma specific activity of lysine or leucine at equilibrium in disintegrations per min/μmol. The data were normalized to body weight and expressed as micromoles per kg/h.

Samples of breath were collected during the last hour of the infusion into a liquid scintillation vial containing 4 mL of a 1:1 dilution (vol/vol) of absolute ethanol 1 mmol hydroxide of hyamine and thymolphthalein indicator for measurement of $^{14}CO_2$ production. When 2 mmol CO_2 have been collected, this mixture turns from blue to clear. Then, the sample was counted in a liquid scintillation counter to determine disintegrations per min/mL. The rate of CO_2 production was determined using the closed circuit Douglas bag method, as previously described. The rate of lysine or leucine oxidation was calculated from the rate of production of ^{14}C radioactivity in the expired breath (E) divided by plasma specific radioactivity (SA_p) times 0.81 to correct for retention of CO_2 in the bicarbonate pool (24): $O = (E/SA_p) \times 0.81$, where O is oxidation in micromoles per h, E is in disintegrations per min/h, and SA_p is in disintegrations per min/μmol. Because at steady state the rates of entry and exit of lysine or leucine from the amino acid pool are the same and equal to flux, lysine or leucine incorporation into protein can be calculated from the following equation: $Q = I + B = O + S$, where B is breakdown, I is dietary intake and equal to zero as the subject was fasting, O is oxidation, and S is incorporation into protein.

Statistical analysis

The data were analyzed using the Statistical Analysis System (SAS Institute, Cary, NJ) in the Windows environment (25). The rate of weight loss was averaged over the last 3 days of the weight maintenance and fast period and the last 2 days of the periods in which T_3 was administered. Statistical significance for T_4, T_3, rT_3, FT_4, and FT_3 was determined by averaging the last 3 days of each period. The general linear models procedure using a two-nested study design was used to determine differences between the periods with and without T_3. Contrasts were used to determine which differences between groups were significant. $P < 0.05$ was considered statistically significant.

Results

Weight was unaffected by T_3 supplementation during the weight maintenance period, as shown in Fig. 1. During the fast, the subjects lost a total of 8.6 ± 0.5 kg, or 7.5% of their prefast weight. T_3 supplementation during the fast significantly ($P < 0.05$) slowed the rate of weight loss (F, 0.99 ± 0.05 kg/day; F + T_3, 0.58 ± 0.5 kg/day). This is further demonstrated by the regression line for each period in Fig. 1; WM and WM + T_3 are combined because there is no difference between the slopes of these lines.

There was no correlation ($P = 0.07$; $r^2 = 0.12$) between BMI and serum T_3 during the WM period (Fig. 2). Also, there was no correlation ($P = 0.17$; $r^2 = 0.07$) between BMI and serum T_3 during F + T_3.

Figure 3 shows the relationship between the change in weight and serum T_3 when T_3 was administered during the fast. Although the subjects with the greatest increase in serum T_3 lost the least amount of weight, the strength of this relationship is questionable because only 18% of the variability in the data can be explained by this correlation. Four individuals had the greatest change in weight but less than a 1 pmol/L change in serum T_3, whereas one individual had the greatest change in serum T_3 with very little change in weight. For 24 of the subjects, there was an inconsistent response in weight change to a change in serum T_3.

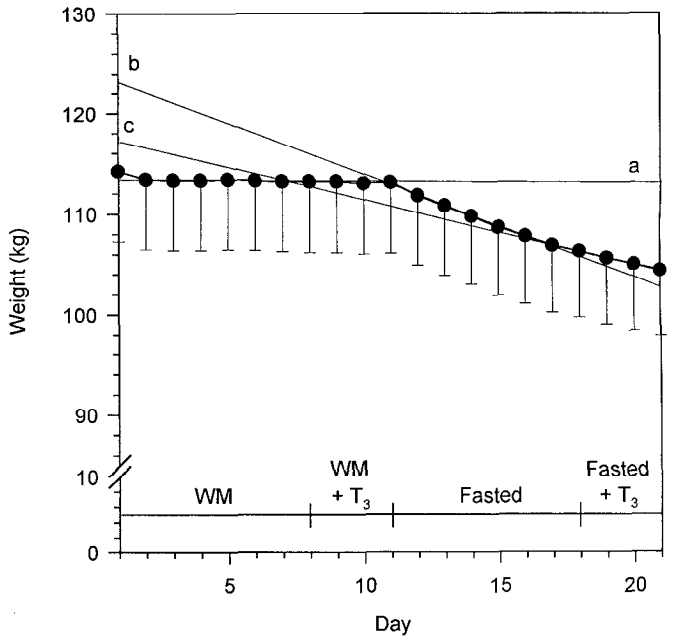

FIG. 1. Weight during WM and F periods with and without T_3 supplementation. a is the regression line ($y = 114 - 0.07x$) for WM and WM + T_3. WM and WM + T_3 were combined because there was no significant change in weight between the two periods. b is the regression line ($y = 123 - 0.94x$) for the F period. c is the regression line ($y = 117 - 0.60x$) for the F + T_3 period. Values are the mean ± SEM.

The changes in serum thyroid hormone levels during the four study periods are shown in Fig. 4. Changes in FT_3 mimicked changes in T_3, whereas changes in FT_4 mimicked changes in T_4. Serum rT_3 changes were the opposite of those in serum T_3 and FT_3.

In Table 1, the average values of the various thyroid hormones for the last 3 days of each period are shown. Values for the WM period were within the normal range. T_3 supplementation during WM significantly increased serum T_3 and FT_3 and slightly, but significantly, decreased serum rT_3, T_4, and FT_4. During fasting serum T_3 and FT_3 fell approximately 40%, whereas serum T_4 and FT_4 were unchanged compared to the WM period. Serum rT_3 increased almost 2-fold. T_3 supplementation during the fast returned serum T_3 levels to values not significantly different from those observed during WM. Serum T_4 levels were unaffected by T_3 supplementation during the fast, whereas serum rT_3, FT_3, and FT_4 increased significantly over WM values.

Glucose appearance was unaffected by T_3 supplementation during WM alone (WM, 7.67 ± 0.17 μmol/kg·min; WM ± T_3, 7.60 ± 0.17 μmol/kg·min), but fell significantly during fasting (WM, 7.67 ± 0.17 μmol/kg·min; F, 4.66 ± 0.17 μmol/kg·min; Fig. 5). T_3 supplementation during fasting increased glucose appearance slightly above fasting levels, but not to

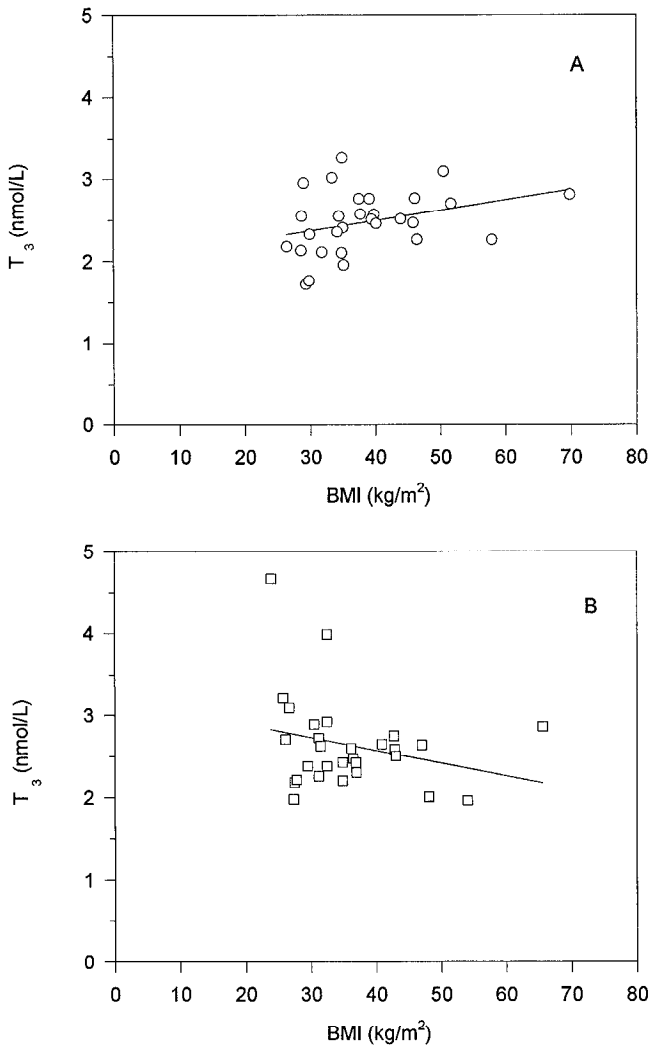

FIG. 2. Relationship between BMI and serum T_3 during the WM (A) and F + T_3 (B) periods. For the WM and F + T_3 periods, values for days 5, 6, and 7 and days 19, 20, and 21, respectively, were averaged. The regression line is $y = 127 + 0.9x$ ($P = 0.08$; $r^2 = 0.12$) for WM and is $y = 210 - 1.0x$ ($P = 0.17$; $r^2 = 0.07$) for F + T_3.

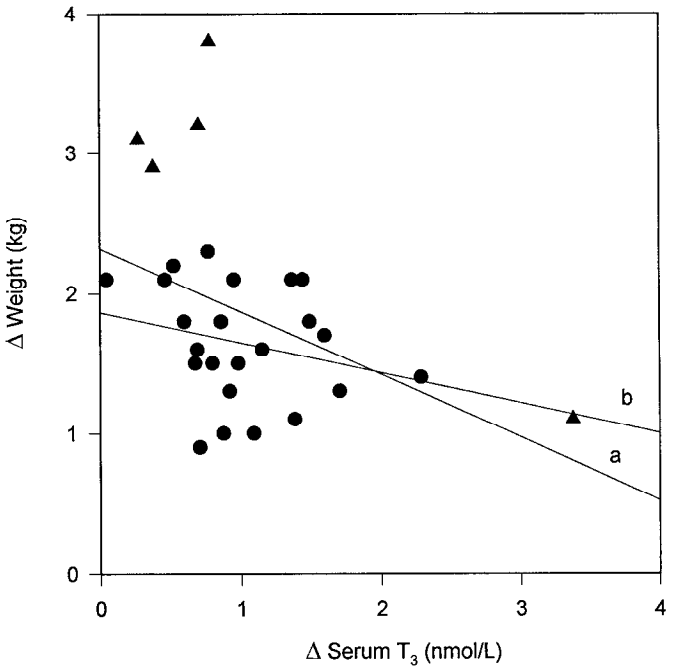

FIG. 3. Change in serum T_3 after T_3 supplementation during F *vs.* the change in weight for the same period. Δ serum T_3 is the difference between the measured value of serum T_3 for day 18 and the measured value for serum T_3 on day 21. Also, Δ weight is the difference between weight on day 18 and that on day 21. a is the regression line ($y = 2.32 - 0.04x$; $P = 0.03$; $r^2 = 0.176$) for all points shown on the graph. b is the regression line ($y = 1.86 - 0.02x$; $P = 0.25$; $r^2 = 0.06$) for all points shown on the graph except the outliers (▲); those individuals who lost more than 2.5 kg during F + T_3 and the one individual with a change in serum T_3 greater than 3 during F + T_3.

prefasting rates (F, 4.66 ± 0.17 μmol/kg·min; F ± T_3, 5.11 ± 0.11 μmol/kg·min). Serum glucose values followed the same trend. Fasting values (F, 3.4 ± 0.2 mmol/L) were significantly lower than prefasting or F ± T_3 values (WM, 4.9 ± 0.2 mmol/L; WM + T_3, 4.8 ± 0.1 mmol/L; F ± T_3, 4.1 ± 0.1 mmol/L). T_3 supplementation significantly increased F ± T_3 glucose values, although not to prefasting levels (F ± T_3, 4.1 ± 0.1 mmol/L; WM, 4.9 ± 0.2 mmol/L).

The amount of oxygen inspired per min did not change with fasting or T_3 supplementation compared to that in the WM and WM + T_3 groups (Table 2). However, the amount of carbon dioxide produced decreased significantly with the absence of caloric intake and did not change with T_3 supplementation during fasting. The respiratory quotient (RQ) was 0.8 during WM and WM + T_3, indicating a mixed fuel diet was being burned, and fell to 0.7 during the fast, indicating that the subjects were burning fat as the primary fuel source. The resting metabolic rate fell with fasting, although the change was not significantly different from the value during the WM period due to a large variation. Thyroid hormone did not affect the resting metabolic rate.

Urinary urea nitrogen excretion was unaffected by thyroid hormone supplementation during weight maintenance (WM, 300 ± 11 mmol/day; WM ± T_3, 289 ± 7 mmol/day; Fig. 6). Nitrogen intake during this time was 914 mmol/day, on the average, which was greater than that excreted in the form of urea nitrogen in the urine. Fasting, a period of zero intake of nitrogen, significantly reduced the excretion of urinary urea nitrogen (200 ± 11 mmol/day) to 67% of the prefasting values. T_3 supplementation further reduced urinary urea nitrogen excretion (168 ± 11 mmol/day) significantly by 16% below the fasting value and to 56% of the measured value for the WM period.

There was no correlation ($y = 1539 + 16x$; $P < 0.43$; $r^2 = 0.03$) between the reduction in serum T_3 from the WM to F period and the cumulative loss of urinary urea nitrogen. Also, there was no correlation ($y = 27.8 - 0.4x$; $P < 0.72$; $r^2 = 0.01$) between the excretion of urea nitrogen in the urine and the change in serum T_3 before starting and after 3 days of T_3 supplementation during the fast.

Neither urinary creatinine (WM, 13.3 ± 0.9 mmol/day; WM ± T_3, 13.3 ± 0.9 mmol/day; F, 12.6 ± 0.8 mmol/day; F ± T_3, 13.0 ± 0.9 mmol/day) nor 3-methylhistidine excretion (WM, 256 ± 29 μmol/day; WM ± T_3, 260 ± 20 μmol/day; F, 271 ± 16 μmol/day; F ± T_3, 279 ± 21 μmol/day) changed with caloric restriction or T_3 supplementation during WM or

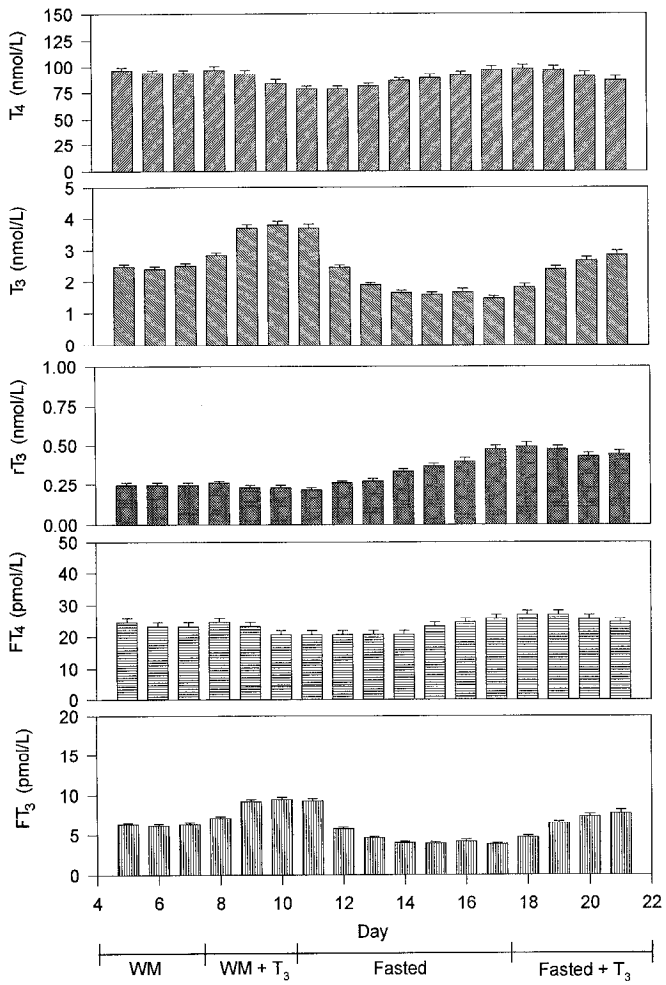

FIG. 4. Serum concentrations of T_4, T_3, rT_3, FT_4, and FT_3 during the WM and F periods with and without T_3 supplementation. Each *bar* is the mean ± SEM. The last three values for each period were averaged.

TABLE 1. Thyroid hormone values for the weight maintenance and fasting periods with and without T_3 supplementation

	WM	WM + T_3	F	F + T_3
T_4 (nmol/L)	95 ± 3[a]	86 ± 3[b]	94 ± 4[a]	94 ± 4[a]
T_3 (nmol/L)	2.5 ± 0.1[a]	3.7 ± 0.1[b]	1.5 ± 0.1[c]	2.7 ± 0.1[a]
rT_3 (ng/dL)	16 ± 1[a]	15 ± 1[b]	28 ± 1[c]	31 ± 1[d]
FT_4 (pmol/L)	24 ± 1[a]	22 ± 1[b]	24 ± 1[a]	26 ± 1[c]
FT_3 (pmol/L)	6.3 ± 0.2[a]	9.3 ± 0.2[b]	3.8 ± 0.2[c]	7.2 ± 0.3[d]

Values are the mean ± SEM. The mean is the average of the last 3 days of each period.

[a,b,c,d] Groups within a row with different superscripts are significantly different ($P < 0.05$).

F periods. Also, the ratio of urinary 3-methylhistidine to creatinine did not change (WM, 18.9 ± 1.8 μmol/mmol; WM ± T_3, 18.8 ± 1.2 μmol/mmol; F, 20.5 ± 1.0 μmol/mmol; F ± T_3, 19.8 ± 1.0 μmol/mmol).

Leucine appearance did not differ significantly among the WM, WM + T_3, and F periods (Table 3). However, T_3 supplementation during the fast significantly decreased the rate of appearance of leucine. Leucine oxidation increased significantly with fasting compared to those during WM and

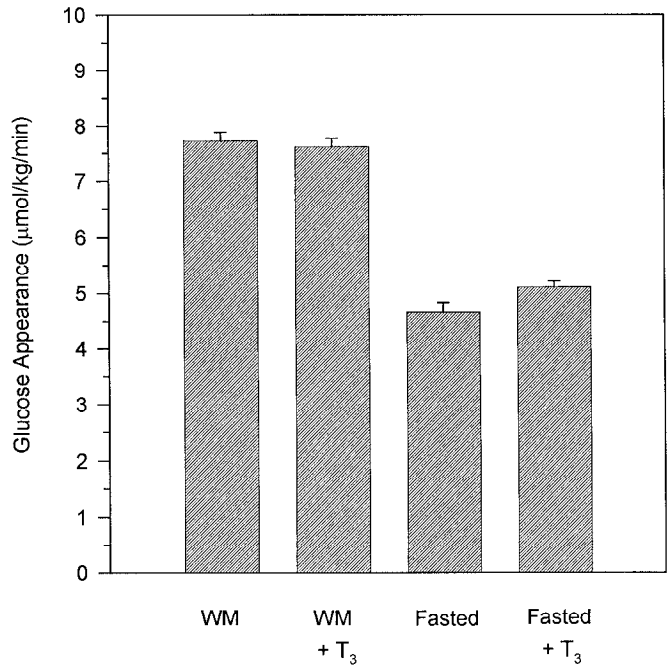

FIG. 5. Glucose appearance during WM and F periods with and without T_3 supplementation. Each *bar* is the mean ± SEM. WM and WM ± T_3 do not differ significantly. F and F ± T_3 are significantly lower than WM and WM ± T_3. F ± T_3 is significantly greater than the F value.

WM + T_3, whereas T_3 supplementation during the fast significantly reduced the rate of oxidation to prefasting levels. Leucine incorporation remained unchanged from that during the WM, WM + T_3, and F periods, but T_3 supplementation during fasting significantly reduced leucine incorporation into protein. Lysine metabolism was unaffected by fasting or T_3 supplementation.

Plasma leucine appearance correlated ($y = 31.1 + 0.13x$; $P < 0.009$; $r^2 = 0.184$) significantly and positively with urinary 3-methylhistidine excretion (Fig. 7). However, there is a lot of scatter of the data points about the line. Leucine oxidation did not correlate ($y = 3.34 + 0.002x$; $P < 0.58$; $r^2 = 0.009$) with urinary urea nitrogen excretion (data not shown).

Plasma levels of tyrosine, ornithine, lysine, histidine, and arginine did not change between WM and F periods or with T_3 supplementation (Table 4). Thyroid hormone supplementation during the WM period did not significantly affect most plasma amino acids, except for serine and cysteine, which increased, and glycine and methionine, which decreased. Fasting compared to WM significantly reduced plasma levels of aspartate, glutamine, and alanine while significantly increasing plasma levels of threonine, serine, glycine, valine, cysteine, isoleucine, and leucine.

Discussion

The goal of thyroid supplementation after adaptation to starvation was to return serum T_3 to normal prefasting values. This was achieved. We chose a short period of T_3 supplementation to avoid the hypothalamic-pituitary adjustments to exogenous hormone supplementation. We are unaware of any other studies with a similar design. Several

TABLE 2. Basal energy expenditure parameters during weight maintenance and fasting with and without T_3 supplementation

	WM	WM + T_3	F	F + T_3
O_2 consumption (L/min)	250 ± 16[a]	241 ± 19[a]	230 ± 19[a]	239 ± 21[a]
CO_2 production (L/min)	216 ± 15[a]	203 ± 17[b]	167 ± 14[c]	170 ± 14[c]
Respiratory quotient	0.86 ± 0.01[a]	0.84 ± 0.01[a]	0.72 ± 0.02[b]	0.71 ± 0.01[b]
Resting metabolic rate (kJ/day)	7357 ± 485[a]	7031 ± 564[a,b]	6496 ± 543[b]	6747 ± 581[a,b]

Values are the mean ± SEM.
[a,b,c] Groups within a row with different superscripts are significantly different ($P < 0.05$).

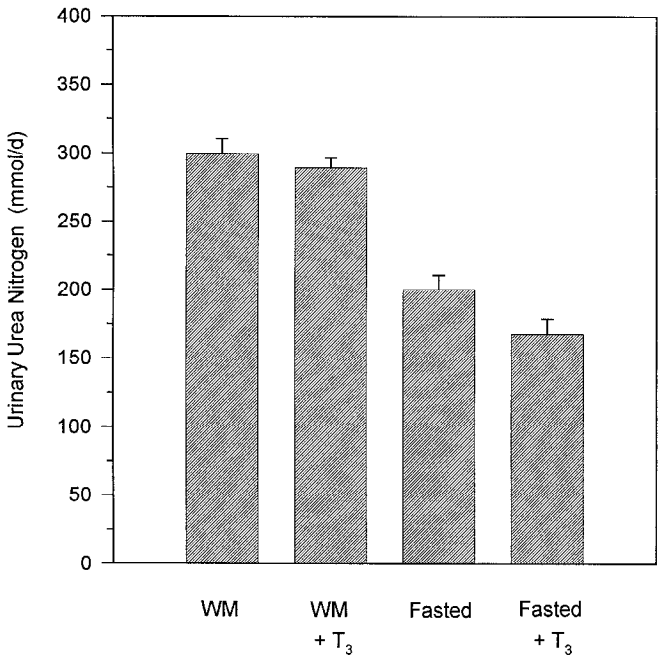

FIG. 6. Urinary urea nitrogen excretion for the various periods. Values are the mean ± SEM.

studies maintained the subject on a very low calorie diet (200–500 Cal/day) (26–28) or fasting (8, 9, 11) and supplemented with a dose of T_3 that ranged from 40–150 μg/day. In these studies, T_3 administration started when the weight loss diet started and continued for the entire weight loss period. Serum T_3 levels were nearly double the preweight loss values. Only Nair *et al.* (11) supplemented T_3 after the first week of fasting, but serum T_3 levels during the F + T_3 period were twice the value in control group before weight loss.

The changes in serum thyroid hormones during fasting in this study were consistent with those noted by other investigators (4, 26, 29–32). Serum T_3 decreased significantly, rT_3 increased, and serum T_4 did not change with caloric deprivation. Small, but statistically significant, changes in thyroid hormone levels were noted after thyroid supplementation with the weight-maintaining diet. However, these small changes were not physiologically significant in our view, because there were no changes noted in the metabolic parameters measured in this study.

Previous studies reported that T_3 administration increases the rate of weight loss during fasting or semistarvation (26, 27, 33). Our data do not agree with this, although we administered T_3 for a relatively short period of time and at physiological levels. In fact, the rate of weight loss slowed with T_3 supplementation, which was probably not the result of thyroid hormone supplementation, but due to the normal slowing of weight loss that occurs with continued caloric deprivation. Fisler *et al.* (34) fasted 22 obese male subjects for 40 days. During the first 5 days, the rate of weight loss was 1.1 kg/day; this slowed to 0.8 kg/day during the second 5-day period. The rate of weight loss continued to slow until about the fifth week of starvation, at which point it plateaued at approximately 0.5 kg/day.

Koppeschaar *et al.* (28) reported a negative correlation between the change in serum T_3 with T_3 supplementation and the amount of weight lost for 10 subjects who consumed 200 Cal for 28 days and 50 μg T_3 (Cytomel) three times a day during the last 14 days. In the present study, using all of the data points, there was a significant negative correlation between the change in serum T_3 after T_3 supplementation and the rate of weight lost during the fast. However, very little of the variation (18%) in the data was explained by regression of change in weight and change in serum T_3. For most of the subjects there was no significant relationship between weight loss during fasting and the change in the T_3 level.

Results from this study suggest that T_3 may not be the primary factor influencing oxygen consumption and resting metabolic rate, as oxygen consumption and resting metabolic rate did not change with weight loss. This agrees with the report by Krotkiewski *et al.* (29), who found no change in oxygen consumption in obese women consuming a very low calorie diet for 3 weeks. Also, Yang and Van Itallie (35) found no correlation between changes in BMR and serum T_3 concentration in six morbidly obese subjects maintained on a hypocaloric diet for 64 days.

Two independent lines of investigation support a strong relationship between carbohydrate and thyroid hormone. First, the carbohydrate content of the diet can regulate serum T_3 concentrations. Burman *et al.* (31) fasted seven obese women for 7 days, then administered 50 g carbohydrate while continuing the fast 5 more days and found that dietary carbohydrate significantly increased serum T_3, decreased rT_3, and had no effect on serum T_4. Danforth *et al.* (36) found that during long term overfeeding studies, serum T_3 was increased when carbohydrate was isocalorically substituted for fat in the diet. The second line of evidence is that changes in plasma glucose parallel changes in serum T_3. During severe caloric restriction and prolonged fasting, plasma glucose and serum T_3 decrease. Increasing serum T_3 during a fast significantly increases the plasma glucose concentration (11, 33). This study confirms these previous observations; plasma glucose decreased with fasting and increased with T_3 supplementation. The rise in plasma glucose during F + T_3 was due to an increase in hepatic glucose appearance. This

TABLE 3. Plasma leucine or lysine appearance, oxidation, and incorporation into protein as measured with L-[1-^{14}C]leucine or [U-^{14}C]lysine during the fed and fasted periods with and without T_3 supplementation

	WM	WM + T_3	F	F + T_3
Leucine				
Appearance (μmol/kg · h)	68.2 ± 8.3^a	64.3 ± 6.5^a	66.8 ± 6.8^a	56.0 ± 4.5^b
Oxidation (μmol/kg · h)	3.4 ± 0.5^a	3.2 ± 0.4^a	4.4 ± 0.4^b	3.4 ± 0.2^a
Incorporation (μmol/kg · h)	64.8 ± 7.9^a	61.0 ± 6.2^a	62.3 ± 6.4^a	52.7 ± 4.4^b
Lysine				
Appearance (μmol/kg · h)	33.6 ± 4.1^a	37.7 ± 4.9^a	32.2 ± 5.5^a	32.7 ± 4.1^a
Oxidation (μmol/kg · h)	3.3 ± 0.5^a	3.7 ± 0.4^a	3.6 ± 0.6^a	3.9 ± 0.6^a
Incorporation (μmol/kg · h)	30.2 ± 3.6^a	34.0 ± 4.7^a	28.5 ± 5.0^a	28.8 ± 3.9^a

Values are the mean ± SEM.
^{a,b} Groups within a row with different superscripts are significantly different ($P < 0.05$).

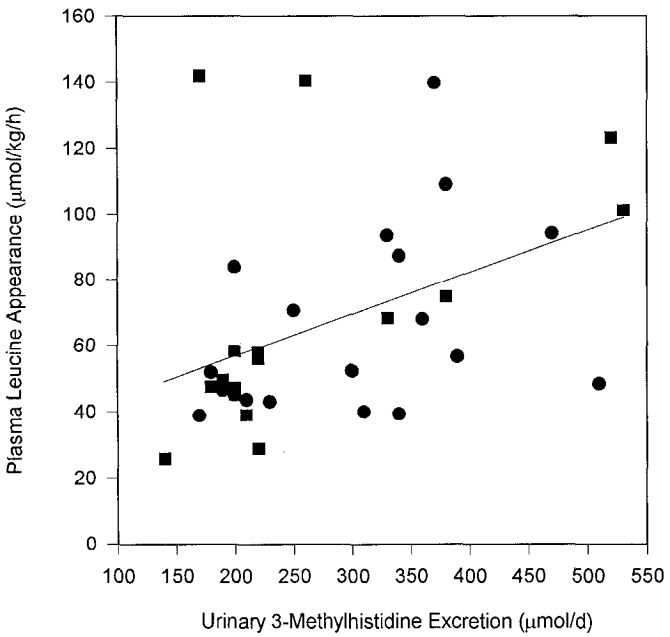

FIG. 7. Plasma leucine appearance *vs.* urinary 3-methylhistidine excretion. ■, values measured during WM; ●, values measured during F.

suggests that thyroid hormone may be involved in regulating hepatic glucose appearance during fasting.

Henson and Heber (23) reported that urea excretion decreases after 7 days of fasting. The results of this study support that finding. However, this study does not agree with other investigators who have reported that T_3 supplementation with fasting increases urea nitrogen excretion. Carter *et al.* (8) administered 300 μg T_3 daily for 144 h, fasting the subjects for the last 72 h. Compared to a control group that was fasted but did not receive T_3 supplementation, urinary urea and creatine excretions were greater in the group administered T_3. Gardner *et al.* (9) administered 5 μg T_3 every 3 h for 72 h before and 72 h during a total fast in normal male volunteers to maintain serum T_3 slightly above baseline values. This resulted in progressively increasing daily urinary excretion of urea nitrogen. For both of these studies, the fasting period was short, and T_3 was administered when fasting was initiated and throughout the fast. Results from our study suggest that T_3 supplementation after adaptation to fasting does not affect protein metabolism once the body has shifted from glycolysis to lipolysis to spare protein. By the seventh day of the fast, RQ had shifted from approximately 0.8, the RQ for a mixed diet, to 0.7, indicating that fat was the main fuel source. The significant decline in urinary urea nitrogen observed in this study probably was not secondary to T_3 supplementation, but due to the continued decline in urinary urea nitrogen observed with caloric deprivation (34).

Heber and Henson (23) previously reported that lysine appearance decreased after 7 days of fasting. In this study, lysine appearance, oxidation, and incorporation into protein did not change despite a shift from glycolysis to lipolysis and a change in serum thyroid hormone concentration. This is in agreement with the report by Winterer *et al.* (37), who reported no change in protein breakdown rates studied with ^{15}N glycine in five obese women after 7 days of fasting compared to rates measured in the fed state on either a weight maintenance or a low calorie, high quality protein diet. The reason for the difference between our current results and previously published results is not clear.

If a decrease in serum T_3 mediates the sparing of muscle protein during fasting, we expected T_3 supplementation to increase the measured parameters of protein metabolism. Instead, leucine appearance, oxidation, and incorporation decreased; lysine appearance, oxidation, and incorporation were unchanged; and urea nitrogen excretion decreased. It cannot be determined from this study whether these changes were due to continued caloric deprivation or increasing serum T_3 to prefasting levels. The changes in leucine metabolism observed in this study contrast with the report of Nair *et al.* (11), who found that 7 days of even greater levels of T_3 supplementation during the last 7 days of a 14-day fast did not change leucine metabolism. Both studies suggest that T_3 supplementation during a fast will not adversely affect protein metabolism. However, it is difficult to administer T_3 for prolonged periods to determine the more chronic effects of changes in thyroid hormone during fasting on protein metabolism without affecting the hypothalamic-pituitary axis, thus reducing endogenous thyroid hormone synthesis and neutralizing exogenous thyroid supplementation. This is supported by the work of Burman *et al.* (38), who found a slight decrease in T_4 levels in normal subjects given 5 μg T_3 every 4 h. This dose administered for 6 days during fasting did not increase 3-methylhistidine excretion, a marker of muscle catabolism. These results are consistent with our obser-

TABLE 4. Plasma amino acid levels before and after fasting with and without T_3 supplementation

Amino acid	WM	WM + T_3	F	F + T_3
Taurine	36 ± 2^a	35 ± 4^a	$45 \pm 5^{a,b}$	51 ± 6^b
Aspartate	3.8 ± 0.3^a	3.7 ± 0.2^a	3.1 ± 0.2^b	3.0 ± 0.2^b
Threonine	103 ± 8^a	102 ± 6^a	132 ± 9^b	135 ± 10^b
Serine	98 ± 5^a	86 ± 4^b	112 ± 6^c	$105 \pm 6^{a,c}$
Glutamine	72 ± 8^a	70 ± 7^a	48 ± 5^b	49 ± 4^b
Glycine	184 ± 10^a	170 ± 9^b	210 ± 15^c	220 ± 12^c
Alanine	333 ± 21^a	316 ± 18^a	218 ± 15^b	168 ± 11^c
Valine	238 ± 22^a	$251 \pm 33^{a,b}$	344 ± 36^c	280 ± 22^b
Cysteine	107 ± 7^a	124 ± 8^b	141 ± 13^b	101 ± 6^a
Methionine	$32 \pm 4^{a,c}$	25 ± 3^b	$36 \pm 4^{a,c}$	30 ± 3^c
Isoleucine	43 ± 2^a	52 ± 5^a	99 ± 6^b	80 ± 5^c
Leucine	106 ± 5^a	112 ± 8^a	202 ± 11^b	158 ± 8^c
Phenylalanine	45 ± 3^a	47 ± 2^a	48 ± 3^a	53 ± 2^b
Tyrosine	64 ± 3^a	66 ± 3^a	65 ± 5^a	67 ± 3^a
Ornithine	38 ± 2^a	40 ± 2^a	41 ± 2^a	39 ± 2^a
Lysine	178 ± 10^a	194 ± 13^a	177 ± 9^a	210 ± 19^a
Histidine	69 ± 2^a	67 ± 3^a	63 ± 3^a	64 ± 3^a
Arginine	66 ± 3^a	65 ± 3^a	62 ± 5^a	62 ± 3^a

Values are the mean ± SEM, expressed as micromoles per L.
^{a,b,c} Groups within a row with different superscripts are significantly different ($P < 0.05$).

vations, but studied fewer subjects and used different methodologies for assessing the metabolic effects of T_3.

In conclusion, we found that fasting for 7 days significantly reduced urea nitrogen excretion and glucose appearance and increased leucine oxidation, but did not affect leucine appearance or the basal metabolic rate. Short term T_3 supplementation during the fast increased glucose appearance, did not alter the basal metabolic rate, and did not increase urinary urea nitrogen excretion or leucine appearance. Thus, our results do not support the hypothesis that a fall in serum T_3 during fasting mediates protein sparing.

Acknowledgments

The authors thank Dr. Lindsey Henson for her expertise in starting this study. Also, the authors thank the nursing, dietary, laboratory, and administrative staff of the Clinical Research Center, particularly Merlyn Dubria, Mario Paredes, Pedro Morales, and Mary Grosvenor.

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