

BRIEF REPORT

Effect of Long-Term Calorie Restriction with Adequate Protein and Micronutrients on Thyroid Hormones

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Context: Caloric restriction (CR) retards aging in mammals. It has been hypothesized that a reduction in T_3 hormone may increase life span by conserving energy and reducing free-radical production.

Objective: The objective of the study was to assess the relationship between long-term CR with adequate protein and micronutrient intake on thyroid function in healthy lean weight-stable adult men and women.

Design, Setting, and Participants: In this study, serum thyroid hormones were evaluated in 28 men and women (mean age, 52 ± 12 yr) consuming a CR diet for 3–15 yr (6 ± 3 yr), 28 age- and sex-matched sedentary (WD), and 28 body fat-matched exercising (EX) subjects who were eating Western diets.

Main Outcome Measures: Serum total and free T_4 , total and free T_3 , reverse T_3 , and TSH concentrations were the main outcome measures.

Results: Energy intake was lower in the CR group (1779 ± 355 kcal/d) than the WD (2433 ± 502 kcal/d) and EX (2811 ± 711 kcal/d) groups ($P < 0.001$). Serum T_3 concentration was lower in the CR group than the WD and EX groups (73.6 ± 22 vs. 91.0 ± 13 vs. 94.3 ± 17 ng/dl, respectively) ($P \leq 0.001$), whereas serum total and free T_4 , reverse T_3 , and TSH concentrations were similar among groups.

Conclusions: Long-term CR with adequate protein and micronutrient intake in lean and weight-stable healthy humans is associated with a sustained reduction in serum T_3 concentration, similar to that found in CR rodents and monkeys. This effect is likely due to CR itself, rather than to a decrease in body fat mass, and could be involved in slowing the rate of aging. (*J Clin Endocrinol Metab* 91: 3232–3235, 2006)

CALORIC RESTRICTION (CR) slows aging in rodents, fish, worms, and insects (1). In addition, CR has beneficial health effects in both primates and humans (2–5). Although the precise mechanisms responsible for the relationship between CR and aging processes are not known, it has been hypothesized that CR-mediated changes in endocrine function and a decrease metabolic rate are important factors (1, 2).

Thyroid hormones influence cell respiration, free radical production, and energy homeostasis (6). Although T_4 is the main product secreted by the thyroid gland, most thyroid actions are mediated by T_3 . Data from studies conducted in long-lived rodents have shown that CR decreases serum T_3 concentrations, whereas serum T_4 and TSH concentrations usually remain unchanged (7, 8).

The purpose of the present study was to evaluate the thyroid hormonal profile in healthy lean and weight-stable volunteers who were consuming CR diets, containing adequate protein and micronutrients, for years. Plasma concentrations of thyroid hormones in subjects consuming a CR diet were compared with values obtained in two comparison

groups: 1) age- and sex-matched sedentary subjects consuming a Western diet (WD), and 2) age-, sex-, and body fat-matched endurance runners consuming a WD.

Subjects and Methods

Study subjects

Three groups of subjects (28 participants/group) were studied. One group (CR group) had been consuming a CR diet with adequate nutrients for a 6 ± 3 yr (range 3–15 yr) and were recruited by contacting the Calorie Restriction Society. The second group [exercising (EX) group] were endurance runners who had been running an average of 48 miles/wk (range 20–90 miles/wk) for 21 ± 11 yr (range 5–35 yr), and were recruited from the St. Louis area. The EX group was matched on age, sex, and percent body fat with the CR group. The third group (WD group) were sedentary (regular exercise < 1 h/wk) subjects, recruited from the St. Louis area who were eating a WD. The WD group was matched on age and sex with the CR and EX groups. The characteristics of the study participants are shown in Table 1. None of the participants had evidence of chronic disease, smoked cigarettes, or were taking medications or nutritional supplements that could affect the outcome variables. All participants reported weight stability, defined as less than a 2-kg change in body weight in the preceding 6 months. Serum C-reactive protein (CRP) and TNF α concentrations from 24 of 28 CR subjects and 20 of 28 WD subjects were reported previously in a study that evaluated the effects of CR on diastolic function (4), and CRP values were reported for 18 of the CR and 18 of the WD subjects in a report of the effect of CR on coronary heart disease risk factors (5). The present study was approved by the Human Studies Committee of Washington University School of Medicine, and all subjects gave informed consent before their participation.

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Abbreviations: BMI, Body mass index; CR, caloric restriction; CRP, C-reactive protein; EX, exercising; FT $_4$, free T_4 ; WD, Western diet.

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TABLE 1. Characteristics of the study subjects

	CR group (n = 28)	EX group (n = 28)	WD group (n = 28)	P value		
				CR vs. EX	CR vs. WD	EX vs. WD
Age (yr)	52.0 ± 12	52.1 ± 12	52.3 ± 10	ns	ns	ns
Sex (M/F)	24/4	24/4	24/4			
Height (m)	1.73 ± 0.1	1.75 ± 0.1	1.77 ± 0.1	ns	ns	ns
Weight (kg)	58.8 ± 5.9	68.2 ± 7.6	81.9 ± 14.6	0.003	0.0001	0.0001
BMI (kg/m ²)	19.7 ± 1.7	22.2 ± 1.9	26.0 ± 3.2	0.0001	0.0001	0.0001
TSH (mIU/liter)	1.27 ± 0.7	1.68 ± 1.0	1.24 ± 0.6	ns	ns	ns
T ₃ (ng/dl)	73.6 ± 22	94.3 ± 17	91.0 ± 13	0.0001	0.001	ns
T ₄ (μg/dl)	5.4 ± 1.5	5.1 ± 0.7	5.7 ± 0.9	ns	ns	ns
FT4 (ng/dl)	0.96 ± 0.14	1.0 ± 0.09	0.97 ± 0.22	ns	ns	ns
hsCRP (mg/liter)	0.23 ± 0.27	0.65 ± 0.76	1.11 ± 1.17	0.001	ns	ns
TNF-α (pg/ml)	0.74 ± 0.5	1.42 ± 1.3	1.54 ± 0.9	0.030	0.009	ns

Normal ranges: TSH, 0.47–5.0 mIU/liter; T₃, 70–165 ng/dl; T₄, 4.5–12 μg/dl; and FT4, 0.71–1.85 ng/dl. Values are means ± SD. M, Male; F, female; ns, not significant; hsCRP, high-sensitivity CRP.

Body composition

Total body fat mass and fat free mass were determined by dual-energy x-ray absorptiometry (QDR 1000/w; Hologic, Waltham, MA).

Dietary assessment

Participants recorded all food and beverage intake for 7 consecutive days. Food records were analyzed by using the Nutrition Data System from the Nutrition Coordinating Center of the University of Minnesota (version 4.03_31).

Thyroid hormones and markers of inflammation

Serum T₄ concentrations were determined by using fluorescence polarization immunoassay. Serum T₃, TSH, free T₃, and free T₄ (FT4) concentrations were determined by using microparticle enzyme immunoassay (Abbott Laboratories, North Chicago, IL). Serum rT₃ concentrations were determined by using RIA (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA). Commercially prepared ELISA kits were used to measure TNFα (Quantakine High Sensitive; R&D Systems, Minneapolis, MN), and high-sensitive CRP (ALPCO, Windham, NH) concentrations. The coefficients of variation of all assays were less than 10%.

Statistical analysis

One-way ANOVA was used to compare group variables, followed by Tukey *post hoc* testing when indicated. One-way ANOVA with Games-Howell was performed for distributions in which equal variances could not be assumed. Statistical significance was set at $P < 0.05$ for all tests. All data were analyzed by using SPSS software (version 13.0; SPSS Inc., Chicago, IL). All values are expressed as means ± SD.

Results

Body composition

Mean body mass index (BMI) values were different among the three groups (Table 1). Data obtained from body weight

TABLE 2. Body composition of the study subjects

		CR group (n = 28)	EX group (n = 28)	WD group (n = 28)	P value		
					CR vs. EX	CR vs. WD	EX vs. WD
Total body fat (%)	Men	6.9 ± 4.7	9.9 ± 4.7	23.7 ± 6.3		0.0001	0.0001
	Women	20.5 ± 9.9	20.0 ± 1.8	28.8 ± 7.0		ns	ns
Trunk fat (%)	Men	3.7 ± 4.4	7.0 ± 6.0	26.0 ± 8.3	ns	0.0001	0.0001
	Women	11.8 ± 8.4	13.2 ± 2.6	22.3 ± 11.0	ns	Ns	ns
Lean mass (%)	Men	92.9 ± 4.6	90.1 ± 4.7	76.3 ± 6.3	ns	0.0001	0.0001
	Women	79.4 ± 9.9	79.9 ± 1.8	71.2 ± 7.1	ns	ns	ns
Lean mass (kg)	Men	52.8 ± 4.9	59.7 ± 5.4	61.3 ± 7.5	0.001	0.0001	ns
	Women	38.9 ± 5.3	40.3 ± 3.0	39.1 ± 8.4	ns	ns	ns

Values are means ± SD. ns, Not significant.

records kept by the CR participants showed that BMI decreased by 17%, from 23.7 ± 3 to 19.6 ± 2 kg/m² during the period of CR. Total body fat and truncal fat were similar in the CR and EX groups and lower than in the WD group (Table 2).

Nutrient intake

The CR subjects consumed a balance of foods that supplied more than 100% of the recommended daily intake for all the essential nutrients. Foods with a high nutrient to energy ratio such as vegetables, fruits, nuts, dairy products, egg whites, wheat and soy proteins, and meat were consumed, whereas processed foods, rich in refined carbohydrates, free sugars, and partially hydrogenated oils, were avoided. Energy intake was lower in the CR group (1779 ± 355 kcal/d; range 1112–2260 kcal/d) than in either the EX (2811 ± 711 kcal/d; range 1935–4459 kcal/d) or WD group (2433 ± 502 kcal/d; range 1756–3537 kcal/d) ($P = 0.0001$ for CR vs. EX or WD; $P = 0.043$ for EX vs. WD). Therefore, energy intake in the CR group was 27 and 37% lower than in the WD and EX groups, respectively. The percentage of total energy intake derived from protein, carbohydrate, and fat was approximately 23, 49, and 28%, respectively, in the CR group; approximately 15, 53, and 32% in the EX group; and approximately 17, 52, and 31% in the WD group. Protein intake was higher in the CR group than the WD and EX groups ($P = 0.0001$).

Thyroid hormones

Mean serum T₃ concentration was significantly lower in the CR group than the EX or WD groups, whereas serum T₄

and FT₄, and TSH concentrations were not significantly different among groups (Table 1). Mean serum free T₃ concentration (normal range 1.45–3.48 pg/dl) was significantly lower in 10 CR subjects who had the lowest serum total T₃ concentrations than in 10 age- and sex-matched sedentary WD subjects (1.08 ± 0.46 vs. 1.68 ± 0.72 pg/ml; $P = 0.04$). However, serum rT₃ concentration (normal range 19–46 ng/dl) in 10 CR subjects who had the lowest serum total T₃ concentrations was normal and not significantly different from 10 age- and sex-matched sedentary WD subjects (26 ± 11 vs. 19 ± 4 ng/dl, respectively).

Markers of inflammation

Mean serum TNF α concentration was lower in the CR group than in the EX and WD groups, and mean serum CRP concentration was lower in the CR group than in the WD group (Table 1). Mean serum albumin concentration was similar in the CR (4.12 ± 0.3 g/dl), EX (4.09 ± 0.2 g/dl), and WD (4.16 ± 0.2 g/dl) groups. Mean serum prealbumin concentration in 10 CR subjects who had the lowest serum total T₃ concentrations was not significantly different from 10 age- and sex-matched sedentary WD subjects (26.9 ± 4.1 vs. 27.5 ± 4.5 mg/dl, respectively).

Discussion

The effect of long-term CR with adequate protein and micronutrient intake on thyroid function has not been carefully evaluated in healthy lean, weight-stable subjects. In this study, we compared the thyroid hormonal profile of men and women who were consuming a self-imposed CR diet, containing more than 100% of the recommended daily intake for all essential nutrients, for 3–15 yr, with age- and sex-matched sedentary subjects who were consuming a WD and age-, sex-, and body fat-matched endurance runners. We found that serum T₃ concentrations were lower in the CR group than sedentary or exercising subjects eating a WD. In contrast, no significant differences in serum TSH, T₄, FT₄, or rT₃ were detected between groups.

Our data suggest that the mechanism responsible for the decrease in serum T₃ concentrations induced by CR is likely related to CR itself, rather than changes in body composition. Serum T₃ concentration was approximately 30% lower in the CR than the EX group, even though percent body fat was low and similar in these groups. However, energy intake was approximately 37% lower in CR than EX subjects. It has been hypothesized that energy deprivation can modulate serum T₃ concentration by reducing the activity or concentrations of iodothyronine deiodinases, which convert T₄ to T₃ (6).

Data from a series of studies have shown that short-term (2 wk to 6 months) fasting or severe CR decreases serum T₃ and transiently increases serum rT₃ concentrations in obese subjects who are actively losing weight (9). Similar findings have been reported in a study of eight nonobese individuals who unintentionally underwent moderate CR and intense physical labor (70–80 h/wk) for 21 months (3). In addition, the results from some studies (9–11) suggest that a low-carbohydrate intake (50–120 g/d) can prevent the fall in serum T₃ and particularly the rise in serum rT₃ concentration induced by CR. Carbohydrate intake in our CR subjects was

approximately 250 g/d, which may have contributed to their normal serum rT₃ concentrations. Therefore, our findings provide evidence that long-term CR in sedentary lean, weight-stable subjects causes similar but persistent changes in thyroid hormones as previously reported during short-term fasting or CR in obese subjects who were continuing to experience active diet-induced weight loss.

Patients who have the sick euthyroid syndrome also have low serum T₃ concentrations (12). However, these patients have systemic nonthyroidal illnesses, such as cancer, myocardial infarction, severe infections, and major injuries (6, 12). Therefore, it is likely that inflammation, rather than decreased calorie intake, is responsible for the reduction in serum T₃ concentrations in patients with sick euthyroid syndrome (13). In fact, infusion of proinflammatory cytokines in human subjects decreases serum T₃ concentration (14, 15). Moreover, the decline in serum T₃ concentration induced by illness is blunted in IL-6 knockout mice, which supports the notion that cytokines are involved in the pathogenesis of the sick euthyroid syndrome (16). The mechanism responsible for this response is probably related to a cytokine-induced reduction in type I iodothyronine-5'-monodeiodinase expression, which results in decreased conversion of T₄ to T₃ in extrathyroidal tissues and decreased serum T₃ concentrations (6, 13–16). In contrast, low serum T₃ concentration was not associated with an increase in inflammatory cytokines in our CR subjects. In fact, markers of systemic inflammation, serum CRP and TNF α concentrations, were low in our CR subjects. These findings are consistent with data from CR studies conducted in rodents and monkeys, which showed that CR caused a marked decrease in markers of inflammation and a reduction in serum T₃ concentration (7, 8, 17, 18). The combination of decreased serum T₃ and reduced systemic inflammation could alter the aging process by reducing metabolic rate, oxidative stress, and systemic inflammation (1, 19, 20).

In conclusion, the results of this study demonstrate that long-term CR, with adequate intake of protein and micronutrients, in healthy lean and weight-stable subjects is associated with sustained low serum T₃ concentration, similar to that found in calorie-restricted rodents and monkeys. This effect is likely due to CR itself, rather than a decrease in body fat mass, and could be involved in slowing the rate of aging.

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L.F. participated in the concept, design, and implementation of the study, undertook plausibility testing, and drafted the report. S.K. participated in the design and drafting of the report. J.O.H. participated in the concept, design, and implementation of the study and drafting of the report. B.N.P. participated in the design and implementation of the study and drafting of the report. All the authors declared that they participated in the study as mentioned above and that they reviewed and approved the manuscript in its final version.

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The authors declare that they have no conflict of interest in connection with this paper.

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