

Dietary Selenium Intake Modulates Thyroid Hormone and Energy Metabolism in Men^{1,2}

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ABSTRACT Most studies of selenium and thyroid hormone have used sodium selenite in rats. However, rats regulate thyroid hormone differently, and selenite, which has unique pharmacologic activities, does not occur in foods. We hypothesized that selenium in food would have different effects in humans. Healthy men were fed foods naturally high or low in selenium for 120 d while confined to a metabolic research unit. Selenium intake for all subjects was 47 $\mu\text{g}/\text{d}$ (595 nmol/d) for the first 21 d, and then changed to either 14 ($n = 6$) or 297 ($n = 5$) $\mu\text{g}/\text{d}$ (177 nmol/d or 3.8 $\mu\text{mol}/\text{d}$) for the remaining 99 d, causing significant changes in blood selenium and glutathione peroxidase. Serum 3,3',5-triiodothyronine (T_3) decreased in the high selenium group, increased in the low selenium group, and was significantly different between groups from d 45 onward. A compensatory increase of thyrotropin occurred in the high selenium group as T_3 decreased. The changes in T_3 were opposite in direction to those reported in rats, but were consistent with other metabolic changes. By d 64, the high selenium group started to gain weight, whereas the low selenium group began to lose weight, and the weight changes were significantly different between groups from d 92 onward. Decreases of serum T_3 and compensatory increases in thyrotropin suggest that a subclinical hypothyroid response was induced in the high selenium group, leading to body weight increases. Increases of serum T_3 and serum triacylglycerol accompanied by losses of body fat suggest that a subclinical hyperthyroid response was induced in the low selenium group, leading to body weight decreases. J. Nutr. 133: 3443–3448, 2003.

KEY WORDS: • selenium • thyroid • thyrotropin • body weight • energy metabolism

Selenocysteine is an essential component of several enzymes. There are four selenium-dependent glutathione peroxidases known in humans (1–4). They are encoded by separate genes and protect against oxidative damage by reducing hydrogen peroxide and other hydroperoxides at the expense of glutathione. In humans, there are three forms of thioredoxin reductase, an antioxidant enzyme important in regulation of the cell cycle that was recently shown to be a selenocysteine-containing enzyme (5), as was selenophosphate synthetase, which forms the activated selenium precursor for synthesis of selenocysteine (6). Many other selenocysteine-containing pro-

teins have been reported, but their functions are not known (7–10).

A major advance in our understanding of selenium's role in metabolism began with the discovery that type I iodothyronine deiodinase, the enzyme responsible in humans for most of the peripheral conversion of thyroxine (T_4)⁴ to the active form 3,3',5-triiodothyronine (T_3), is a selenoenzyme (11,12). More recently it was discovered that the type II deiodinase (responsible for T_4 conversion to T_3 in the brain) and the type III deiodinase (the inner ring deiodinase responsible for deactivating T_4 and T_3) are also selenocysteine enzymes (13,14). Extracellular glutathione peroxidase is expressed at a high level in thyroid follicular cells and is excreted into the follicular lumen, where indirect control of thyroglobulin iodination is thought to occur by regulation of hydrogen peroxide concentrations (15). Thus, selenoenzymes may modulate or control many aspects of thyroid hormone metabolism: iodination of thyroglobulin in the thyroid gland; peripheral synthesis of T_3 from T_4 ; degradation of T_4 to 3,3',5'-triiodothyronine ("reverse T_3 ", rT_3); inactivation of T_3 to 3,3'-diiodothyronine; and regulation of thyroidal activity by the pituitary-hypothalamic axis (16). Thyroid hormone metabolism is sensitive to the total energy content of the diet and the amount of carbo-

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⁴ Abbreviations used: rT_3 , 3,3',5'-triiodothyronine ("reverse T_3 "); T_3 , 3,3',5-triiodothyronine; T_4 , thyroxine; TSH, thyroid-stimulating hormone, thyrotropin.

hydrate (17), and it may also be affected by other dietary components, including iodine (18), iron (19), zinc (20), copper (21), manganese (22) and methionine (23), as well as certain goitrogenic substances such as cyanide compounds in cassava (24).

In adults, the major effects of thyroid hormone are on energy metabolism. In the hyperthyroid state, thermogenesis, and thus the basal metabolic rate, is increased and body weight tends to decrease. In the hypothyroid state, thermogenesis and basal metabolic rate are decreased and body weight tends to increase. There are effective treatments available for both hyperthyroidism and hypothyroidism (25), but the significance and treatment of subclinical hyperthyroidism and hypothyroidism remain controversial (26,27). Genes related to energy metabolism whose expression is known to be regulated by T_3 include malic enzyme in the liver (28), α -myosin heavy chain in heart (29), uncoupling protein-3 in skeletal muscle (30), type I deiodinase in liver (31) and thyroid-stimulating hormone (thyrotropin, TSH) in the pituitary (32).

Studies of selenium and thyroid hormone metabolism have been conducted in rats. Unfortunately, studies in rats cannot be extrapolated to humans because circulating T_3 is produced mainly by deiodination of T_4 in liver in humans (17), but comes primarily by release from the thyroid in rats (16). The human and rat type I deiodinase enzymes are also different in several respects, including amino acid sequence, molecular weight (33) and sensitivity to inhibition with propylthiouracil (34). Most studies of selenium and thyroid hormone have used sodium selenite as the source of dietary selenium. However, selenite is an insignificant source of selenium in human diets (except for some selenium supplements) and possesses potent pharmacologic activities unrelated to the nutritional requirement for selenium, including prooxidant (35), insulin-mimetic (36), glutathione-depleting (37) and RNA synthesis-inhibiting (38) activities. On the other hand, selenium in food occurs primarily as derivatives of the amino acids selenocysteine and selenomethionine (39–41). Selenium is catabolized to Se^{-2} and used for de novo synthesis of selenocysteine-containing proteins, which express selenium's nutritional effects as enzyme activities (41). Selenium from selenomethionine in excess of nutritional requirements is incorporated nonspecifically into proteins in place of methionine, apparently without regulation, whereas excess selenium in other forms tends to be excreted efficiently in the urine. We hypothesized that the effects of selenium in food in humans would be different from the reported effects of selenite in rats.

We fed 11 men a controlled diet of conventional foods with naturally high or low selenium contents for 120 d while confined to a metabolic research unit to identify the metabolic effects in humans of dietary selenium as it occurs naturally in foods. In this report, we present results describing novel effects of these diets on thyroid hormone and energy metabolism, body weight and body composition.

SUBJECTS AND METHODS

Subjects and diets. Healthy male volunteers ($n = 12$) were recruited and confined in a metabolic research unit for 120 d under 24-h supervision by staff members and fed a diet composed of conventional foods naturally high or low in selenium as previously described (42). Because this study was not originally intended to study energy metabolism, if body weight subsequently changed by $>1\%$ from baseline, energy intake was adjusted in increments of 0.4 MJ/d. When energy intake was changed, all components of the diet were adjusted proportionally such that the relative composition of the diet did not change. This led to the following energy intake changes in the low selenium group during the intervention period: 3 subjects'

energy intakes were held constant throughout; one subject's energy intake was increased by 0.4 MJ/d on d 63 and then increased by a further 0.4 MJ/d on d 92; another subject's energy intake was decreased by 0.4 MJ/d on d 63 and then decreased by a further 0.4 MJ/d on d 92; and, a third subject's energy intake was increased by 0.4 MJ/d on d 39, for a net increase of 0.07 MJ/d in the low selenium group's mean energy intake. In the high selenium group during the intervention period, 3 subject's energy intakes were held constant throughout, one subject's energy intake was decreased by 0.4 MJ/d on d 70, and another subject's energy intake was increased by 0.8 MJ/d on d 96, for a net increase of 0.08 MJ/d in the high selenium group's mean energy intake. These deliberate changes in energy intake were similar in magnitude to the random weighing errors and menu variations in energy content, which caused daily energy intake to vary with a SD of 0.075 MJ/d.

Because the amounts and chemical form of selenium given to the subjects as stable isotopes on d 110 were different from the selenium in the foods, a blood sample was obtained from all subjects the morning of d 110 before the stable isotope was administered, and only the biochemical data up to d 110 are presented here. The weight changes were already significant before the stable isotope administration and continued the same trend afterwards; thus, these data are presented for the entire 120-d study period.

Laboratory measurements. Blood samples were collected at 0700 h, after an overnight fast of 12 h. Blood samples for clinical chemistries were clotted, and the serum was separated by centrifugation at $1200 \times g$ for 15 min and refrigerated until analyzed each night at a reference laboratory ("Chemzyme Plus," Smith-Kline Beecham, Santa Cruz, CA). Aliquots of serum were stored at -70°C . Total T_4 , total T_3 and total rT_3 were measured by RIA in 12 mm $\times$ 75 mm tubes (Coat-A-Count, Diagnostic Products, Los Angeles, CA), and TSH was measured by immunoradiometric assay (Coat-A-Count IRMA, Diagnostic Products).

Body composition measurements. Subjects were weighed daily between 0700 and 0715 h, after urinating to empty their bladders and before eating breakfast. Subjects wore standard hospital gowns for all weight measurements. Body composition was determined in wk 3, 10 and 17 by total body electrical conductivity using the HA-2 body composition analyzer (EM-SCAN, Springfield, IL). A prediction equation specific for healthy men was used to estimate fat-free mass from body conductivity (43). Body fat mass was calculated by subtracting fat-free mass from body weight.

Energy expenditure and utilization. Resting metabolic rate was measured in wk 3, 10 and 17. All measurements were made in the morning before breakfast after a 12-h overnight fast. Subjects rested quietly in a semireclined position for 20 min, and then measurements of oxygen consumption and carbon dioxide production were taken during the next 15 min using an automated respiratory gas exchange system (Model 2900, SensorMedics, Anaheim, CA). The system was calibrated with standard gas mixtures. Resting metabolic rate was calculated using the Weir equation, including a correction for urinary nitrogen (44). Pulse, respiration and temperature were measured once a week in the morning before rising. Heart rates were measured for 24 h periods during wk 3, 10 and 17 using Holter electrocardiograph recorders (Del Mar Avionics, Irvine, CA).

Statistical analysis. Effects of dietary selenium were determined using repeated-measures ANOVA, controlling for each subject's baseline value, or by a t test of the within-subject changes when only a baseline and final measurement were made. The ANOVA calculations were performed with BMDP 7.0 program 2V, Analysis of Variance and Covariance with Repeated Measures (Los Angeles, CA). The ANOVA included a complete model: Selenium, Time, Covariate (baseline value), and Selenium $\times$ Time. A two-tailed probability ≤ 0.05 was considered significant. Effects of food selenium were detected as significant Selenium main effects or Selenium $\times$ Time interactions, and the Student-Newman-Keuls multiple comparison test was used to identify significant differences between the groups at individual time points. The estimates of data variability are SD, unless otherwise noted.

RESULTS

Experimental diets. Controlling the geographic source of the rice and beef in otherwise identical diets resulted in

TABLE 1

Effects of low selenium and high selenium diets on thyroid status and body composition in men^{1,2}

	Low Se diet		High Se diet		Statistical analysis ³		
	Baseline ⁴	Final	Baseline	Final	Se	Time	Se × Time
T ₃ , nmol/L	1.57 ± 0.25	1.64 ± 0.16	1.82 ± 0.36	1.57 ± 0.07	0.013	—	0.048
T ₄ , nmol/L	118 ± 26	90.3 ± 6.6	113 ± 15	86.8 ± 12.7	—	0.033	—
rT ₃ , nmol/L	0.43 ± 0.10	0.30 ± 0.05	0.42 ± 0.07	0.30 ± 0.08	—	<0.001	—
TSH, mU/L	1.69 ± 0.30	1.77 ± 0.46	2.25 ± 0.81	2.96 ± 1.05	—	0.011	0.031 ⁵
Triacylglycerol (as triolein), mmol/L	1.13 ± 0.58	1.22 ± 0.89	1.04 ± 0.23	0.88 ± 0.20	0.025	—	—
Body weight, kg	74.9 ± 9.8	74.4 ± 9.0	73.5 ± 12.6	74.2 ± 12.7	—	0.030	0.0001
Fat-free mass, kg	63.0 ± 8.2	64.3 ± 8.0	59.8 ± 7.2	61.2 ± 7.7	—	0.045	—
Fat mass, kg	12.1 ± 4.9	10.5 ± 4.3	13.8 ± 11.7	13.3 ± 11.6	—	—	0.021 ⁶

¹ Values are means ± SD, *n* = 6 (low Se) and 5 (high Se).² Abbreviations: T₃, 3,3',5'-triiodothyronine; T₄, thyroxine; rT₃, 3,3',5'-triiodothyronine ("reverse T₃"); TSH, thyroid-stimulating hormone, thyrotropin.³ Values are *P*-values from repeated-measures ANOVA controlling for baseline values, BMDP Program 2V.⁴ Values at the end of the 21-d stabilization period.⁵ NS in repeated-measures ANOVA, *P* from *t* test of within-subject changes in high selenium group vs. low selenium group.⁶ Paired *t* test in low selenium group; test was not significant in the high selenium group.

constant daily selenium intakes of 47, 14 and 297 µg/d (595, 177 and 3760 nmol/d) from the stabilization, low selenium and high selenium diets, respectively, at the mean energy intake of 11.7 MJ/d (42). These intakes are similar to the amount required to maintain glutathione peroxidase activity in plasma (45), less than the minimum human requirement to prevent Keshan disease (46) and similar to typical human intakes in South Dakota (47), respectively. The low selenium diet and the high selenium diet significantly altered tissue selenium concentrations and glutathione peroxidase activities, reflecting physiologic changes in selenium nutritional status (42). The low selenium diet decreased selenium concentration by 34% in plasma, 26% in RBC and 19% in skeletal muscle, whereas the high selenium diet increased selenium concentrations 85, 66 and 38%, respectively, in these tissues (42).

Thyroid hormone status. The high and low selenium diets led to changes in serum T₃ that were significantly different between groups (*P* = 0.013; Table 1) (Fig. 1). The maximum serum T₃ response was observed at d 45, the first measurement after the diet change, with a 14% increase in the low selenium group and a 23% decrease in the high selenium group. The changes in serum T₃ remained significantly different throughout the study, ending 8% higher and 11% lower in the low selenium and high selenium groups, respectively. Serum TSH increased significantly only in the high selenium group (*P* = 0.031, *t* test of within-subject changes) (Fig. 2). The increase of TSH on d 45 was significant in the high selenium group. T₄ and rT₃ concentrations in serum did not differ between groups, although the concentrations of these iodothyronines declined by 14 and 30%, respectively, in both groups during the study (Table 1). All thyroid variables measured were within the normal range in all subjects at all time points. There was no difference between groups in total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, glucose, urea nitrogen or total protein in serum (data not shown). The clinical blood chemistry values were within normal ranges for all subjects at all time points and did not show any other changes attributable to the diet (42).

Body weight and composition. (Table 1). Body weights were stabilized during the 21-d baseline period and remained stable through d 57 (Fig. 3). However, by d 64, body weights in the high selenium group began to increase relative to the low selenium group. This difference became significant by d 92

and remained so for the rest of the study (*P* = 0.0001, Se × Time interaction). Body fat mass decreased by a mean of 1.7 ± 1.2 kg in the low selenium group (paired *t* test, *P* = 0.021). Fat-free mass increased by a mean of 1.3 ± 0.8 kg in both groups. Serum triacylglycerol concentrations (Fig. 4) increased when T₃ concentrations increased in the low selenium group and decreased when T₃ concentrations decreased in the high selenium group (*P* = 0.025, Se main effect), indicating a difference in fat metabolism between the high and low selenium groups. The groups did not differ in energy intake, resting oxygen consumption, respiratory exchange ratio, 24-h heart rate, mean arterial blood pressure or urinary nitrogen excretion (data not shown).

DISCUSSION

The results of this study suggest that dietary selenium modulated thyroid hormone metabolism and serum T₃ concentrations, which led to changes in energy metabolism and

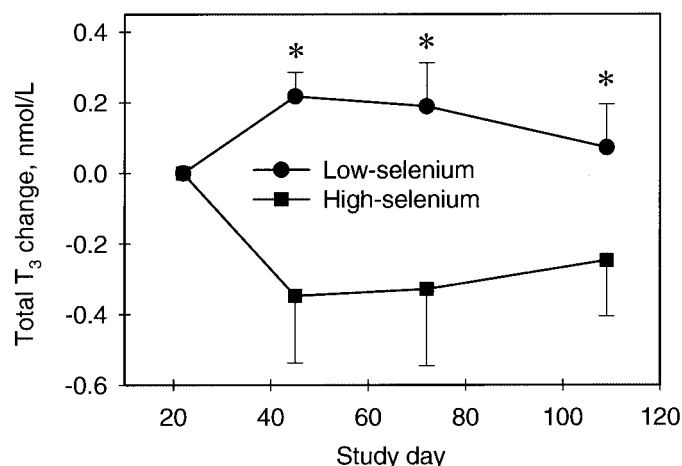


FIGURE 1 Changes in serum 3,3',5'-triiodothyronine (T₃) concentration in men consuming the high selenium diet (*n* = 5) or the low selenium diet (*n* = 6). Points represent the group mean ± SEM. *Significantly different from the high selenium group at that time point, *P* < 0.05.

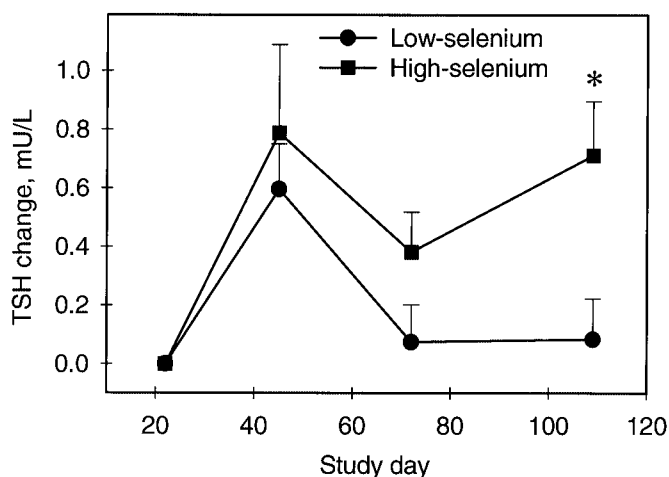


FIGURE 2 Changes in serum thyroid-stimulating hormone (TSH) concentration in men consuming the high selenium diet ($n = 5$) or the low selenium diet ($n = 6$). Points represent the group mean $\pm$ SEM. *Significantly different from the low selenium group at that time point, $P < 0.05$.

subsequent changes in body weight and composition. In the high selenium group, decreases in serum T_3 and compensatory increases in serum TSH suggest that a subclinical hypothyroid response was induced by the high selenium diet and that decreased energy expenditure caused the observed weight gain. In the low selenium group, increases in serum T_3 and serum triacylglycerols accompanied by losses of body fat suggest that a subclinical hyperthyroid response was induced by the low selenium diet and that increased energy expenditure caused the observed weight loss. Although a role for selenium in thyroid hormone metabolism has been known for some time, this appears to be the first report of dietary selenium altering energy metabolism in humans. The increased TSH in the high selenium group and the body fat loss in the low selenium group indicate that these were physiologically important changes in thyroid status.

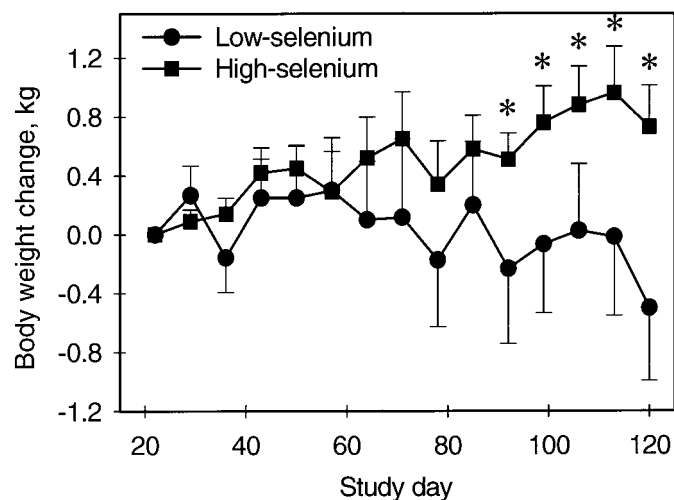


FIGURE 3 Changes in body weight in men consuming the high selenium diet ($n = 5$) or the low selenium diet ($n = 6$). Points represent the group mean $\pm$ SEM. Weights were recorded weekly on Monday mornings. *Significantly different from the low selenium group at that time point, $P < 0.05$.

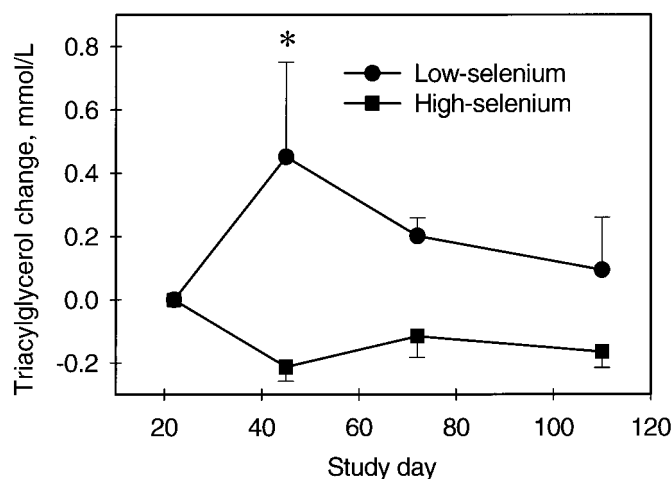


FIGURE 4 Changes in serum triacylglycerol concentration (as triolein) in men consuming the high selenium diet ($n = 5$) or the low selenium diet ($n = 6$). Points represent the group mean $\pm$ SEM. *Significantly different from the high selenium group at that time point, $P < 0.05$.

Most selenium depletion studies in rats have noted increased T_4 and rT_3 , and decreased T_3 (34,48). In the present study, only T_3 responded to selenium and in the opposite direction, increasing when selenium was restricted and decreasing when selenium was increased. This difference may be due to the fact that serum T_3 in humans comes mainly by deiodination of T_4 in liver (17), whereas serum T_3 in rats is released mainly preformed from the thyroid (16), which may be related to differences in the regulation of type I activity by selenium in thyroid and liver (49) as well as differences in the properties of the type I enzymes in humans and rats (33,34). There is, however, one report that high dietary selenium increased thyrotropin in rat serum (50), consistent with our observations in humans. It is also relevant that most rat studies induced a state of profound selenium deficiency, with glutathione peroxidase activities suppressed by as much as 90%, whereas we induced only a mild depletion, with only a 12% decrease in plasma glutathione peroxidase activity in the low selenium group.

The effects of selenium depletion in rats have been rationalized as reflecting decreased expression of the peripheral type I selenodeiodinase enzyme responsible for converting T_4 to T_3 and rT_3 to 3,3'-diiodothyronine (11). However, there is at least one human study that suggests that type I selenodeiodinase may be regulated by selenium oppositely from rats. Mothers in seleniferous areas of Venezuela with higher blood selenium tended to have lower T_3 , which was attributed to a depression of type I selenodeiodinase activity by high dietary selenium intakes (51), consistent with the decrease in T_3 in our high selenium group. There are also a few reports of effects of selenium on thyroid hormones different from what we observed, but only in persons with health problems, i.e., severely selenium-deficient phenylketonuric children (52), cystic fibrosis patients (mean age 13.8 y) (53) and institutionalized elderly (54).

Circulating concentrations of T_4 and rT_3 decreased by ~ 14 and 30%, respectively, in both groups during the study. Decreased thyroid hormone concentrations are frequently observed in confined human metabolic studies and may be related to the carbohydrate content of the experimental diets (55) or to the decreased dietary iodine intake compared with the subjects' estimated prestudy diets (18). However, the T_3 , T_4 , rT_3 , TSH (Table 1) and other clinical chemistry values

(42) were within normal ranges for all subjects at all times. Because we used real foods from different parts of the world, we cannot completely exclude the possibility that some constituent of the diet other than selenium influenced the effects we observed. However, each of the three experimental diets was analyzed for all of the dietary components known to affect thyroid hormone or energy metabolism and no differences exceeding the analytical error of the methods were found (42).

The weight changes observed in this study were consistent with the known metabolic effects of thyroid hormone and with the observed changes in serum T_3 . Treatment of hypothyroidism (56,57) and T_3 administration (58,59) cause weight loss in humans, and hypothyroidism (57) and treatment of hyperthyroidism (56) cause weight gain. Although there were some minor changes made to the subjects' energy intakes that might have affected T_3 (17), there is little basis to suspect this was responsible for the observed weight changes. The increase in mean energy intake was similar in both groups (0.07–0.08 MJ/d) and is too small to account for the different weight changes between groups. In addition, the low selenium group lost weight despite a slight increase in energy intake, and the weight gains in the high selenium group were already significant before any subject's energy intake was increased. Furthermore, the mean cumulative energy intake increase in the high selenium group of 1.92 MJ per subject was considerably smaller than the energy equivalent of the mean weight gain of 0.7 kg, even if it were all lean tissue.

We observed no changes in resting metabolic rate or body temperature that would indicate a change in the basal rate of energy expenditure. However, the daily energy balance would have had to change by only ~ 0.25 MJ/d to account for the mean weight gain of 0.7 kg (even if it were all fat tissue), a shift that would not have been detectable with the energy expenditure measurements used. There have been other reports of T_3 -induced weight losses in humans without significant changes in basal metabolic rate (58) or oxygen consumption (59). The study protocol prescribed two 3.2-km walks every day and prohibited any other form of exercise. Nevertheless, the subjects may have had significantly different energy expenditures due to voluntary activities that were not reflected in the resting oxygen consumption rates or the 24-h heart rates. Any such effect would have been randomly distributed between the two groups, unless it was due to the diets. There is at least one possible mechanism whereby selenium could change voluntary activity levels and affect energy balance independently of thyroid hormone, i.e., a beneficial effect of dietary selenium on mood, including anxiety, depression and tiredness (60,61). No significant changes in mood scores were observed in our subjects (62). However, we cannot rule out the possibility that high selenium intakes decreased anxiety, leading to less voluntary activity ("fidgeting"), a positive energy balance and weight gain, or vice versa.

Although the increased serum triacylglycerol and decreased body fat in the low selenium group suggest that lipolysis was increased by serum T_3 as has been reported by others (57,63,64), the respiratory gas exchange ratio did not indicate any significant changes in carbohydrate or fat utilization rates after the baseline period. This was not unexpected, however, because the changes in daily carbohydrate and fat utilization rates required to explain the observed changes in body composition would have been too small to detect by the indirect calorimetry methods used. Increased serum triacylglycerol driven by thyroid hormone has also been reported in selenium-deficient rabbits fed a high fat diet (49). Our observation that weight loss in the low selenium group was not accompanied by loss of lean tissue is consistent with previous reports of T_3 -induced weight loss without changes in nitrogen balance (59) or protein turnover (58).

These small changes in body weight seem unimportant for healthy adult men. It is tempting to speculate that the body's homeostatic mechanisms would eventually adapt to a high selenium intake and restore energy balance and weight maintenance. On the other hand, a simple extrapolation suggests that if the effect of high dietary selenium were to persist, it could cause a weight gain of up to 12 kg in 5 y. This would be a noteworthy rate of weight increase compared with the typical weight gain in men of 1.4–4 kg in 5 y (65–67). This potential adverse effect of dietary selenium deserves further attention in light of the report of dramatic cancer-preventive benefits from supplementation with $200 \mu\text{g/d}$ ($2.5 \mu\text{mol/d}$) of selenium (68), corresponding to a total selenium intake similar to that of our high selenium group. On the other hand, selenium-induced weight gain might be an unanticipated benefit of selenium treatment in current and proposed clinical trials for AIDS (69) and cancer (68).

The results suggest that changes in the activities of selenoenzymes involved in thyroid hormone metabolism led to shifts in circulating T_3 concentrations and perturbations of the pituitary-thyroid axis. The resulting subclinical hypothyroid and hyperthyroid responses caused changes in the expenditure of metabolic energy leading to gradual changes in body weight and composition, which, over time, might be physiologically important. Much more work will be required to determine the significance of these clinical observations to the general population and to adequately assess the potential risks and benefits of high intakes of selenium from food.

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