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Serum thyroid hormone concentrations may increase during carotenoid depletion of healthy adult women

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Vitamin A and thyroid hormone status are interrelated. Much of vitamin A is derived from dietary carotenoids, so we investigated whether carotenoid depletion might influence thyroid hormone status. Nine healthy adult women were fed a low carotenoid diet (58 μg/day) for 68 days then repleted with the same diet supplemented with 15,000 μg of carotenoid/day for 28 days. The women lived on a metabolic unit, where their dietary intakes, exercise, and activities were controlled. Serum thyroid stimulating hormone (TSH) increased significantly after 39 days of carotenoid depletion ($P < 0.02$), followed by significant increases in serum thyroxine (total T4), then free thyroxine (FT4), and free triiodothyronine (FT3; all $p < 0.02$). TSH and total T4 concentrations stabilized during carotenoid repletion but remained significantly elevated over baseline. Serum triiodothyronine (total T3), transthyretin, and total protein concentrations were not influenced by carotenoid depletion. Thus, carotenoid depletion may induce increases in some serum thyroid hormone concentrations in healthy young adult women. (J. Nutr. Biochem. 6:613–617, 1995.)

Keywords: thyroxine; triiodothyronine; thyroid stimulating hormone; women; carotenoids

Introduction

Thyroid function appears to be influenced by vitamin A status. For example, vitamin A deficiency increases serum thyroxine and triiodothyronine concentrations.[1–4] Triiodothyronine nuclear receptors and messenger RNA content are influenced by vitamin A status[5] as well as thyroid-stimulating hormone messenger RNA.[6] Furthermore, transthyretin (prealbumin) can transport both retinol-binding protein (the major carrier of vitamin A from its storage site in the liver to the tissues) and thyroxine.[7–9] Conversely, diseases of the thyroid influence retinol-binding protein concentrations, vitamin A metabolism, and status.[10–12] Finally, thyroid hormones facilitate vitamin A uptake into some tissues such as the testis.[13]

A few studies have reported that thyroid hormone concentrations may influence carotenoid status. Aktuna et al.[14] reported that hyperthyroidism accelerated the conversion of beta-carotene to vitamin A and that the skin yellowing characteristically seen in hypothyroidism is caused by accumulations of beta-carotene. They found that people with hypothyroid function had significantly higher serum carotene concentrations than euthyroid or hyperthyroid patients. Serum retinol concentrations were not different in these groups. Concentrations of carotenoid pigments in salmon are influenced by thyroxine.[15] Thyroxine and thiouracil influence carotenoid utilization in chicks.[16] We measured total T4, total T3, free T4 (FT4), free T3 (FT3), thyroid-stimulating hormone (TSH), vitamin A, carotenoid, and transthyretin concentrations to investigate the influence of carotenoid depletion on hormone status in a controlled metabolic unit study of healthy young adult women.

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Methods and materials

Details of the study protocol and diet are reported elsewhere.[17] Briefly, nine healthy adult women (aged 18–42 years) lived on the metabolic research unit of the Western Human Nutrition Research Center for 100 days during May through August, 1992. They were fed a low carotenoid diet that contained approximately 58 μg/day of total carotenoids (mostly as beta-carotene). This diet was supplemented with 1,500 μg/day of beta-carotene (in the form of Dry Carotene Beadlets, Hoffmann-LaRoche, Inc., Nutley, NJ USA) for the first 4 days of the study (called the baseline period), and 15,000 μg/day of beta-carotene (as Dry Carotene Beadlets) for the last 28 days of the study (called the repletion period). An additional supplement of mixed carotenoids (Mixed Carotenoid Complex, Neo-Life Company of America, Fremont, CA USA) was given to the subjects during the last 12 days of the study. The diet was a 4-day rotational diet of natural foods that contained adequate levels of nutrients, including vitamin A (content estimated as 1,100 retinol/Eq/day by reversed-phase high performance liquid chromatography [HPLC] analysis). The diet contained 58% carbohydrate, 33% fat, and 14% protein, with a polyunsaturated/saturated fat ratio of 1.4. The women lived on the metabolic unit 24 hr a day, 7 days a week. Their diet, exercise, and activities were controlled so that no significant variations occurred throughout the study.

Total T4, total T3, free T4, free T3, and TSH were measured by radioisotope kits (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA USA) on a Packard 500 gamma counter (Packard Instruments, Grove Hill, IL USA) according to manufacturer's specifications. Total T4 and total T3 concentrations were assayed in duplicate. Free T3, free T4, and TSH concentrations were assayed once, because we did not have enough serum left for duplicate assays. Normal ranges and CVs for these kits were: 58–161 nmol/L, CV 6% for total T4; 0.3–4 μIU/mL, CV 4% for TSH IRMA; 9–25 nmol/L, CV 10% for FT4; 3–9 pmol/L, CV 6% for total T3; and 1.8–6 pmol/L, CV 5% for FT3. Lyophilized human serum controls (RIATRACPLUS tri-level assayed controls; Becton-Dickinson Advanced Diagnostics, Sparks, MD USA) were used for quality control measurements of the accuracy and precision of these assays. Transthyretin (prealbumin) concentrations were measured by automated immunoprecipitin analysis (Antibody Reagent Set II for Prealbumin, SPQ Test System, Atlantic Antibodies, INCSTAR, Stillwater, MN USA) on a COBAS FARA centrifugal analyzer (Roche Diagnostic Systems, Hoffmann-LaRoche Inc.) according to manufacturer's guidelines. Serum samples were frozen and stored at −70°C, then thawed and assayed randomly. Serum total T3 and total T4 assays were conducted within 3 months of sample collection. Serum FT4, FT3, and TSH were assayed later, after approximately 18 months of storage.

Serum retinol and carotenoid concentrations were measured by reversed-phase HPLC as previously described.[18] This HPLC method measures the six most common carotenoids found in human serum: beta-carotene, alpha-carotene, lycopene, lutein-zeaxanthin, and cryptoxanthin. "Total serum carotenoids" were calculated by adding together the concentrations of the six individual carotenoids. Retinol-binding protein concentrations were measured by size-exclusion HPLC as previously described.[19] Body stores of vitamin A were estimated by stable isotope dilution of d-4-retinyl acetate using GC mass spectrometry as previously described.[20]

Data were plotted graphically with Sigmaplot 5.0 (Jandel Scientific, San Rafael, CA USA). Simple statistics, ANOVA, and Pearson correlation coefficients (r) were calculated with SAS version 6.03 for microcomputers (SAS Inst., Cary, NC USA). Differences were considered to be statistically significant at the $P < 0.05$ level.

Results

All six serum carotenoid concentrations decreased significantly from baseline during the carotenoid depletion phase of the study: first rapidly, then very slowly. All carotenoids measured were still present in serum at the end of the depletion phase. All carotenoids increased significantly after carotenoid repletion,[17] especially beta- and alpha-carotene which were present in the highest concentrations in the Mixed Carotenoid supplement. Serum TSH increased significantly over baseline values by the 39th day of carotenoid depletion, then stabilized during carotenoid repletion (*Figure 1*). Mean serum TSH concentrations were negatively correlated with mean serum total carotenoid concentrations during the baseline and carotenoid depletion phases of the study ($r = -0.77$). This correlation lost its significance when assay values from the carotenoid repletion phase of the study were included, probably because serum carotenoid concentrations increased rapidly during repletion, while TSH concentrations were stable.

Serum total T4 concentrations also increased steadily during the carotenoid depletion phase of the study, but at a slower rate than TSH (*Figure 2*). Total T4 concentrations were significantly different from baseline by the 60th day of depletion. Total T4 concentrations also stabilized after carotenoid repletion. Mean total T4 concentrations correlated inversely with mean total carotenoid concentrations during the baseline and depletion phases of the study ($r = -0.79$).

The increased total T4 concentrations were probably not caused by nonspecific stress or activities associated with living on a metabolic research unit, because total T4 concentrations remained stable or decreased in the three prior metabolic unit studies during which this test was administered. Total T4 concentrations measured in 9 young adult women fed a nutritionally complete diet for 52 days and 8

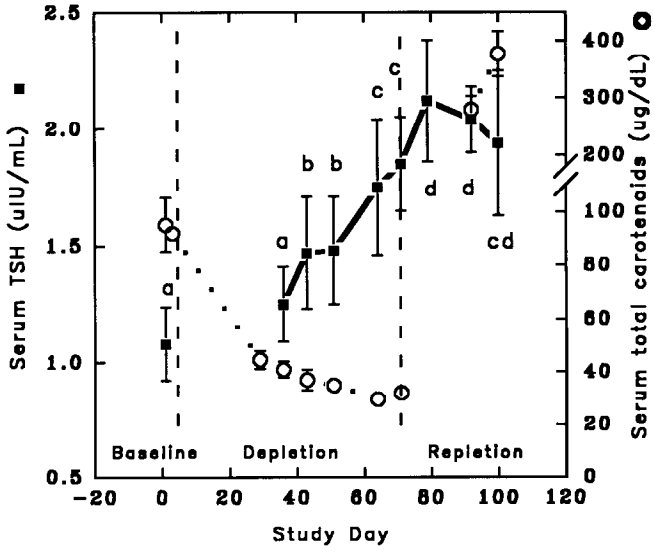

Figure 1 The influence of dietary carotenoids on serum TSH concentrations. ■ = mean (±SEM) of TSH concentrations (n = 9). Different super- and subscripts indicate significant concentration differences at the $P < 0.05$ level. O = mean (±SEM) of serum total carotenoids.

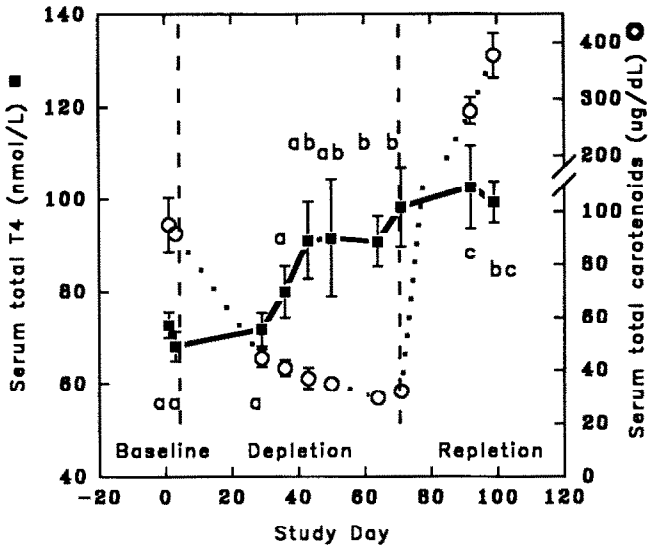

Figure 2 The influence of dietary carotenoids on total T4 concentrations. ■ = mean (±SEM) of serum total T4 concentrations (n = 9). Different superscripts indicate significant differences at the $P < 0.05$ level. O = mean (±SEM) of serum total carotenoids.

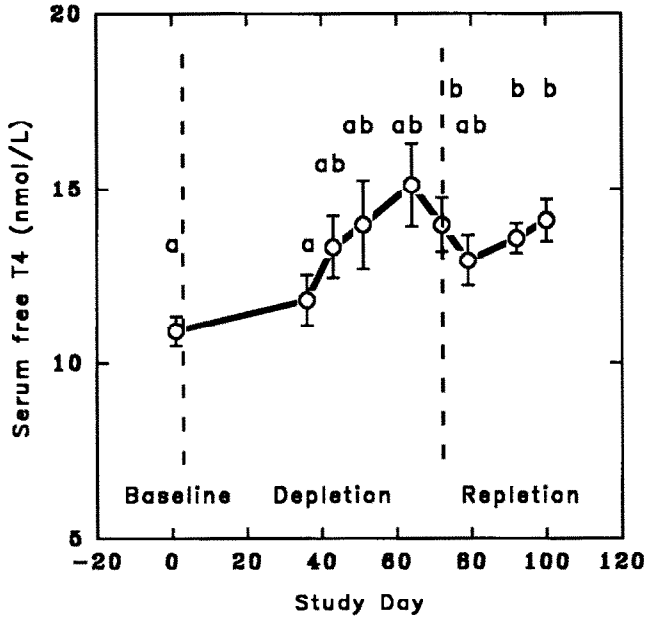

Figure 3 The influence of dietary carotenoids on serum FT4 concentrations. Each point represents the mean (±SEM) of FT4 concentrations (n = 9). Different superscripts show significant differences at the $P < 0.05$ level.

young adult men fed a low fat, low vitamin E diet for 100 days remained stable throughout the studies. Total T4 concentrations of one study of 12 young adult men fed a nutritionally complete diet for 52 days showed a gradual but eventually significant decrease.

Serum FT4 concentrations increased over baseline values, but this increase was not significant until study day 72 (*Figure 3*). Serum FT3 concentrations appeared to parallel serum FT4 concentrations but did not attain significance over baseline values until study day 92, during the repletion phase of the experiment (*Figure 4*). Mean serum FT4 and FT3 concentrations were negatively correlated with mean serum total carotenoid concentrations during the baseline and depletion phases of the study ($r = -0.81$ and $r = -0.77$, respectively).

Total T3 and transthyretin concentrations did not change significantly during this study. Furthermore, serum carotenoid concentrations were not correlated significantly with total T3 or transthyretin concentrations. Thus, it is not surprising that none of the women showed specific physiological symptoms of increased thyroid metabolism during this study.

Thyroid hormone concentrations are influenced by estrogen concentrations and pregnancy. Carotenoid depletion appears to have influenced the menstrual cycles in these women.[21,22] Approximately 65% of the 42 women fed our carotenoid depletion diets developed menstrual cycle abnormalities (typically long menstrual cycles of >35 days and skipped luteinizing hormone peaks), compared with approximately 5% of women on our metabolic unit who are not fed low carotenoid diets. These menstrual cycle abnormalities persisted in a majority of the women throughout the relatively short carotenoid repletion phase of the study (16 days of β-carotene followed by 12 days of mixed carotenoids), but normalized within 3 months after the women left the study. However, we found that TSH and total T4 concentrations were not significantly correlated with menstrual cycle length, the luteinizing hormone spike, luteinizing hormone, or prolactin concentrations. Serum total T4 concentrations were negatively correlated to serum follicle stimulating hormone concentrations during carotene repletion ($r = -0.89$). More details of our studies on the influence of low carotenoid diets on menstrual cycles will be published elsewhere.

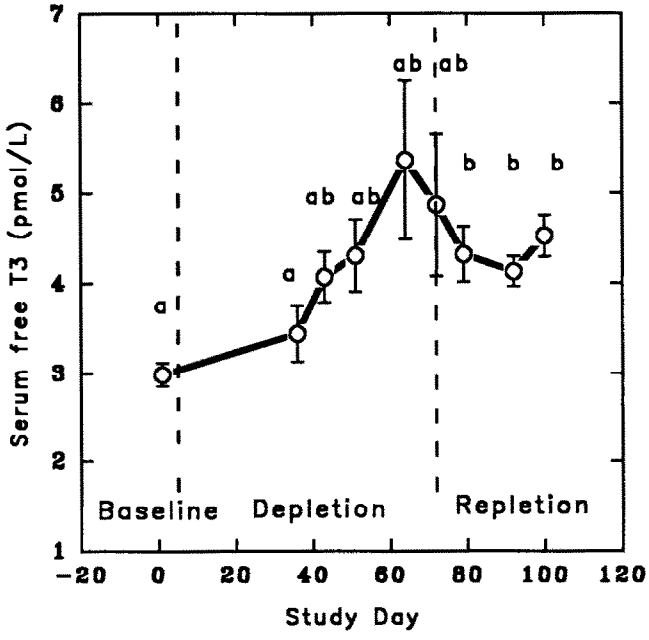

Figure 4 The influence of dietary carotenoids on serum FT3 concentrations. Each point represents the mean (±SEM) of FT3 concentrations (n = 9). Different superscripts show significant differences at the $P < 0.05$.

Vitamin A status, as estimated by stable isotope dilution, decreased in most subjects but increased in others. Vitamin A status was positively correlated with serum total T4 concentrations, but only on study days 43 ($r = 0.70$) and 51 ($r = 0.92$).

Discussion

Serum TSH concentrations increased significantly in women who were fed a diet adequate in all established nutrients but low in carotenoids. This was followed by slow but significant increases in total T4, FT4, and FT3. Typically, increases in total T4 are paralleled by decreases in TSH, but this is not the case when the increases in thyroid hormone concentrations are caused by TSH. This suggests that the low carotenoid diet stimulated TSH secretion, which caused eventual increases in the other thyroid hormones.

Several changes in nutritional status have previously been reported to cause changes in thyroid hormone concentrations: iodine depletion, vitamin A depletion, and anorexia nervosa or starvation. However, we believe that these factors did not cause the changes in thyroid hormone concentrations we saw in this study. Thyroiditis can be caused by iodine deficiency,[24] but there is no reason to suspect that iodine deficiency occurred on this study. The study diet was supplemented with vitamins and minerals as needed and was not grossly deficient in any nutrient including iodine. No indications of goiter or iodine deficiency occurred during this study. Vitamin A deficiency can be ruled out, because the study diet contained approximately 1,100 retinol equivalents of preformed vitamin A. Serum retinol and retinol-binding protein concentrations did not change during the study. Body stores of vitamin A, as estimated by stable isotope dilution, decreased in most women but increased or remained stable in others. Anorexia nervosa is associated with elevated serum carotenes and depressed serum T3 and T4,[23] but severe changes in energy intake did not occur in this study. The subjects' weight did not vary more than 2 kg over the 100 day experiment. The gross dietary composition, though lower in fat than the typical American diet, was similar to that found in most of our metabolic unit studies. Typically, total T4 concentrations show a nonsignificant decrease during our metabolic unit studies. Thus, it is likely that these changes in thyroid hormones were caused by carotenoid depletion.

Thyroid hormone concentrations continued to increase during the beginning of carotenoid repletion but stabilized by the end of carotenoid repletion. In fact, TSH and total T4 concentrations may have begun to decrease from there highest levels (*Figures 1 & 2*). The serum concentrations of these thyroid hormones were negatively correlated with total serum carotenoid concentrations during carotenoid depletion, but not during carotenoid repletion, again suggesting that thyroid hormone concentrations were being stabilized by carotenoid repletion. We do not know why thyroid hormone concentrations were not normalized during carotenoid repletion, but suggest two possible reasons. First, the increases in thyroid hormones during this study was slow, suggestive of an indirect effect. If thyroid hormone concentrations increased by a slow and indirect effect, it is likely that decreases would also be slow. Second, it is possible that a carotenoid other than β-carotene is involved in thyroid status regulation. Subjects were not repleted with mixed carotenoids until 12 days before the end of the study, and the mixed carotenoid capsule contained relatively small amounts of some major carotenoids such as lycopene and lutein. Thus, the subjects might not have been repleted until after they left the study.

Our study provides little information as to the mechanism by which carotenoid concentrations could affect thyroid status. A possible explanation for the influence of carotenoid depletion on thyroid hormones is that it is an indirect effect caused by changes in menstrual cycle hormone concentrations. Estrogens and pregnancy are known to influence thyroid hormones significantly.[24–26] High estrogen concentrations (such as seen when birth control pills are taken) can increase total T4 and total T3 concentrations.[25,26] Carotenes appear to be sequestered and metabolized by the bovine corpus luteum.[27] Progesterone concentrations in dairy cattle were positively correlated to beta-carotene concentrations and negatively correlated to vitamin A concentrations.[27] Carotenoids can also influence fertility in sows.[28] Menstrual cycle and hormonal abnormalities were frequent on this study and appear to be associated with several of our low carotenoid diets. It is possible that changes in the menstrual cycles of these women stimulated TSH secretion, which in turn stimulated total T4, FT4, and FT3. Furthermore, menstrual cycle abnormalities normalized slowly, with some subjects not completely normalized until 3 months after the metabolic unit study ended. Thus, an indirect mechanism involving changes in menstrual cycle hormones would also explain why thyroid hormone concentrations were not normalized by the end of the study. However, it should be noted that none of the menstrual cycle abnormalities we measured were highly correlated with thyroid hormone concentrations.

A second possibility is that carotenoid depletion may influence the peripheral metabolism of preformed vitamin A, to effectively decrease its concentration at sites that regulate thyroid hormones. Vitamin A deficiency is known to influence thyroid hormone status similarly to the changes that we report for carotenoid depletion.[1,6] Although preformed vitamin A was provided in the diet at constant and presumably adequate amounts (of 1,100 RE/day), the influence of carotenoid depletion on the peripheral metabolism of vitamin A is largely unknown but probably significant. We have initiated a series of projects to investigate the influence of vitamin A status on carotenoid metabolism and the influence of carotenoid status on vitamin A metabolism. We believe that the possible relationship between carotenoids and thyroid hormone concentrations deserves further study.

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