〈Original Article〉

Changes in serum levels of retinol and ascorbic acid in response to amounts of vitamins taken from the diets in young Japanese women

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Summary The aim of the present study was to evaluate the effects of dietary amounts of retinol and ascorbic acid on the levels of these vitamins in serum. After a diet history questionnaire, 54 healthy female volunteers (aged 18 and 19 years old) received formula diets (containing 466 μgRE/d of retinol and 149 mg/d of ascorbic acid) for 3 days. Venous blood was collected before and after intake of formula diets, and serum concentrations of retinol and ascorbic acid were determined.

Before intake of formula diets, subjects' serum concentrations of retinol did not correlate with the amounts of retinol observed by diet history questionnaire. However, serum concentration of ascorbic acid was significantly correlated with the dietary amounts of ascorbic acid ($r= 0.377$, $p= 0.005$). After intake of formula diets for 3 days, subjects' serum concentrations of retinol were decreased significantly (from 423±67 μg/L to 349±69 μg/L, $p= 0.003$), but no significant increase was observed in serum concentrations of ascorbic acid (from 9.8±2.6 mg/L to 10.0±1.5 mg/L, $p= 0.473$). Changes (Δ) in serum levels of retinol and ascorbic acid before and after intake of formula diet, as compared with the corresponding changes (Δ) in amounts of intakes of these vitamins, were significantly correlated in both vitamins. We calculated that 650 μgRE/d of dietary retinol changed serum retinol levels by 32 μg/L, and that 100 mg/d of dietary ascorbic acid changed serum ascorbic acid levels by 0.52 mg/L.

Key words: Vitamin A, Vitamin C, Nutritional assessment, Diet history questionnaire, Hypovitaminosis

1. Introduction

Vitamins are essential nutrients in all living organisms. Lack of vitamins is not compensated for by taking other nutrients. Humans need to take in most of their vitamins from food, and a shortage of vitamins in the diet occasionally results in vitamin deficiency, sometimes causing serious illnesses. Recent studies show that vitamin deficiency can be assessed by manifest symptoms caused by deficient vitamins, and

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by anthropometric characteristics and the physical strength of the subjects. A diet history questionnaire and laboratory tests using blood and urine specimens have also been important for assessing marginal vitamin deficiency, i.e., the "gray area" of nutrition[1-3]; herein subjects manifested no clinical signs of vitamin deficiency, but their vitamin levels in blood were significantly lower than the reference value. Once vitamin deficiency was determined, subjects were treated to maintain vitamin blood levels within the reference value with the supplementation of vitamins and/or the dietary control. However, in the dietary control, we naturally considered how vitamin blood levels change in response to amounts of vitamin taken from diets, and knowledge that how much vitamins from diets could alter the nutritional status of vitamins was actually required.

Because blood levels are decided by body pools of vitamins and their half-lives (the time they stay in the body), in addition to their amounts taken in the diets, amounts of water-soluble vitamins are generally small in the body pool and have a short half-life. By contrast, fat-soluble vitamins have large amounts in the body pool and have a long half-life. Therefore, blood levels of fat-soluble vitamins may change slowly (i.e., slowly decrease in depletion and slowly increase in repletion) in response to their amounts taken in from the diets. However, accurate characterization of each vitamin's response is limited. Therefore, we conducted experiments that focused on the effects of dietary amounts of fat- and water-soluble vitamins on the levels of these vitamins in serum. In this study, we chose retinol as a typical example of a fat-soluble vitamin and ascorbic acid as a water-soluble vitamin.

2. Materials and methods

2.1. Subjects

The study was carried out with 54 healthy volunteers. They were Japanese female university students (aged 18 and 19 years old) having a normal BMI (body mass index) of 20±2. Informed written consent was obtained from all volunteers, and the studies were approved by the guidelines established by the Protection of Human Subjects Committee of Tokyo Kasei University.

2.2. Study design

During the control period, all subjects received formula diets for three days. Except for receiving the formula diets, subjects were allowed to carry on with life as usual. The formula diets (natural-food meals) were prepared by the nutrition staff at Tokyo Kasei University under the direction of dietitians. The formula diets provided 1,800-1,850 kcal/d of energy, 65 g/d of protein, fat (20-25% of the energy was from fat sources), 600 mg/d of calcium, 11-12 mg/d of iron, 1,800 IU/d of vitamin A (466 μ gRE/d of retinol by chemical analysis), 4.6 mg/d of vitamin E, 0.7 mg/d of vitamin B_1, 1.1 mg/d of vitamin B_2 and 200 mg/d of vitamin C (149 mg/d of ascorbic acid by chemical analysis).

The formula diets provided 72% of the Japan Dietary Reference Intake (DRIs) for retinol and 149% of DRI for ascorbic acid. The DRI for women of this age group was 650 μ gRE/d for retinol and 100 mg/d for ascorbic acid. The formula diets were made up of three cuisines (i.e., breakfast, lunch and supper), and the amounts of retinol and ascorbic acid in each cuisine were ca. 150 μ gRE and 50 mg, respectively[4, 5].

All subjects completed a diet history questionnaire (3-day diet recall) prior to examination. They did not habitually smoke, drink, or use dietary supplements. Venous blood after a 12-hour overnight fast was collected from the subjects before and after intake of formula diets (for 3 days), and serum samples were used for analyses of retinol and ascorbic acid. In this study, we chose retinol as a typical example of fat-soluble vitamin and ascorbic acid as a water-soluble vitamin, because the physiology of absorption, metabolism and excretion for these vitamins is near fully understood[1, 2, 6]. Moreover, these vitamins were easy to measure in our laboratory.

2.3. Assays

Serum concentration of retinol was determined by a normal-phase high-performance liquid chromatography (HPLC) with UV detection after extraction with hexane[7]. The reference range was 288 to 602 μ g/L. Serum concentration of ascorbic acid

(total ascorbic acid concentrations, e.g., sum of ascorbic acid and dehydroascorbic acid) was determined by the 4-hydroxy-2,2,6,6-tetramethyl-piperidinyloxy, free radical (TEMPO) method[8]. The reference range was 7.0 to 13.8 mg/L.

2.4. Statistics

Serum concentrations of vitamins before and after intake of formula diets were compared using Wilcoxon signed-rank test, and differences in a two-tailed probability of less than 0.05 were considered to be statistically significant. The regression equation was calculated by Passing-Bablok procedure.

3. Results

3.1. Diet history questionnaire

According to their diet history questionnaires, subjects on average ingested 739±715 μ gRE/d of retinol and 98.9±75.8 mg/d of ascorbic acid. Because some of the 54 subjects showed low intake of one or both vitamins, they were separately classified as 34 subjects with low retinol intake and 39 subjects with low ascorbic acid intake at levels below the DRIs (Fig. 1). Thus, with retinol, 34 subjects (63%) participated in the depletion test and 20 subjects (37%) participated in the repletion (but, intakes were lower than DRI) test. With ascorbic acid, 46 subjects (85%)

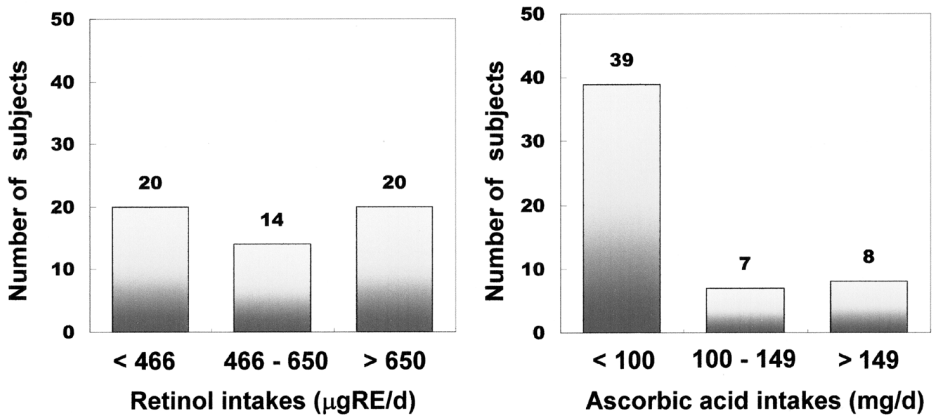

Fig. 1 Results of diet history questionnaire in 54 healthy female volunteers.
The DRI for women of this age group was 650 μ gRE/d for retinol and 100 mg/d for ascorbic acid. After all subjects completed a diet history questionnaire, they received formula diets containing 466 μ gRE/d of retinol and 149 mg/d of ascorbic acid.

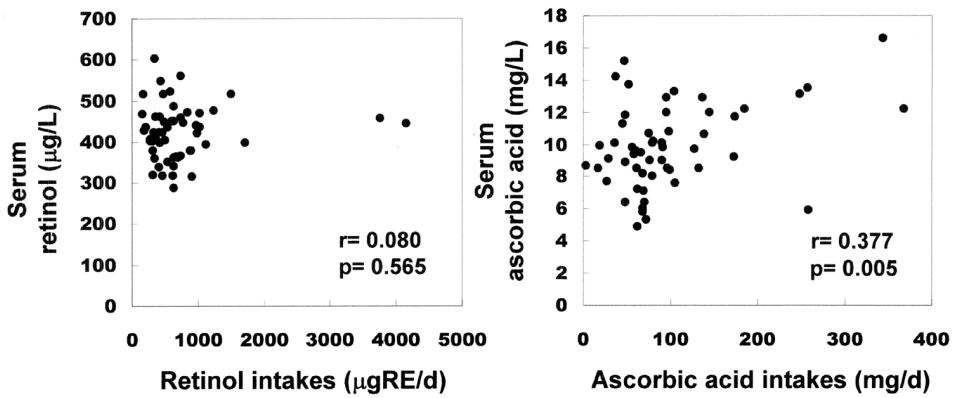

Fig. 2 Relationship of intakes of retinol and ascorbic acid (mean intake over 3 days) with serum retinol and ascorbic acid in 54 healthy female volunteers.

participated in the repletion test and 8 subjects (15%) in the depression (but, intakes were higher than DRI) test.

3.2. Effect of formula diets on serum vitamin concentrations

Before intake of formula diets, subjects' serum retinol concentrations did not correlate with the amounts of retinol observed by diet history questionnaire (Fig. 2). However, serum concentration of ascorbic acid was significantly correlated with the dietary amounts of ascorbic acid (r= 0.377, p= 0.005).

After intake of formula diets for 3 days, subjects' serum concentrations of retinol were decreased significantly (from 423±67 μg/L to 349±69 μg/L, p= 0.003: Fig. 3), but no significant increase was observed in serum concentration of ascorbic acid (from 9.8±2.6 mg/L to 10.0±1.5 mg/L, p= 0.473: Fig. 3). Although diet history showed that 20 subjects had taken amounts of retinol less than that contained in formula diets, serum concentration of retinol did not increase after the intake of formula diets (p= 0.136). Furthermore, no significant decrease was observed in serum ascorbic acid (p= 0.342) in the eight subjects who had taken amounts of this vitamin more than that contained in formula diets.

When changes (Δ: i.e., differences) in serum levels of retinol and ascorbic acid before and after

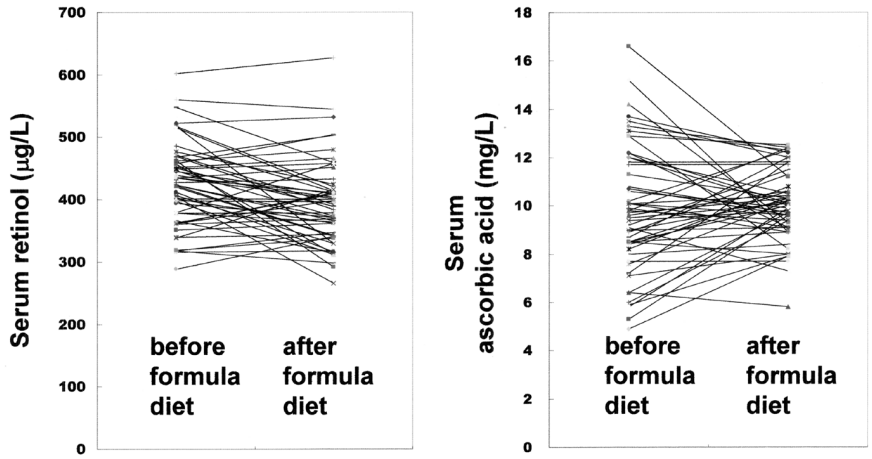

Fig. 3 Effect of formula diets on serum vitamin concentrations in 54 healthy female volunteers.

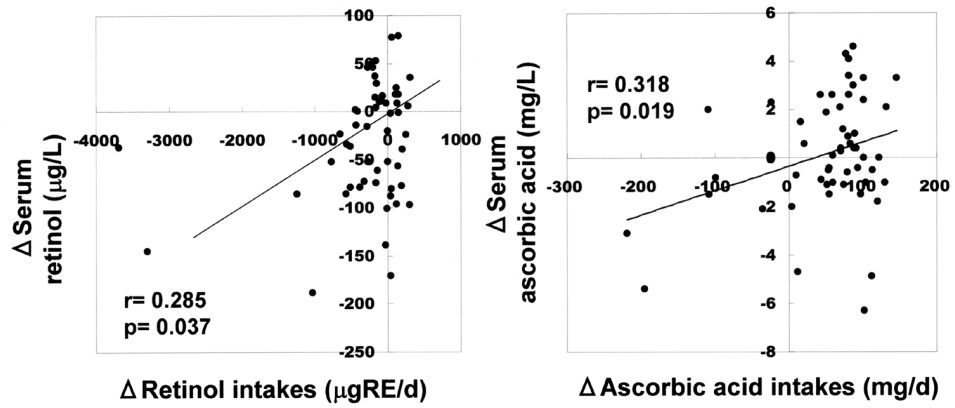

Fig. 4 Relationship of changes (Δ) in serum levels of retinol and ascorbic acid before and after intakes of formula diet with the corresponding changes (Δ) in amounts of intakes of these vitamins in 54 healthy female volunteers.

intakes of formula diet were compared with the corresponding changes (Δ) in amounts of dietary intakes of these vitamins, significant correlations were observed in both vitamins (Fig. 4). The regression equation of retinol was Y (Δ serum retinol)= 0.052X (Δ retinol intakes) − 1.72. That of ascorbic acid was Y (Δ serum ascorbic acid)= 0.0097X (Δ ascorbic acid intakes) − 0.45. Of the 54 subjects, 19 (35%) showed decreases in serum levels paralleling retinol intakes. However, 25 subjects (46%) showed parallel increases of ascorbic acid.

4. Discussion

Nowadays, serum retinol is reported to be maintained constantly in the body. Liver stores retinol, and releases retinol as a form of RBP-4 (retinol-binding protein) into blood circulation in order to maintain normal body functioning[9]. Thus, retinol is controlled in a homeostatic manner, and serum levels do not drop until the body store is significantly exhausted[10]. We previously reported the case of a patient treated with plasmapheresis using an anion-exchange resin[11]. The patient's plasma retinol before perfusion was 423 μg/L. It was decreased to a deficient level (152 μg/L) after perfusion since plasma retinol was adsorbed on the anion-exchange resin, but returned to a normal level on the next day; presumably the stored retinol in the liver was secreted into the circulation. Our results obtained here agreed with the above three reports. Although participating with the depletion test of retinol, only one subject was revealed to have vitamin A deficiency. Her serum retinol concentration was decreased from 404 μg/L to 265 μg/L after receiving formula diets. In the remaining 53 subjects, serum retinol concentrations remained within reference range, although serum levels dropped from 423±67 μg/L to 349±69 μg/L. It was suggested that the liver pool would compensate for the deficit amounts of retinol in the formula diets. Calculating from the above regression equation (Fig. 4), we could ascertain that 650 μgRE/d (amounts corresponding to DRI) of dietary retinol could change serum retinol levels by 32 μg/L in a day.

Although serum ascorbic acid levels were significantly correlated with the dietary amounts of ascorbic acid, as observed by the diet history questionnaire, a significant increase was not observed in the levels after the intakes of formula diets containing amounts of ascorbic acid that were 149% higher than DRI (149 mg/d). This was explained by the saturation concentration of ascorbic acid in leukocytes and plasma[12, 13]. Ascorbic acid in leukocytes reached a maximum at the intake of 100 mg/d, and an amount greater than 100 mg/d would be excreted in urine. Sequentially, serum levels were no longer increased although subjects took in higher amounts from their diet. Levine et al.[12, 13] reported a depletion-repletion study in which seven healthy male volunteers were depleted of ascorbic acid by being fed a diet containing less than 5 mg/d ascorbic acid. Subjects' plasma ascorbic acid levels fell to 2 mg/L. For repletion, they were given 30, 60, 100, 200, 400, 1,000 and 2,000 mg/d of ascorbic acid. Plasma ascorbic acid levels were increased and paralleled the given doses. Plasma levels were increased on average 5 mg/L for those given doses between 60 and 100 mg/d, and 2 mg/L for those given doses between 100 and 200 mg/d. In our study, 100 mg/d (amounts corresponded to DRI) of dietary ascorbic acid could change serum levels by 0.52 mg/L (calculated from the egression equation in Fig. 4), and was incompatible with Levine's report. Our lower concentrations were explained by the small number of subjects with hypovitaminosis C (serum ascorbic acid, < 7.0 mg/L). Of the 54 subjects, only seven revealed hypovitaminosis C. However, except for one subject, serum concentrations of ascorbic acid in the remaining six were increased after intakes of formula diets. The increases ranged from 2.0 to 4.3 mg/L (average, 3.35 mg/L), similar to Levine's report. We concluded that the lower response of serum levels to formula diets in subjects with normovitaminosis C would reflect the usual status observed generally in our dietary life.

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Declaration of Interest

The authors declare no potential conflicts of interest.

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