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Vitamin Contents in Rat Milk and Effects of Dietary Vitamin Intakes of Dams on the Vitamin Contents in Their Milk

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Summary Studies of factors that affect milk vitamin contents are important. We investigated the vitamin contents in rat milk and the effects of dietary vitamin intakes of dams on the vitamin contents in their milk. A low-vitamin diet (0.2%) and a high-vitamin diet (4.0%) based on a diet containing 1% AIN-93-VX (normal diet) was given to female rats from pregnancy to lactation. Regarding the effects of the vitamin intakes, the concentrations of vitamins B_1, B_2, B_6, B_{12} and E were decreased with the low-vitamin diet, but were not increased with the high-vitamin diet. The concentrations of niacin, pantothenic acid and biotin were not decreased with the low-vitamin diet, but were increased with the high-vitamin mixture diet. The folate concentration remained constant regardless of the intake of folate. These findings clearly indicate that the levels of certain vitamins in milk are easily affected by the dietary vitamin intakes.

Key Words content, lactation, milk, rat, vitamin

It is generally believed that milk contains all of the nutrients for the proper development of infants. However, this is not the case, at least with regard to vitamins. Therefore, studies of factors that affect milk vitamin contents are important. Kirchgessner et al. (*1*) reported that the vitamin B_1 content in rat milk was lower in rats fed with a low-vitamin B_1 diet than in rats fed with a sufficient vitamin B_1 diet. Duerden and Bates (*2*) reported that the vitamin B_2 concentration in rat milk was extremely low when dams were fed a vitamin B_2-restricted diet compared with control dams. Regarding the vitamin B_6 content in rat milk, Kirksey and Susten (*3*) reported that the vitamin B_6 level was a more sensitive indicator than liver or muscle of chronically low intakes of vitamin B_6 by dams, while other investigators also reported that the level of vitamin B_6 in the milk of dams changed according to their intake of vitamin B_6 (*3–6*). Two groups reported that the concentration of vitamin B_{12} in milk was affected by the dietary intake of vitamin B_{12} (*7*, *8*). Meanwhile, O'Connor et al. (*9*) reported that the folate content of rat milk was increased according to increases in dietary folate. These reports clearly indicate that the levels of certain vitamins in rat milk are easily affected by the dietary vitamin intakes. For other vitamins, there is no available information.

In this study, we investigated nine kinds of vitamin contents in rat milk, the changes in the vitamin contents during lactation, and the effects of dietary vitamin intakes of the dams on the vitamin concentrations in their milk.

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MATERIALS AND METHODS

Chemicals. Vitamin-free milk casein, sucrose and L-methionine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, a mineral mixture (AIN-93-M-MX) (*10*) and a vitamin mixture (AIN-93-VX containing chorine bitartrate) (*10*) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Thiamin hydrochloride (vitamin B_1, $C_{12}H_{17}ClN_4OS$-HCl=337.27), thiamin diphosphate chloride ($C_{12}H_{19}ClN_4O_7P_2S$=460.77), riboflavin (vitamin B_2, $C_{17}H_{20}N_4O_6$=376.37), cyanocobalamin (vitamin B_{12}, $C_{63}H_{88}CoN_{14}O_{14}P$=1,355.40), nicotinamide ($C_6H_6N_2O$=122.13), calcium pantothenate (PaA-Ca, $C_{18}H_{32}N_2O_{10}$-Ca=476.54), folic acid ($C_{19}H_{19}N_7O_6$=441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$=244.31), (±)D-α-tocopheryl acetate ($C_{31}H_{52}O_3$=472.74), pyridoxal 5′-phosphate monohydrate ($C_8H_{10}NO_6P$-H_2O=265.169) and pyridoxal hydrochloride ($C_8H_9NO_3$-HCl=203.62) were purchased from Wako Pure Chemical Industries.

Nembutal (2.5 g/50 mL) was obtained from Dainippon Sumitomo Pharma (Osaka, Japan). Oxytocin (50 IU/mg) and lumiflavin ($C_{13}H_{12}N_4O_2$=256.3) were obtained from Sigma-Aldrich Japan K.K. (Tokyo, Japan).

All other chemicals used were of the highest purity available from commercial sources.

Animals and diets. Male and female rats of the Wistar strain (8 wk old) were obtained from CLEA Japan, Inc. (Tokyo, Japan). The rats were immediately placed in individual cages and fed a 20% casein diet containing 1% vitamin mixture (Table 1) for 1 wk to

Table 1. Compositions of the experimental diets.

	0.2% VX[1] (%)	1.0% VX (%)	4.0% VX (%)
Casein	20	20	20
L-Methionine	0.2	0.2	0.2
Gelatinized cornstarch	46.9	46.9	46.9
Sucrose	24.2	23.4	20.4
Corn oil	5	5	5
Mineral mixture (AIN-93-MX)	3.5	3.5	3.5
Vitamin mixture (AIN-93-VX)	0.2	1	4
(mg/100 g of diet)			
All-*trans*-retinyl palmitate (500,000 IU/g)	0.16	0.8	3.2
Cholecalciferol (400,000 IU/g)	0.05	0.25	1
All-rac-α-tocopheryl acetate (500 IU/g)	3	15	60
Phylloquinone	0.015	0.075	0.3
Thiamin-HCl	0.12	0.6	2.4
Riboflavin	0.12	0.6	2.4
Pyridoxine-HCl	0.14	0.7	2.8
Cyanocobalamin	0.0005	0.025	0.01
Nicotinic acid	0.6	3	12
Ca pantothenate	0.32	1.6	6.4
Folic acid	0.04	0.2	0.8
D-Biotin	0.004	0.02	0.08
Choline bitartrate	5	25	100
Sucrose	up to 200	up to 1,000	up to 4,000

[1] VX: vitamin mixture.

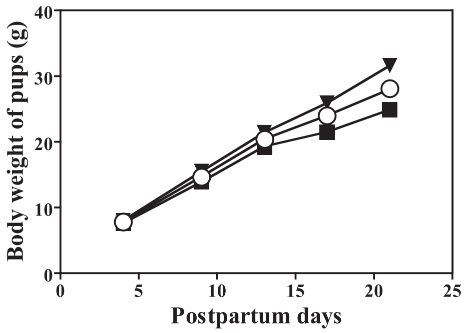

Fig. 1. Effect of feeding with the three levels of vitamin mixture diets to dams on the body weight gains of pups. ■, 0.2% vitamin mixture diet; ○, 1.0% vitamin mixture diet (normal diet); ▼, 4.0% vitamin mixture diet. Values are expressed as means±SE for 5–7 rats.

Table 2. The vitamin contents in rat milk.

Vitamins	Values	*n*
Vitamin B_1 (μg/mL)	0.204±0.082	16
Vitamin B_2 (μg/mL)	4.67±0.78	16
Vitamin B_6 (μg/mL)	1.49±0.23	13
Vitamin B_{12} (μg/mL)	0.032±0.008	16
Niacin (μg/mL)	7.02±2.28	16
Pantothenic acid (μg/mL)	15.2±6.6	16
Folate (μg/mL)	2.91±0.38	16
Biotin (μg/mL)	0.154±0.047	13
Vitamin E (μg/mL)	15.3±0.55	16

The values are means±SD for postpartum days 9, 13, 17, and 21.

allow them to acclimatize to their new circumstances. The female rats were then divided into three groups and fed one of three experimental diets containing 0.2, 1.0 or 4.0% vitamin mixture (Table 1) during mating, gestation and lactation. After the female rats delivered their pups, the pups that the mother rat brought up was adjusted to six. The day of postpartum was designated day 1. For collection of milk, the mother rat was separated from the litter at 09:00. At 15:00, the dam was anesthetized with 0.1 mL of nembutal (2.5 g/50 mL) and intraperitoneally injected with 2.5 IU of oxytocin. After confirming the effectiveness of the anesthesia, milk (about 2 mL) was collected using a special milking machine (Automatic Milker WAT-2001; Little Leonard Co. Ltd., Tokyo, Japan). The milk collection was carried out on days 4, 9, 13, 17 and 21. The collected milk was diluted by 1 : 2 by the addition of saline (0.85% NaCl), and the diluted milk was stored at −20˚C until analysis.

The animal room was maintained at a temperature of about 22˚C and a humidity of about 60% with a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle. The body weight of each pup was measured when it was separated from the mother. The care and treatment of the experimental animals conformed to The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Analyses. Vitamin B_1 (*11*), vitamin B_2 (*12*), vitamin B_6 (*13*), vitamin B_{12} (*14*), nicotinamide (*15*), pantothenic acid (*16*), folate (*17*), biotin (*18*) and vitamin E (*19*) in milk were measured as described in the cited references.

Statistical analysis. Each value was expressed as the mean±SE. The statistical significance of differences was determined by ANOVA and subsequent Tukey-Kramer multiple-comparison tests. Differences with values of $p<0.05$ were considered to be statistically significant. Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA) was used for all analyses.

RESULTS

Effects of feeding the three vitamin diets to the dams on the body weight gains of pups

The low-vitamin (0.2%) and high-vitamin (4.0%) diets based on the AIN-93 diet (*10*) (1.0% vitamin diet; normal diet) were given to the female rats from pregnancy to lactation. Figure 1 shows the effects of feeding the three vitamin diets to the dams on the body weight gains of the pups. The weights of the pups were almost the same among the three groups.

Vitamin contents in milk of dams fed the normal diet

The reference values of rat's milk vitamin contents were assumed to be the average of the values on the days 9, 13, 17, and 21, because there were quite different values for vitamin B_1, vitamin B_2, and vitamin E on day 4. Table 2 shows the average vitamin contents in

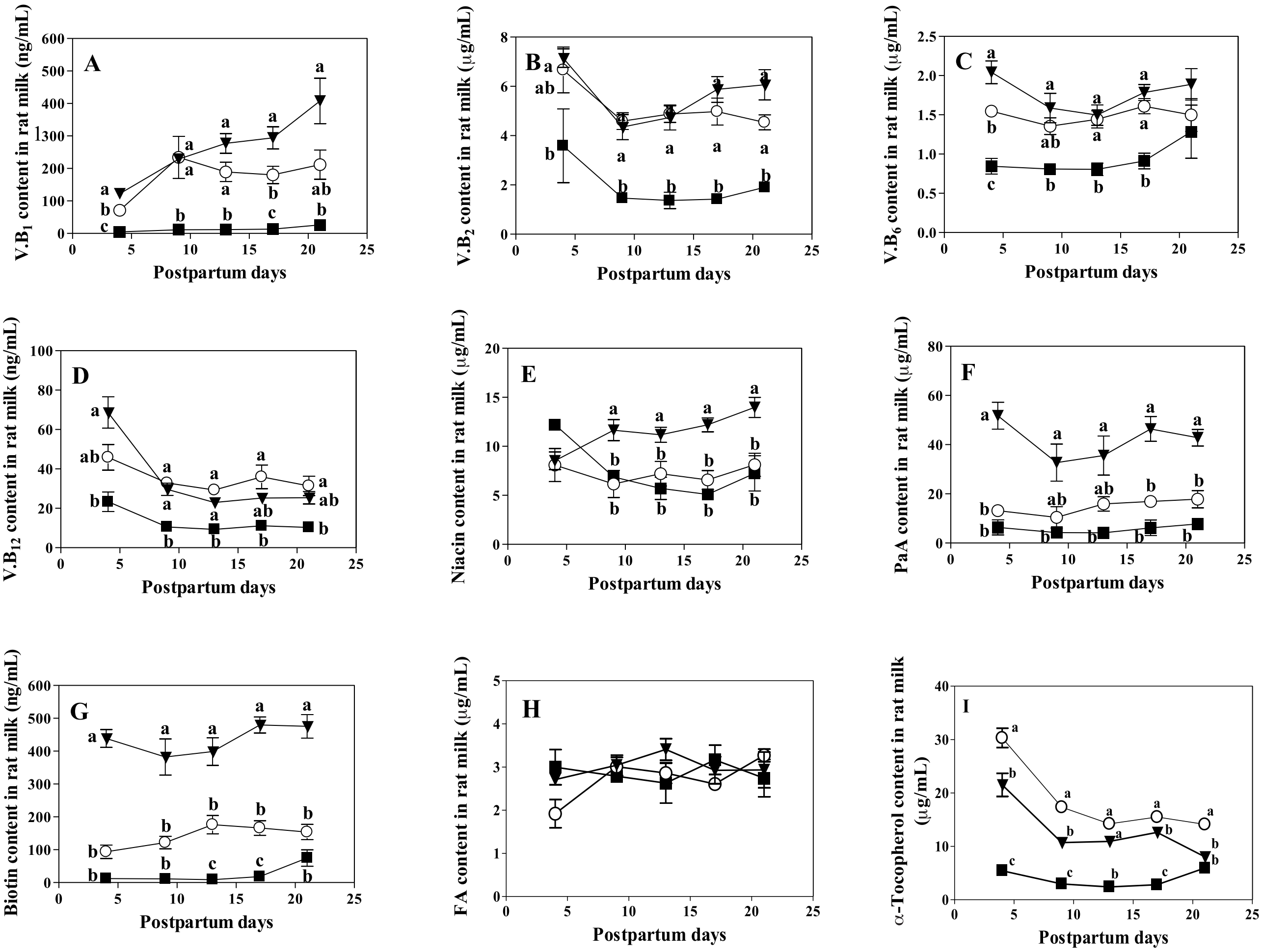

Fig. 2. Effect of feeding with the three levels of vitamin mixture diets to dams on the milk contents of vitamin B_1 (A), vitamin B_2 (B), vitamin B_6 (C), vitamin B_{12} (D), niacin (E), pantothenic acid (PaA) (F), biotin (G), folate (H), and vitamin E (I) contents in rat milk. ■, 0.2% vitamin mixture diet; ○, 1.0% vitamin mixture diet (normal diet); ▼, 4.0% vitamin mixture diet. Values are expressed as means±SE for 3–5 rat milks. Different letters on the symbols denote statistically significant differences (as determined by Tukey-Kramer multiple-comparison tests, $p<0.05$) in milk vitamin content among the groups of the three vitamin mixture diets in the same postpartum day.

rat milk collected on postpartum days 9, 13, 17 and 21 when the dams were fed the normal diet (1% vitamin mixture diet).

Changes in the vitamin contents during lactation of the dams fed the normal diet

Figure 2 shows the changes in the vitamin contents on specific postpartum days. The vitamin B_1 (Fig. 2A) content was lower on postpartum day 4 than on the other days. On the other hand, the contents of vitamins B_2 (Fig. 2B), B_{12} (Fig. 2D) and E (Fig. 2I) were higher on postpartum day 4 than on the other days. The contents of vitamin B_6 (Fig. 2C), niacin (Fig. 2E), biotin (Fig. 2G) and folate (Fig. 2H) remained relatively constant during lactation.

Effects of feeding the three vitamin diets to the dams on the vitamin contents in their milk

Figure 2 also shows the effects of the intake of the dietary vitamins on the vitamin contents in the rat milk during lactation. Although the concentrations of vitamins B_1 (Fig. 2A), B_2 (Fig. 2B), B_6 (Fig. 2C) and B_{12} (Fig. 2D) did not increase with the high-vitamin diet compared with the normal vitamin diet, these vitamin concentrations decreased with the low-vitamin diet.

The concentrations of niacin, pantothenic acid and biotin were not decreased by feeding the low-vitamin diet to the dams, although these vitamin concentrations were increased by feeding the high-vitamin mixture diet to the dams (Fig. 2E, 2F and 2G, respectively).

The folate concentration remained constant regardless of the amount of folate intake (Fig. 2H). The concentration of vitamin E was decreased with the low-vitamin diet, but was not increased with the high-vitamin diet compared with the normal vitamin diet (Fig. 2I).

DISCUSSION

The body weight gains of the pups were almost the same among the three groups regardless of whether the dams were fed diets containing low (0.2%), normal (1.0%) or high (4%) levels of the vitamin mixture. The present findings mean that the milk of lactating rats fed the low-vitamin diet (0.2%) was able to maintain normal growth of the pups. This finding is likely to have arisen because the AIN-vitamin mixture (*10*) contains around five-fold higher vitamin contents than the required amounts.

The first purpose of the present study was to analyze the milk vitamin concentrations of rats fed the normal diet. As summarized in Table 2, we measured 9 kinds of vitamins, namely vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, pantothenic acid, folate, biotin and vitamin E.

We did not measure the contents of vitamins A, D and K in rat milk. The reported values for vitamins A and D in rat milk are around 800 ng/mL (*20*) and 2 ng/mL (*21*), respectively. We could not find any data for the vitamin K content in rat milk.

The concentration of vitamin B_1 in the milk of dams fed the normal diet was around 200 ng/mL (Fig. 2A and Table 2). There is one previous report about the vitamin B_1 content in rat milk. Kirchgessner et al. (*1*) reported that the content increased according to the postpartum days, with values of 840, 1,600 and 2,500 ng/mL on days 2, 6 and 13, respectively, when the dams were fed a diet containing 0.67 mg/100 g diet (the same concentration in the 1% vitamin mixture diet as the normal diet). These values were about 10-fold higher than the present data for the 1% vitamin mixture diet. Kirchgessner et al. (*1*) used Sprague-Dawley rats as the experimental animals and fed a relatively high-fat diet (8.7% fat), while we used Wistar rats and fed a 5% fat diet. These differences may be reasons why the milk vitamin B_1 concentrations were so different.

The concentration of vitamin B_2 in the milk of dams fed the normal diet was around 5,000 ng/mL (Fig. 2B and Table 2). There is one previous report about the vitamin B_2 content in rat milk. Duerden and Bates (*2*) reported that the content was around 8,000 ng/mL when the dams were fed a diet containing 1.5 mg/100 g diet. The concentration of dietary vitamin B_2 was 2.5-fold higher than that in the present normal diet, and the content of vitamin B_2 in the milk was 1.6-fold higher than that in the present study. Duerden and Bates (*2*) also reported that the vitamin B_2 content in milk was significantly lower when the dams were fed a vitamin B_2-restricted diet. In the present study, the vitamin B_2 content in milk was lower in the dams fed the low-vitamin diet than in the dams fed the normal and high-vitamin diets.

The concentration of vitamin B_6 in the milk of dams fed the normal diet was around 1,500 ng/mL (Fig. 2C and Table 2). In previous reports, a constant value for the vitamin B_6 content in rat milk was not obtained. Thomas and Kirksey (*6*) reported that the vitamin B_6 content was 500 ng/mL when the dams were fed a diet containing 0.3 mg pyridoxine-HCl/100 g diet (this concentration is three-sevenths of that in our normal control diet). Felice and Kirksey (*5*) reported that the content was around 900 ng/mL when the dams were fed a diet containing 1.0 mg pyridoxine-HCl/100 g diet (this concentration is 1.4-fold higher that the concentration in our normal control diet). Debes and Kirksey (*4*) reported that content was around 500 ng/mL when the dams were fed a diet containing 2.0 mg pyridoxine-HCl/100 g diet (this concentration is 2.85-fold higher than the concentration in our normal control diet). In the present study, the vitamin B_6 content increased according to the change in diet from the low-vitamin (0.14 mg pyridoxine-HCl/100 g diet) to the normal diet (0.7 mg pyridoxine-HCl/100 g diet). However, the concentration did not increase when the dietary vitamin intake was increased from the normal diet to the high-vitamin diet (2.8 mg pyridoxine-HCl/100 g diet) (Fig. 2C). This finding is consistent with those in Kirksey and Susten (*3*) and Pang and Kirksey (*22*). They fed five levels of pyridoxine-HCl (0.12, 0.24, 0.48. 0.96 and 1.92 mg/100 g diet) to female rats, and found that the vitamin B_6 concentration in the milk reached a plateau of around 300 ng/mL with the 0.48 mg pyridoxine-HCl/100 g diet.

The concentration of vitamin B_{12} in the milk of dams fed the normal diet was around 30 ng/mL (Fig. 2D and Table 2). Regarding previous reports, the vitamin B_{12} content in rat milk was dramatically increased according to the intake of vitamin B_{12} of the dams. When the dams were changed from a diet containing 0.2 μg/100 g diet to a diet containing 20 μg/100 g diet, the concentration of vitamin B_{12} increased from about 7 ng/g milk curd to 120 ng/g milk curd (*22*). When the dams were fed a vitamin B_{12}-deficient diet, the content was around 5 ng/mL (*8*).

Regarding previous reports of the folate content in rat milk, values of 150 ng/mL milk (*9*) and 440 ng/mL milk (*23*) were observed when the dams were fed a diet containing 0.2 mg/100 g diet. In the present study, the folate concentration in the milk of dams fed the normal diet was around 3 μg/mL (Fig. 2H and Table 2). This value was about 10-fold higher than the previously reported values (*9*, *23*). The previously reported values were obtained using Sprague-Dawley rats as the experimental animals, while we used Wistar rats. This difference may be the reason why the folate concentrations in the milk were so different.

No data for the contents of niacin, pantothenic acid, biotin and vitamin E in rat milk have been reported. The concentration of niacin in the milk of dams fed the normal diet was around 7 μg/mL (Fig. 2E and Table 2). The concentration of pantothenic acid in the milk of dams fed the normal diet was around 15 μg/mL (Fig. 2F and Table 2). The concentration of biotin in the milk of dams fed the normal diet was around 150 ng/mL (Fig. 2G and Table 2). The concentration of vitamin E in the milk of dams fed the normal diet was around 15 μg/mL (Fig. 2I and Table 2).

The second purpose of the present study was to evaluate the changes in the vitamin contents during lactation. The content of vitamin B_1 was remarkably increased from day 9 (Fig. 2A). A similar phenomenon has already been reported by Kirchgessner et al. (*1*). On the other hand, the contents of vitamin B_2 (Fig. 2B) and vitamin E (Fig. 2I) remarkably decreased from day 9. These phenomena would be associated with the vitamin requirement in the pups and regulated through the expression of carrier proteins for the vitamins in the mammary glands. The other vitamins remained at relatively constant concentrations during lactation. Regarding vitamin B_{12}, Williams and Spray (*8*) already reported a similar phenomenon to the present study. However, Felice and Kirksey (*5*) reported that the content of vitamin B_6 was significantly higher on day 21 than earlier in the lactation period. For vitamin B_2, niacin, pantothenic acid, biotin, folate and vitamin E, the changes in the vitamin contents during lactation are reported here for the first time.

The final purpose of the present study was to clarify the effects of dietary vitamin contents on the milk vitamin contents in rats. In previous reports, the milk contents of vitamins B_1 (*1*) and B_2 (*2*) were decreased when the dams were fed on corresponding vitamin-restricted diets, and the vitamin B_6 (*3*) and B_{12} (*7*) contents in the rat milk reflected the intakes of the respective vitamins. In the present study, the concentrations of vitamins B_1, B_2, B_6, B_{12} and E were decreased with the low-vitamin diet, but were not increased with the high-vitamin diet. The present findings for vitamin B_1 are similar to the findings of Kirchgessner et al. (*1*), who also found that the vitamin B_1 content was decreased by feeding a low-vitamin B_1 diet, but was not increased by feeding an excess vitamin B_1 diet. The concentrations of niacin, pantothenic acid and biotin were not decreased with the low-vitamin diet, but were increased with the high-vitamin diet. These results indicate that the concentrations of niacin, pantothenic acid and biotin in milk are not easily decreased, even with low intake, while the concentrations of vitamins B_1, B_2, B_6, B_{12} and E in milk are affected by their intakes. The folate concentration remained constant regardless of the folate intake. It is known that there is a well-developed epithelial folate transport system for the regulation of normal folate homeostasis (*24*, *25*). Therefore, the concentrations of the vitamins could also be well-regulated by transport systems in intestinal absorption and in secretion to the milk. However, the present findings suggest there is a specific regulation mechanism for each of the vitamins to maintain the milk vitamin contents. Regarding the vitamin E concentration in human milk, its concentration is associated with the total fat intake by mothers, while the vitamin E intake seems to have no effect (*26*).

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