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Effects of Multivitamin/Mineral Supplementation, at Nutritional Doses, on Plasma Antioxidant Status and DNA Damage Estimated by Sister Chromatid Exchanges in Lymphocytes in Pregnant Women

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Abstract: The purpose of this study was to evaluate the effect of multivitamin/mineral-supplementation during pregnancy on plasma levels of antioxidants and sister chromatid exchange (SCE) rate – an indicator of damage to DNA. A controlled, semi-randomized, prospective trial was performed comparing the supplement group, who received multivitamin/mineral tablet once daily for 10 weeks, to the control group. Plasma levels of antioxidants and SCE in lymphocytes were measured initially (20 wk gestation) and at the end of the intervention (34 wk gestation). In the control group, SCE rates increased significantly at 34 wk gestation compared to 20 wk gestation, whereas there was no change in the supplement group. Plasma retinol, β-carotene and ascorbate decreased significantly in the control group. In the supplement group, a significant increase in plasma β-carotene (55.6%), coenzyme Q10 (40.2%), folic acid (15.9%) and zinc (24.2%) was observed after 10 weeks of supplement. Increased plasma levels of antioxidants in the supplement group could not decrease SCE rates, however, they could prevent an increase in SCE rates which may be induced by reactive oxygen species generated from the enhanced steroid hormones in the last trimester, suggesting that multivitamin/mineral-supplement during pregnancy may prevent DNA damage due to the altered hormonal profile.

Key words: Pregnancy, multivitamin/mineral supplementation, sister chromatid exchange, sex hormones, antioxidants

Abbreviations used: SCE, sister chromatid exchange; HFC, high frequency cell; *H* index, heterogeneity index; EMEM, eagle's minimum essential medium.

Introduction

Interest in maternal nutritional status as a critical factor for prenatal development has increased [1]. Although an adequate intake of most nutrients can be obtained from a well-balanced diet, such diets do not typically provide pregnant women with all nutrients at current RDA levels. So, it is often recommended for women with childbearing potential to take multivitamin-mineral preparation.

Normal pregnancy is accompanied by anatomic and physiologic changes that affect almost every function of the body. Changes in the level of certain sex hormones such as progesterone and estrogen have major effects on maternal physiology during pregnancy [2]. In published studies, enhanced frequency of SCEs has been noted in pregnant women, and this has been attributed to the high levels of sex hormones present during the last trimester of pregnancy [3, 4].

The SCE technique promises to be a sensitive indicator of DNA damage in mammalian cells [5]. Oxidative damage to DNA is considered a crucial mechanism in cancer development [6]. Thus, increased cytogenetic damage *in vivo,* as measured by SCE in lymphocytes, may reflect increased cancer risk. It is well reviewed that dietary and blood antioxidants play an important role in preventing cancers by inhibiting DNA damage [7, 8].

The aim of this study is to evaluate the changes in SCE rate during the early pregnancy and the late pregnancy and the effect of a 10 week-multivitamin/mineral supplementation during pregnancy on plasma antioxidant status and SCE rate.

Materials and Methods

Subjects: The total study population consisted of 39 healthy pregnant women who were recruited from Ignaz-Semmelweis-Frauenklinik, Vienna. They were in their 19–22 weeks of gestation and the age of the participants ranged from 21 to 39 years. Information on individual characteristics, smoking habits, and lifestyle factors were obtained by questionnaire.

Intervention design: The design adopted was a controlled, semi-randomized, prospective trial. Thirty-nine subjects participated in the intervention study. They were assigned at random to the control (18 subjects) or supplement group (21 subjects). The supplement group took multivitamin/mineral tablet once daily for a period of 10 weeks (from 24 to 34 weeks gestation). The composition of the multivitamin/mineral tablet was as follows: 11 vitamins = β-carotene 3 mg, thiamin 1.6 mg, riboflavin 1.8 mg, pyridoxin 2.2 mg, cobalamin 0.0026 mg, niacin 20 mg, pantothenate 7 mg, biotin 0.1 mg, C 95 mg, E 12 mg, folic acid 0.4 mg; 2 minerals = calcium 130 mg, magnesium 75 mg; 3 trace elements = iron 15 mg, zinc 10 mg, iodine 0.1 mg. The preparation was provided by Milupa AG, Friedrichsdorf, Germany. Dietary information provided by the subjects was recorded using a 7-d weighed dietary intake, 24 h-recall, and food-frequency questionnaire, on the basis of which, and by means of database, the dietary nutrient intake including vitamins and trace elements was calculated. Blood samples (9 ml in heparinized plastic tubes) were collected in the 20th and 34th week from all participants to determine various plasma parameters and to measure the DNA damage in peripheral blood lymphocytes.

SCE assay: Heparinized blood for lymphoyte culture was obtained by venipuncture at the clinic and transferred to our laboratory and processed within 4–6 h. Evaluation of SCE in peripheral lymphocytes was performed as described elsewhere [9]. Briefly, duplicate 0.8 ml volumes of whole blood were added to 9.5 ml EMEM supplemented with 100 units/ml penicillin-streptomycin solution, 2 mM of L-glutamine and 15% v/v heat-inactivated fetal bovine serum. 0.1 ml of phytohemagglutinin and 0.1 ml of lithium heparin and 0.05 ml of 5 mM 5-bromo-2-deoxyuridine, to give 25 μM, were added and incubated at 37°C in 5% CO_2 for 72 h. Colchicine (10 μg/ml) was added 2 h before harvesting. The cells were hypotonized using 0.075 M KCl solution and fixed with fresh methanol : acetic acid (3 : 1, v/v). Metaphase slides were prepared and were stained with 5% Giemsa solution in a freshly made Sörenson's buffer. For each subject, 50 metaphases were scored to determine the mean SCE frequency. SCE rate was represented in three different ways: (1) mean number of baseline SCE per cell, (2) the highest five SCE means (Top5 HFC), (3) heterogeneity index (*H,* variance/mean). HFC and *H* index have been suggested for the detection of a subpopulation of lymphocytes with high SCE rates by Carrano and Moore [10] and Margolin and Shelby [11]. They also suggested that the statistical analysis on HFC and *H* index is more powerful than that based on SCE means of individuals.

Blood parameters: Plasma was obtained from heparinized blood samples by centrifugation (1000 rpm, for 10 min). An appropriate portion of platelet-rich-plasma was collected for estimation of ascorbic acid. The rest of the platelet-rich-plasma was centrifugated at 3000 rpm for 15 min and the supernatant fraction (platelet-poor-plasma) was separated. The plasma was divided in appropriate aliquots and stored at –20°C until further analysis. Plasma ascorbic acid was measured photometrically by the

method of Denson and Bowers [12]. The HPLC procedure developed by Jakob and Elmadfa [13] was used to measure retinol, β-carotene, tocopherol and coenzyme Q10 concentrations in plasma. Folic acid was measured using Amersham Dual Radio Immuno Assay (RIA) kit from the Johnson & Johnson Clinical Diagnostics Ltd (Amersham, UK). Plasma zinc concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer 5100) according to Speitling *et al* [14].

Statistical analyses: The statistical significance of differences between the control and supplement groups was determined by the non-parametric Mann-Whitney U-test and comparisons for results within a group at 20 and 34 weeks gestation were performed using the Wilcoxon matched pairs test. We then used MANOVA to compare the groups, adjusting for age, smoking status, alcohol consumption, use of multivitamin during the intervention, and baseline (20 wk gestation) plasma concentrations of analyte. Correlations between SCE rates were analyzed by Spearman's method. All statistical analyses were conducted using SPSS for Windows.

Table I: Characteristics of subjects[a]

	Control group (n = 18)	Supplement group (n = 21)
Age	28.61 ± 1.06	27.90 ± 0.82
BMI (kg/m^2) at 20 wk	24.08 ± 0.72	23.18 ± 0.51
% increase of BMI at 34 wk	12%	12%
Smoking status		
Non-smokers (%)	10 (56)	12 (57)
Current-/Stop-smokers during pregnancy (%)	2 (11)/6 (33)	2 (10)/7 (33)
Cigarettes/day	12.78 ± 2.82	8.10 ± 1.47
Smoked year	10.75 ± 2.11	9.27 ± 1.46
Packyear[b]	6.57 ± 1.84	3.64 ± 0.68

[a] Values represent mean ± SE

[b] Packyears, an indicator of cumulative smoking dose, was defined as packs smoked daily multiplied by years of smoking.

Results

The characteristics of subjects at study entry in the control and supplement group are shown in Table I. There

Table II: Plasma antioxidant concentrations of the control and supplement group from pregnant women at 20 wk gestation (before intervention) and 34 wk gestation (after intervention)[a]

Parameters and group	Plasma concentration 20 wk	34 wk	% change[b]	*P* value[c]
Retinol (μmol/L)				
Control	1.18 ± 0.08	0.86 ± 0.06	–27.1**	0.33
Supplement	1.09 ± 0.08	0.94 ± 0.06	–13.8	
β-carotene (μmol/L)				
Control	0.68 ± 0.09	0.53 ± 0.05	–22.1*	**0.04**
Supplement	0.63 ± 0.09	0.98 ± 0.16[f]	+55.6*	
Tocopherol (μmol/L)[d]				
Control	34.30 ± 2.15	39.53 ± 1.59	+15.3**	0.49
Supplement	32.85 ± 2.45	38.31 ± 2.06	+16.3	
Coenzyme Q10 (μmol/L)				
Control	1.91 ± 0.13	1.72 ± 0.14	–10.0	0.11
Supplement	1.37 ± 0.21[e]	1.92 ± 0.17	+40.2*	
Ascorbic acid (μmol/L)				
Control	99.52 ± 5.77	80.22 ± 5.33	–19.4**	0.85
Supplement	89.64 ± 3.89	79.21 ± 6.37	–11.6	
Folic acid (nmol/L)				
Control	19.71 ± 2.41	22.66 ± 2.10	+15.0	0.85
Supplement	21.73 ± 2.05	25.19 ± 1.31	+15.9*	
Zinc (μmol/L)				
Control	17.53 ± 1.00	16.27 ± 0.91	–7.2	**0.04**
Supplement	15.77 ± 1.01[e]	19.59 ± 1.33	+24.2*	

[a] Values represent mean ± SE.

[b] Values represent the percentage of the mean baseline (20 wk gestation) concentration (* $P < 0.05$, ** $P < 0.01$).

[c] *P* values from MANOVA comparing changes between the two groups. Adjusted of age, smoking status, alcohol consumption, use of multivitamin during the intervention, and baseline (20 wk gestation) plasma concentration.

[d] Tocopherol was calculated as α-tocopherol + 0.25 × γ-tocopherol.

[e] Significantly different from the control and supplement group at baseline (20 wk gestation).

[f] Significantly different from the control and supplement group at end of the study (34 wk gestation).

Normal ranges for micronutrients: retinol, > 0.7 μmol/L [37]; β-carotene, > 0,4 μmol/L [37]; coenzyme Q10, 0.5–2.1 μmol/L [38]; ascorbic acid, > 46 μmol/L [39]; folic acid, > 13.6 nmol/L [37]; Zinc, 12–26 μmol/L [14].

were no significant differences between the groups with respect to age, body mass index at 20 wk and 34 wk, and smoking status during pregnancy. Although the smoking habits (cigarettes/day, smoked years and packyears), combined from current-smokers and stop-smokers during pregnancy, were higher in the control than in the supplement group, there was no significant difference between the two groups.

The plasma antioxidants listed in Table II were within the normal range during the intervention in both of the groups. Plasma concentrations did not differ significantly between the two groups at baseline, except for coenzyme Q10 and zinc. The baseline plasma concentrations of coenzyme Q10 and zinc were significantly higher in the control group than in the supplement group. For the control group a significant decrease in plasma concentrations of retinol, β-carotene and ascorbic acid and a significant increase in tocopherol were observed at 34 weeks gestation, compared to 20 weeks gestation. The plasma concentrations of coenzyme Q10 and zinc showed a tendency to decrease without statistical significance. The 15% increase in folic acid was also not statistically significant by Wilcoxon matched pairs test. In the supplement group, a significant increase (55.6%) in plasma β-carotene from 0.63 ± 0.09 to 0.98 ± 0.16 μmol/L ($P < 0.01$) was observed after ten weeks of 3 mg supplement of β-carotene/day (Fig. 1). Coenzyme Q10 was increased by 40.2% and this increase was statistically significant ($P < 0.05$). The supplementation of 0.4 mg of folic acid and 10 mg of zinc/d increased significantly the plasma levels of folic acid and zinc by 15.9% and 24.2%, respectively. However, there was no significant effect of multivitamin/mineral supplement on plasma levels of retinol, tocopherol and ascorbic acid. Only the plasma β-carotene concentration was significantly higher in the supplement group than in the control group at the end of the trial. *P* values for the changes in the plasma concentrations of antioxidants after multivariate adjustment for age, smoking status, alcohol consumption, use of multivitamin during the intervention, and baseline (20 wk gestation) plasma concentration are given in Table II. In this model supplementation with multivitamin/mineral during pregnancy was associated with a change significantly larger than the change in the control group only for β-carotene and zinc.

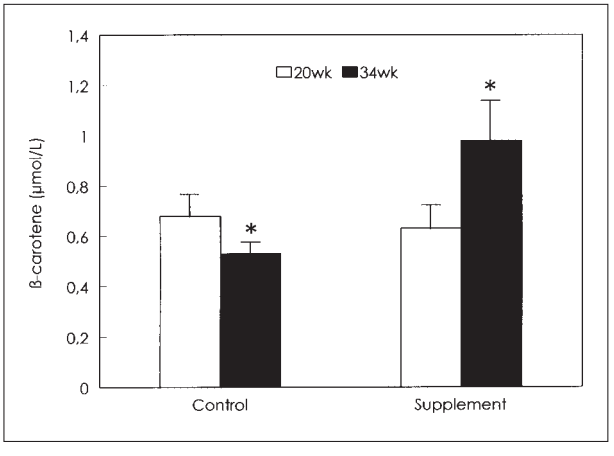

Figure 1: Plasma concentration of β-carotene of the control and supplement group at 20 wk (□) and 34 wk (■) gestation. Error bars represent the standard errors of the mean. * $P < 0.05$ significantly different from 20 wk gestation in the control and supplement group. n = 18 (control); n = 21 (supplement group).

The effect of multivitamin/mineral supplement during pregnancy on SCE rate in peripheral lymphocytes is shown in Table III. There was no statistically significant difference in the frequency of SCE, Top5 HFC and *H* index at 20 weeks gestation between the two groups. For mean SCE frequency, 4.5% increase in the control grop and 3.1% decrease in the supplement group were noted after the intervention, but this difference did not reach the statistical significance ($P > 0.1$). Top5 HFC, computed the mean SCE frequency in the highest 5 cells in the SCE distribution for each individual, was increased significantly in the control group from 13.90 ± 0.70 to 15.31 ± 0.68 ($P = 0.02$). The heterogeneity index *(H)*, a ratio of the sample variance to sample mean SCE, showed a significant increase with the advancement of pregnancy in the control group (from 0.95 ± 0.06 to 1.27 ± 0.09, $P = 0.001$) (Fig. 2). In the supplement group Top5 HF and *H* index did not change during the intervention. After adjustment of changes in the SCE rate for age, smoking status, alcohol consumption, use of multivitamin during the intervention, and initial (20 wk gestation) value of each SCE rate, using MANOVA, the control group showed a significantly larger change in *H* index than the supplement group ($P = 0.03$).

Discussion

Elevated SCE frequency has been noted in pregnant women [3, 4] and in oral contraceptive users [4, 15]. The relationship was found between SCE frequencies and the menstrual cycle [16–18]. These findings suggest that the increased frequency of spontaneous SCE may be due to the altered hormonal profile in these women, compared to a normal control. Although Hill and Wolff [19] did not observe a significant difference between pregnant women in the early pregnancy (3–6 months) and non-pregnant women, Sharma and Das [3] and Ghosh and Ghosh [4] found a highly significant difference between pregnant in late pregnancy (8–9 months) and non-pregnant women.

Table III: Mean SCE frequency, Top5 HFC[a] and *H* index[b] of the control and supplement group from pregnant women at 20 wk gestation (before intervention) and 34 wk gestation (after intervention)[c]

SCE rates and group	20 wk	34 wk	% change[d]	*P* value[e]
Mean SCE				
Control	8.60 ± 0.43	8.99 ± 0.49	+4.5	0.46
	(6.40 ~ 10.94)	(6.68 ~ 13.28)		
Supplement	9.06 ± 0.31	8.81 ± 0.44	–3.1	
	(6.88 ~ 11.64)	(5.34 ~ 11.94)		
Top5 HFC				
Control	13.90 ± 0.70	15.31 ± 0.68	+10.1*	0.13
	(11.00 ~ 19.00)	(11.80 ~ 20.40)		
Supplement	14.99 ± 0.57	14.64 ± 0.61	–2.3	
	(10.60 ~ 21.40)	(9.60 ~ 20.20)		
H index				
Control	0.95 ± 0.06	1.27 ± 0.09	+33.7**	**0.03**
	(0.53 ~ 1.36)	(0.67 ~ 1.85)		
Supplement	1.12 ± 0.07	1.13 ± 0.06	+0.9	
	(0.77 ~ 1.91)	(0.67 ~ 1.62)		

[a] Top5 HF, mean of high SCE frequency cells which were calculated from the highest five metaphases for each study subject.
[b] *H* index; heterogeneity index, variance/mean SCE.
[c] Values represent mean ± SE (ranges).
[d] Values represent the percentage of the mean baseline (20 wk gestation) concentration (* $P < 0.05$, ** $P < 0.01$).
[e] *P* values from MANOVA comparing changes between the two groups. Adjusted of age, smoking status, alcohol consumption, use of multivitamin during the intervention, and initial (20 wk gestation) value of each SCE rate.

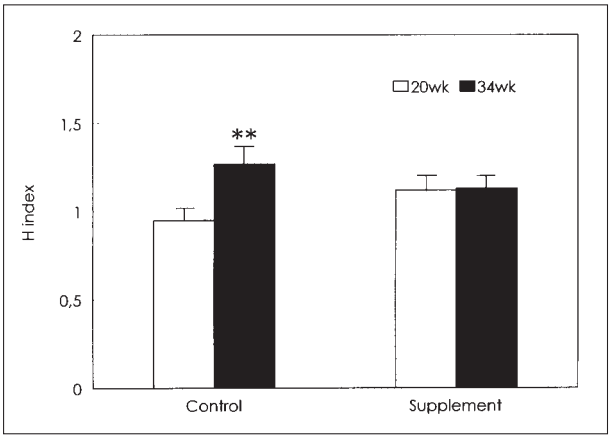

Figure 2: H index (variance/mean SCE) of the control and supplement group at 20 wk (□) and 34 wk (■) gestation. Error bars represent the standard errors of the mean. ** $P < 0.01$ significantly different from 20 wk gestation in the control group. n = 18 (control); n = 21 (supplement group).

This may be because of the difference expected in the overall hormonal and physiological status between the early pregnancy and the late pregnancy.

A profound change in the levels of certain sex hormones such as progesterone, estrogen and human chorionic gonadotropin (HCG) occurs in females during pregnancy, particularly during the last trimester [20]. There are indications that the higher level of these hormones present in the blood of pregnant women and women using oral contraceptives may play an important role in increasing the sensitivity of their lymphocytes to genetic damage [15, 21].

Joseph-Lerner *et al* [18] have found a positive correlation of SCE and 17 β-estradiol, and increased SCE frequency after HCG administration in the group undergoing ovulation induction.

A possible explanation for the effects of hormones could be oxidative DNA damage. Several of the most common cancers in western societies occur in hormonally responsive tissues, including breast, endometrium, and ovary in women and prostate in men. These cancers have been causally linked to exposure to synthetic or endogenous steroidal hormones or their metabolites [22]. Steroid hormones, particularly estrogens, have been suspected for many years of being carcinogens [23]. The most widely appreciated and investigated effect of estrogens is increased cell proliferation [24, 25]. More recently, it has become clear that several metabolites can directly, or indirectly through redox cycling processes that generate reactive oxygen species, cause oxidative DNA damage [26, 27]. Lukic and Barjaktarovic [28] observed that newborns from progesterone-treated pregnant women had significantly higher frequency of SCE than newborns from a control group, suggesting that progesterone might induce formation of DNA-polymerase, exonuclease or some other proteins, thus producing an initial lesion in the DNA.

Therefore, the increased Top5 HFC and *H* index in the control group in the present study may be due to the increased oxidative DNA damage resulting from the en-

hanced steroid hormones in the last trimester. Moreover, the significantly decreased plasma concentration of antioxidants in the control group may aggravate the oxidative damage in DNA. Although there was no change in the mean SCE during the intervention, Top5 HFC and *H* index were significantly increased in the control group and there was a statistically significant correlation between SCE and Top5 HFC (r = 0.921) and between SCE and *H* index (r = 0.396). This result indicates that the analysis of Top5 HFC and *H* index, representing the subpopulation of lymphocytes with high SCE rates, are more sensitive in detecting an effect of pregnancy than the analysis of mean SCE.

In the supplement group, changes in plasma β-carotene and zinc levels were significantly different from changes in the control group after adjustment for multiple covariates. The importance of zinc in cellular antioxidant and free-radical defence mechanisms has been recognized for more than a decade [29] during which time increased lipoperoxidation (expressed as TBARs) has been demonstrated in a variety of tissues from zinc-deficient animals [30]. Zinc can function as an antioxidant by involving a role for the metal as a prosthetic group of Cu, Zn-SOD [29] or associating with the sulphur-rich protein metallothionein (Mt), which may function to scavenge active oxygen species [31].

Of all compounds with vitamin activity that have been studied with regard to cancer prevention, β-carotene may very well be the compound most intensively studied during recent years [32]. β-Carotene, which quenches singlet oxygen, can also function as a chain breaking antioxidant in the lipid phase by neutralizing peroxyl radicals [33]. Coincubation of eukaryotic cells with β-carotene prior to or during exposure to activated phagocytes o to a cell-free, oxidant-generating system prevented oxidant-induced chromosomal damage, and a biologic role for this agent in cytoprotection against phagocyte-derived oxidants has been proposed [34]. In mouse mammary cell culture, β-carotene has been shown to reduce SCEs induced by chemical carcinogens [35]. However, the effect of β-carotene supplementation on oxidative DNA damage *in vivo* is somewhat controversial [36].

In conclusion, increased plasma concentrations of β-carotene and zinc in the supplement group could not decrease high SCE rates, however, they could prevent an increase in high SCE rates which may be induced by reactive oxygen species generated from the enhanced steroid hormones in the last trimester. Therefore, the result in the present study suggests that multivitamin/mineral supplements, particularly β-carotene and zinc, during pregnancy may prevent DNA damage due to the altered hormonal profile by scavenging reactive oxygen species.

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