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Effects of Multivitamin-Mineral Supplementation, at Nutritional Doses, on Plasma Antioxidant Status, Erythrocyte Antioxidant Enzyme Activities and Lipid Peroxidation during Pregnancy

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ABSTRACT

The aim of this study was to evaluate the effect of multivitamin-mineral supplementation during pregnancy on plasma levels of antioxidants, erythrocyte antioxidant enzyme activities, and lipid peroxidation. A controlled, semi-randomized, prospective trial was performed by comparing the supplement group, which received multivitamin-mineral tablets once daily for 10 weeks, with the control group. Plasma levels of β-carotene, tocopherol, coenzyme Q10, ascorbate, folate, zinc, and selenium and malondialdehyde (MDA), as well as the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in erythrocytes were measured initially (20 wk gestation) and at the end of the intervention (34 wk gestation). In the control group, plasma ascorbate and selenium levels decreased and tocopherol levels increased. In the supplement group, a significant increase in plasma β-carotene (46%), coenzyme Q10 (42%), and zinc (24%) was observed after 10 weeks of supplementation. No changes were observed in the plasma levels of MDA, and erythrocyte GSH-Px activity, while SOD activity increased in both control group and the supplement group during the intervention. These data suggest that multivitamin-mineral supplementation during pregnancy produced moderate increases in plasma β-carotene, coenzyme Q10, and zinc concentrations but the enhancement of these plasma antioxidants had no direct effect on the plasma level of MDA, erythrocytes SOD or GSH-Px activities

KEY WORDS pregnancy, multivitamin-mineral supplementation, plasma antioxidant, antioxidant enzymes, lipid peroxidation.

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, especially some vitamins and minerals (vitamins B6, D, E, and folate, iron, calcium, zinc and magnesium), at current RDA levels.[2] So, it is often recommended for women with childbearing potential to take multivitamin-mineral preparation. There is a significant body of evidence that vitamin-mineral supplement use can be associated with a reduced risk of pregnancy complications, and also of birth defects.[3-5]. Another potential supplementation benefit includes improved antioxidant and immune defense systems.[6]

Lipid peroxidation and oxygen radicals are reactive and damaging compounds involved in the etiology of a wide variety of diseases including atherosclerosis and cancer.[6] To control lipid peroxidation and oxygen radicals there is a defensive system in our body that consists of endogenous antioxidant defenses such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and diet-derived antioxidative free radical scavenging molecules such as vitamin C, E, and various carotenoids.[7] Lipid peroxidation has been suggested as a causative factor in pregnancy-induced hypertension, and pre-eclampsia in pregnant women.[8,9] Serum lipid peroxidation breakdown products are elevated with the advancement of normal pregnancy, this being associated partly with the increasing content of lipoproteins.[10,11] Uotila *et al.*[12] have reported that in normal pregnancy lipid peroxidation process seems to be controlled by adaquate antioxidative process. However, scanty and controversial information is available on the antioxidative capacity in normal pregnancy. Moreover, little is known about the effect of multivitamin-mineral supplementation on the antioxidative system and lipid peroxidation during pregnancy.

Therefore, we evaluate the changes in the antioxidative system and lipid peroxidation with the advancement of normal pregnancy and the effect of a 10 week-multivitamin-mineral supplementation during pregnancy on the antioxidative system and lipid peroxidation. For the an-

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tioxidative system, we measured the concentrations of β-carotene, tocopherol, coenzyme Q10, ascorbic acid, folate, zinc, and selenium in plasma, as well as the activity of SOD and GSH-Px in erythrocytes. With regard to the lipid peroxidation, malondialdehyde (MDA) was measured in plasma.

MATERIALS AND METHODS

1. Subjects

The total study population consisted of 35 healthy, non-smoking pregnant women who were recruited from Ignaz-Semmelweis-Frauenklinik, Vienna. They were in their 15 – 20 weeks of gestation and the age of the participants ranged from 21 to 39. Information on individual characteristics and lifestyle factors were obtained by questionnaire.

2. 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 (16 subjects) or supplement group (19 subjects). The supplement group took multivitamin-mineral tablets once daily for a period of 10 weeks (from 24 to 34 weeks gestation). The composition of the multivitamin-mineral tablet is shown in Table 1. Dietary information provided by the subjects was recorded using a 7-d weighed dietary intake, 24h-recall, and food-frequency questionnaire, on the basis of which, and by means of database, the dietary intake of nutrients including vitamins and trace elements was calculated.

Table 1. Composition of preparation

Content substances	Amount per tablet (mg)	German recommended dietary allowances for pregnancy (mg/d)
Vitamin		
C	95.0	100.0
Niacin	20.0	17.0
E	12.0	14.0
Pantothenate	7.0	6.0
B6	2.2	2 6
B2	1.8	1 8
B1	1.6	1 5
Folic acid	0.4	0.6
Biotin	0.1	0.003 – 0.1
B12	0.0026	0.0035
β-carotene	3.0	
Mineral		
Calcium	130 0	1200.0
Magnesium	75.0	300.0
Iron	15.0	30.0
Zinc	10.0	15.0
Iodine	0.1	0.23

3. Blood parameters

Blood samples (9 ml in heparinized plastic tubes) were collected at the 20th and 34th gestational week from all participants. 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 centrifuged 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 −80℃ until further analysis. Erythrocytes were washed three times with isoosmotic phosphate buffered saline (PBS), pH 7.4, and resuspended to the original volume. The erythrocyte-suspensions were filled with nitogen gas and frozen at −80℃ before final analysis.

β-Carotene, tocopherol and coenzyme Q10 were determined by a reversed-phase HPLC method.[13] Plasma proteins were precipitated with ethanol and lipids were extracted with n-hexane. After evaporation, residues were reconstituted with the eluent (dichloromethane and methanol, 15 : 85) and 150 μl were injected into a guard-column (Merck LiChrospher 100 RP-18, 250×4.0 mm i.d., 5 μm). Samples were run at a flow rate of 0.8 ml/min on a Merck Hitachi Pump L-7100 and detected on a Merck Hitachi UV Detector 7400 with an Integrator D-7500 (all from Merck, Germany). Absorption was monitored at 295 nm for tocopherol, at 450 nm for β-carotene and at 270 nm for coenzyme Q10. Concentrations were calculated from areas under the curve using an external calibration curve.

Total cholesterol in plasma was measured by the method of Siedel *et al.*[14] photometrically as an increase in the absorbance at 546 nm, using a colorimetric method from boehringer-mannheim GmbH.

Plasma ascorbic acid was measured photometrically by the method of Denson and Bowers.[15] Trichloroacetic acid is used to precipitate the protein in the samples and to stabilize the ascorbic acid. Copper-sulfate catalyzes the oxidation of ascorbic acid to dehydroascorbic acid. The dehydroascorbic acid is then hydrolyed to diketogluconic acid, which when dissolved in sulfuric acid and treated with 2,4-dinitrophenylhydrazine, forms an osazone. The osazone forms a stable reddish-brown product that can be measured photometrically at 520 nm.

Folic acid was measured using an Amersham Dual Radio Immuno Assay (RIA) kit from the Johnson & John-

son Clinical Diagnostics Ltd (Amersham, UK).

Plasma zinc and selenium concentration were determined by atomic absorption spectrophotometry and by electrothermal atomic absorption spectrophotometry, respectively, according to Speitling *et al.*[16] All measurements were performed on a Perkin-Elmer Model 5100 ZL (Zeeman Furnace Module) spectrometer equipped with Zeeman effect background correction, transversely heated graphite tubes, hydride generator (Perkin Elmer FIAS 400), AS-70 and AS-90.

MDA was determined by HPLC using the method of Wong *et al.*[17] Plasma samples were diluted in phosphate buffer and heated together with a TBA solution in a boiling water bath for 60 min. The tubes were then cooled, and 20 μl of sample injected into HPLC column (Merck, LichroCART 250-4 Lichrospher 100 RP-18). Samples were run at a flow rate of 1.3 ml/min and detected with fluorescence detector. Absorption was monitored at 532 nm and 1,1,3,3-tetracthoxypropane was used as a standard.

The SOD activity was assayed in erythrocyte-suspension by the procedure of Marklund and Marklund.[18] To 500 μl of the hemolysate were added 3.5 ml of water, 1 ml of ethanol and 0.6 ml chloroform. After the centrifugation at 3000 Unit/min for 2 min, various dilutions were prepared from the supernatant. 20 μl pyrogallol were added to each dilution after incubation at 37℃ for 10 min. The reaction was monitored spectrophotometrically at 320 nm for 2 min. The unit of the enzyme was defined as the amount which inhibits the autoxidation of pyrogallol by 50%.

The GSH-Px was determined using the method described by Beutler.[19] To 10 μl of a 1 : 20 dilution of the hemolysate/blank (H_2O) were added 100 μl of 1 M Tris-HCl-5 mM EDTA buffer (pH 8.0), 20 μl of glutathione, 100 μl of glutathione reductase, 100 μl of NADPH and filled with H_2O up to 1 ml. After the incubation at 37℃ for 10 min, 10 μl of t-butyl hydroperoxide was added to that solution. The reaction was run for 90 sec, and the loss of NADPH was monitored at 340 nm by the change in $A_{340\ nm}$/min. GSH-Px was expressed as I.U. (international units)/g of hemoglobin.

Table 2. Characteristics of subjects[1]

	Control group (n = 16)	Supplement group (n = 19)
Age (yrs)	28.81 ± 1.05	28.16 ± 0.85
Height (cm)	167.1 ± 1.1	165.4 ± 1.4
BMI (kg/m^2) at 20 wk	24.22 ± 0.80	23.21 ± 0.52
% increase in BMI at 34 wk	12	12

1) Values represent mean ± SE

4. Statistical analyses

The statistical significance of differences between the control and supplement group was determined by the nonparametric 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. All statistical analyses were conducted using SPSS for Windows. Values were expressed as mean ± SE and differences were considered as significant at a value of $p < 0.05$.

Table 3. Plasma antioxidant concentrations and lipid peroxidation levels of pregnant women of control and supplement group at 20 wk gestation (before intervention) and 34 wk gestation (after intervention)[1]

	Control group (n=16)			Supplement group (n=19)		
	20 wk	34 wk	% change[2]	20 wk	34 wk	% change[2]
Plasma antioxidant status						
β-carotene (μmol/L)	0.65 ± 0.08	0.53 ± 0.05	−18	0.59 ± 0.08	0.86 ± 0.12[6]	+46*
Tocopherol (μmol/L)[3]	34.69 ± 2.40	40.17 ± 1.72	+16**	33.38 ± 2.54	37.50 ± 2.02	+12
Tocopherol/Cholesterol	6.20 ± 0.48	5.62 ± 0.30	−9	5.97 ± 0.49	5.27 ± 0.29	−12
Coenzyme Q10 (μmol/L)	1.91 ± 0.15	1.78 ± 0.15	−7	1.39 ± 0.22[5]	1.97 ± 0.18	+42*
Ascorbic acid (μmol/L)	96.77 ± 5.22	77.54 ± 5.22	−22**	88.65 ± 4.02	77.45 ± 6.54	−13
Folic acid (μnmol/L)	19.09 ± 2.60	21.78 ± 2.14	+14	21.36 ± 2.21	24.60 ± 1.28	+15
Zinc (μmol/L)	17.38 ± 1.11	15.78 ± 0.84	−9	16.00 ± 1.05	19.85 ± 1.39	+24*
Selenium (μmol/L)	0.72 ± 0.04	0.62 ± 0.03	−14*	0.96 ± 0.03	0.72 ± 0.06	−25**
Plasma lipid peroxidation						
MDA (μmol/L)[4]	1.75 ± 0.09	1.56 ± 0.12	−11	1.67 ± 0.14	1.47 ± 0.11	−12

1) Values represent mean ± SE
2) Values represent the percentage of change of the mean baseline (20 wk gestation) concentration after intervenstion (*$p < 0.05$, **$p < 0.01$)
3) Tocopherol was calculated as α-tocopherol + 0.25 × γ-tocopherol
4) Malondialdehyde
5) Significant difference between control and supplement group at the baseline (20 wk gestation)
6) Significant difference between control and supplement group at the end of the study (34 wk gestation)
Normal ranges for micronutrients: β-carotene > 0.4 μmol/L,[32] tocopherol > 16.3 μmol/L,[32] coenzyme Q10: 0.5 − 2.1 μmol/L,[33] ascorbic acid > 46 μmol/L,[34] folic acid > 13.6 nmol/L,[32] zinc 12 − 26 μmol/L,[16] selenium 0.8 − 1.5 μmol/L[35]

RESULTS

The characteristics of subjects in the control and supplement group are shown in Table 2. There were no significant differences between the two groups with respect to age, height, and body mass index at 20 wk and 34 wk of gestation.

Plasma concentrations of various antioxidants are listed in Table 3. Plasma β-carotene, tocopherol and coenzyme Q10, ascorbic acid, folate, and zinc levels were within the normal range during the intervention in both groups. Plasma selenium level was lower than published normal values of between 0.8 and 1.5 μmol/L. Plasma concentrations did not differ significantly between two groups at the baseline, except for coenzyme Q10. The baseline plasma concentration of coenzyme Q10 was significantly higher in the control group than in the supplement group.

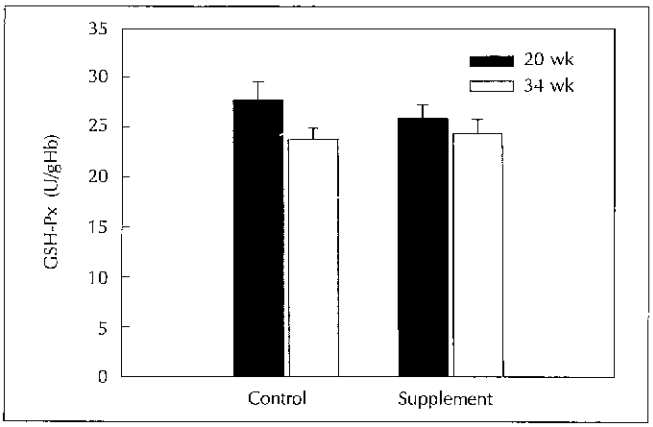

Fig. 1. Activity of erythrocyte glutathione peroxidase of control and supplement group at 20 wk (■) and 34 wk (□) gestation. Error bars represent the standard error of the mean. n = 16 (control), n = 19 (supplement group).

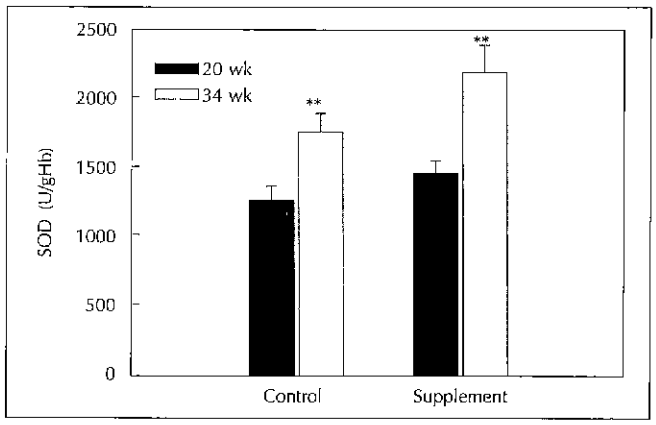

Fig. 2. Activity of erythrocyte superoxide dismutase of control and supplement group at 20 wk (■) and 34 wk (□) gestation. Error bars represent the standard error of the mean $^{**}p < 0.01$ significantly different from 20 wk gestation in the control and supplement group. n = 16 (control), n = 19 (supplement group).

For the control group a significant decrease in plasma concentrations of ascorbic acid and selenium was observed at 34 weeks gestation, compared to 20 weeks gestation. The plasma concentration of tocopherol showed an increase with the advancement of pregnancy in the control group, but the significance disappears if it is expressed as the tocopherol/cholesterol ratio as there was an increase in the plasma cholesterol status of the control group throughout the intervention. The plasma concentrations of β-carotene, coenzyme Q10 and zinc showed a tendency to decrease without statistical significance. The 14% increase in folic acid was also not statistically significant by Wilcoxon matched pairs test.

In the supplement group, a significant increase (46%) in plasma β-carotene from 0.59 ± 0.08 to 0.86 ± 0.12 μmol/L ($p < 0.05$) was observed after ten weeks of 3 mg supplement of β-carotene/day. Coenzyme Q10 increased by 42% and this increase was statistically significant ($p < 0.05$). The supplementation of 10 mg of zinc/d increased significantly the plasma levels of zinc by 24%. However, there was no significant effect of multivitamin-mineral supplement on plasma levels of tocopherol, ascorbic acid, and folic acid. There was a significant decrease in the plasma selenium concentration in the supplement group as in the control group.

No significant changes were observed in the plasma levels of MDA as a marker of lipid peroxidation (Table 3) and erythrocyte GSH-Px activity (Fig. 1), while erythrocyte SOD activity increased significantly in both groups during the intervention (Fig. 2). The changes of the plasma MDA, erythrocyte GSH-Px and SOD activities were not different between the two groups.

DISCUSSION

Normal pregnancy is accompanied by anatomic and physiologic changes that affect almost every function of the body. Plasma volume begins to increase near the end of the first trimester of pregnancy and if the availability of nutrients or the synthesis of normal blood constituents does not keep pace with the expansion of plasma volume, their concentrations per 100 ml of blood will decrease, even though the total amount may rise.[20] This is apparently what happens with plasma levels of ascorbic acid, zinc, and selenium in the control group of our study (Table 3). Slightly but non-significantly elevated levels of plasma folic acid may be caused by iron supplementation containing folic acid in some subjects of the control

group, which was prescribed by their doctors.

In contrast to water-soluble nutrients, fat-soluble nutrients show increased serum concentrations during pregnancy.[20] We observed that the significant increase in plasma tocopherol concentrations with advancing pregnancy in control group vanished if the increasing levels of cholesterol (from 5.46 ± 0.26 to 7.02 ± 0.43 mmol/L, $p<0.01$) were taken into account (Table 3). Haga and Lunde[21] suggested that a better assessment of vitamin E ststus is to measure the tocopherol level as related to β-lipoprotein because of their interdependency in circulation.

The activity of SOD in erythrocytes increased significantly at 34 weeks gestation (3rd trimester), compared to 20 weeks gestation (2nd trimester) (Fig. 2). The increased SOD activity may reflect an increased spontaneous generation of superoxide in the cells.[22] However, there was no alteration in plasma MDA levels, lipid peroxidation breakdown product, with advancing pregnancy in our study (Table 3). In relation to this result, Uotila *et al.*[23] have found that thiobarbituric acid reactive substances (TBARS) failed to show any change seen in the course of pregnancy, but conjugated dienes, another marker of lipid peroxidation, increased significantly in the 2nd trimester, after which a decrease to the same level as in the 1st trimester was noted. Loverro *et al.*[24] reported that in normal pregnancy, longitudinal observation evidenced a significant increase of TBARS level and a rise in the activity of SOD in 3rd trimester. Controversies on the levels of lipid peroxidation during pregnancy can be due to unspecification and inaccuracy of these methods. Therefore, more reliable method for measurement of the lipid peroxidation should be developed.

Mean plasma concentrations of β-carotene, coenzyme Q10 and zinc increased significantly after 10 weeks of supplementation in the group receiving multivitamins and minerals at nutritional doses (Table 3). β-Carotene, which quenches singlet oxygen, can also function as a chain breaking antioxidant in the lipid phase by neutralizing peroxyl radicals.[25] The importance of zinc in cellular antioxidant and free-radical defense mechanisms has been recognized for more than a decade[26] during which time increased lipoperoxidation (expressed as TBARS) has been demonstrated in a variety of tissues from zinc-deficient animals.[27] Zinc can function as an antioxidant by involving a role for the metal as a prosthetic group of Cu, Zn-SOD[26] or associating with the sulphur-rich protein metallothionein (Mt), which may function to scavenge active oxygen species.[28] Coenzyme Q10 is closely related to cellular energy metabolism, which is a prominent endogenous source of reactive oxygen species (ROS),[29] and has furthermore been suggested as a scavenger of ROS.[30]

Despite of improvement of the antioxidative potential in the supplement group, the changes of plasma MDA, erythrocytes SOD, and GSH-Px activities were not different from those of the control group (Table 3) (Fig. 1, 2). In agreement with our results, Hilbert and Mohsenin[31] found that augmentation of micronutrient antioxidants (vitamin E, C and β-carotene) had no effect on antioxidant enzyme activities, despite of much more amounts of supplementation (500 mg of ascorbate, 400 IU of α-tocopherol, 7.5 mg of β-carotene), compared to ours. There are potentially several reasons as to why multivitamin-mineral supplementation was not effective in altering antioxidant enzymes: 1) the multivitamins and minerals may not be enough to quench the oxidants. 2) the mechanism of antioxidant adaptation to oxidative stress is different for plasma antioxidative nutrients and erythrocyte enzymatic antioxidants.

In summary, our data suggest that multivitamin-mineral supplementation during pregnancy produced moderate increases in plasma β-carotene, coenzyme Q10, and zinc concentrations but the enhancement of these plasma antioxidants has no direct effect on the plasma level of MDA, erythrocyte SOD or GSH-Px activities. Further studies are needed to clarify the relationship between lipid peroxidation and antioxidative function and the effect of antioxidative nutrients supplementation on these variables during pregnancy.

Literature cited

1) Keen CL, Zidenberg-Cherr S Should vitamin-mineral supplements be recommended for all women with childbearing potential? *Am J Clin Nutr* 59 532S-539S, 1994

2) National Research Council. Recommended dietary allowances. 10th ed. Washington DC National Academy Press, 1989

3) Gonzalez MJ, Schmitz KJ, Matos MI, Lopez D, Rodriguez JR, Gorrin JJ Folate supplementation and neural tube defects. a review of a public health issue. *P R Health Sci J* 16 387-393, 1997

4) Simmer K, Lort-Phillips L, James C, Thompson RPH A double blind trial of zinc supplementation in pregnancy. *Eur J Clin Nutr* 45 139-144, 1991

5) Belizan JM, Villar J, Gonzalez L, Campodonico L, Bergel E. Calcium supplementation to prevent hypertensive disorders of pregnancy *N Engl J Med* 325 1399-1405, 1991

6) Halliwell B, Gutteridge JMC. Free radicals in Biology and Medicine, 2nd ed. Clarendon press, Oxford, 1989

7) Halliwell B. Antioxidants in human health and disease. *Annu Rev Nutr* 16 33-50, 1996

8) Uotila JT, Tuimala RJ, Aarnio TM. Findings on lipid peroxidation and antioxidant function in hypertensive complication of pregnancy. *Brit J Obstet Gynaecol* 100: 270-276, 1993
9) Mutlu-Turkoglu U, Ademoglu E, Ibrhimoglu L, Aykac-Toker G, Uysal M. Imbalance between lipid peroxidation and antioxidant status in preeclampsia. *Gynecol Obstet Invest* 46: 37-40, 1998
10) Maseki M, Nigishigaki I, Hagihara M, Tomoda Y, Yagi K. Lipid peroxide levels and lipid content of serum lipoprotein fractions of pregnant subjects with or without preeclampsia. *Clin Chim Acta* 115 155-161, 1981
11) Wickens D, Wilkins MH, Lunec J, Ball G, Dormandy TL. Free-radical oxidation (peroxidation) products in plasma in normal and abnormal pregnancy. *Ann Clin Biochem* 18: 158-162, 1981
12) Uotila R, Tuimala R, Aarnio T, Pyykko K, Ahotupa M. Lipid peroxidation products, selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. *Eur J Obstet Gynecol Reprod Biol* 42 95-100, 1991
13) Jakob E, Elmadfa I. Rapid HPLC-Assay for assessment of vitamin K1, A, E and beta-carotene status in children (7 – 19 years). *Internat J Vit Nutr Res* 65 31-35, 1995
14) Siedel J, Hagele EO, Ziegenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem* 29: 1075, 1983
15) Denson YW, Bowers EF. The determination of vitamin C in white blood cells. *Clin Sci* 21 157, 1961
16) Speitling A, Hppe R, Kohlemeier M, Matiaske B, Stalte W, Thefeld W, Wetzel S. Methodenhandbuch der Verbundstudie Ernhrungserhebung und Risikofaktoren Analytik. In: Kber W, Anders HJ, Heeschen W, Kohlmeier M, eds.VERA-Schriftenreihe, Band 1, pp.123-124, Wissenschaftlicher Fachverlag, Niederkleen, 1990
17) Wong SHY, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. *Clin Chem* 33 214-220, 1987
18) Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and convenient assay for superoxide dismutase. *Eur J Biochem* 47: 469-474, 1974
19) Beutler E. Glutathione peroxidase. In: Red cell metabolism: A manual for biochemical methods, pp.71-73, Verlag Grune and Straton, New York, 1984
20) Worthing-Roberts B. Physiology of Pregnancy. In: Worthing-Roberts B, Williams SR, eds. Nutrition in pregnancy and lactation, 5th ed. pp. 64-86, Mosby Year Book, Inc, St. Louis, Missouri, 1993
21) Hg P, Lunde G. Selenium and vitamin E in cord blood from preterm and full term infants. *Acta Paediatr Scand* 67: 735-739, 1978
22) Scarpa M, Viglino P, Contri D, Rigo A. Generation of superoxide ion in human red cell lysates. *J Biol Chem* 259 10657-10659, 1984
23) Uotila J, Tuimala R, Aarnio T, Pyykko K, Ahotupa M. Lipid peroxidation products, selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. *Eur J Obstet Gynecol Reprod Biol* 42: 95-100, 1991
24) Loverro G, Greco P, Capuano F, Carone D, Cormio G, Selvaggi L. Lipid peroxidation and antioxidant enzymes activity in pregnancy complicated with hypertension. *Eur J Obstet Gynecol* 70: 123-127, 1996
25) Burton GW, Ingold KV. Beta-carotene: An unusual type of lipid peroxidant. *Science* 224 569-573, 1984
26) Sullivan JF, Jetton MM, Hahn KJ, Burch RE. Enhanced lipid peroxidation in liver microsomes of zinc-deficient rats. *Am J Clin Nutr* 33 51-56, 1980
27) Zidenberg-Cherr S, Keen CL. Essential trace elements in antioxidant processes. In: Dreosti IE, ed. Trace element, Micronutrients and Free radical, pp.107-127, Humana Press, New Jersey, 1991
28) Dreosti IE. Free radical damage and the genome. In: Dreosti IE, ed. Trace element, Micronutrients and Free radical,.pp.149-169, Humana Press, New Jersey, 1991
29) Reilly PM, Schiller HJ, Bulkley GB. Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. *Am J Surg* 161 488-503, 1991
30) Forsmark-Andree P, Ernster L. Evidence for a protective effect of endogenous ubiquinol against oxidative damage to mitochondrial protein and DNA during lipid peroxidation. *Mol Aspects Med* 15 S73-81, 1994
31) Hilbert J, Mohsenin V. Adaptation of lung antioxidants to cigarette smoking in humans. *Chest* 110: 916-920, 1996
32) Sauberich HE, Skala JH, Dowda RP. Laboratory Tests for the Assessment of Nutritional Status. CRC Press, Cleveland, 1973
33) Noia F, Littarru G, De Santis M, Oradei A, Mactromarino C, Trivellini C, Caruso A. Coenzyme Q 10 in pregnancy. *Fetal Diagn Ther* 11(4): 264-270, 1996
34) Moser U, Bendich A. Vitamin C. In: Machlin LJ ed. The Handbook of Vitamins, pp.195-232, Marcel Dekker Inc., New York and Basel, 1991
35) Van Kampen EJ, Deelstra H. Selenium. *Int J Vit Nutr Res* 63 313-316, 1993