Glutathione Peroxidase Activity Modulates Fatty Acid Profiles of Plasma and Breast Milk in Chinese Women

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Abstract:

Since little is known about the effect of selenium on the fatty acid profiles (FAP) of human breast milk, the purpose of this study was to measure the effect of habitual dietary selenium (Se) intake on this profile in plasma and breast milk. Subjects were lactating women from three locations in China where habitual selenium intakes are extremely low (Xichang), adequate (Beijing), or extremely high (Enshi). Plasma and milk samples were obtained within seven days of parturition (early samples) or within eighteen months postpartum (mature samples) and analyzed for selenium concentration, glutathione peroxidase (Gpx) activity and FAP. Plasma and milk selenium concentrations were significantly lower in the samples from women from Xichang and significantly higher in those from Enshi when compared to those from Beijing. Plasma Gpx activity, however, was higher in samples from Beijing than Xichang or Enshi. In contrast, the early breast milk samples had similar Gpx activity regardless of location. The mature samples, however, followed the same trend as plasma with the samples obtained from the women in Beijing having the highest activity. Of the unsaturated fatty acids examined, the concentration of linoleic acid, 18:2(n-6), in both plasma and milk was greater in the samples from Beijing when compared to those from Xichang or Enshi. Thus dietary selenium appears to influence the fatty acid composition in human breast milk, but influences Gpx activity only in mature milk samples.

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Article:

INTRODUCTION

Although the total lipid concentration of mature human breast milk remains fairly constant, the fatty acid profile (FAP) can be modified by a number of factors including time postpartum, maternal nutritional status, and the maternal diet (1). Recent studies have suggested that dietary selenium (Se) may also influence the FAP of human breast milk and plasma. Higher concentrations of polyunsaturated and monounsaturated fatty acids were found in breast milk samples obtained from women from New Zealand supplemented with selenium as compared to samples obtained from a similar group of women not given a supplement (2). Dotson et al. (3) found that milk selenium concentration was negatively correlated with milk arachidonic, eicosapentaenoic, and docosahexaenoic acid concentrations. In a healthy human population from Spain the serum selenium concentration was found to be directly related to percent essential fatty acids (linoleic and linolenic acids) and the sum of the (n-6) polyunsaturated fatty acids but inversely related to the sum of the percent saturated fatty acids in plasma phospholipids. Selenium was also a predictor of the unsaturation index (4). A positive correlation was found between concentrations of selenium and long-chain polyunsaturated fatty acids in serum from Finnish subjects (5) but was not seen in a Dutch population where serum selenium concentrations were higher (6).

Both lower glutathione peroxidase (Gpx) activity and selenium concentrations have been found in plasma of pregnant women when compared to nonpregnant ones (712). For instance, in a healthy Polish population, Zachara et al. (12) reported that plasma levels of selenium and Gpx activity were 42% and 20% lower, respectively, in pregnant women than in nonpregnant women. In contrast. Butler et al. (13) found that Gpx activity gradually increased during pregnancy even though whole blood and plasma selenium levels decreased. The selenium concentrations in erythrocytes of pregnant and nonpregnant women were similar, suggesting that plasma levels are more sensitive to selenium intakes than erythrocytes (7, 12, 14). Lactating women also have lower plasma selenium levels than nonlactating women (10).

The selenium content in the soil in the People's Republic of China varies markedly, resulting in intakes that range from 7 to 38.000 µg per day (9). In cities such as Shanghai and Beijing, selenium intakes are adequate and average 110 µg per day, and plasma concentrations average 1,22 µmol/L (15). Sichuan Province is an area characterized by low intakes of selenium averaging 11 µg per day and plasma selenium concentrations of 0,22 µmol/L (15). People living in Hubei Province average 759 jig per day and plasma concentrations average 6,26 µmol/L (15). The use of subjects from these areas allows one to examine the effect of selenium nutriture without depletion or supplementation. Consequently, the purpose of this study was to determine whether plasma and breast milk FAP differed in lactating women from the Hubei and Sichuan Provinces and Beijing, women whose habitual diets differed markedly in selenium content.

MATERIALS AN DMETHODS

Subjects

This study is a subset of a larger study investigating selenium nutriture and its physiologic relevance in China. Samples of breast milk and plasma were obtained from 60 lactating Chinese women from three geographic regions. Twenty-one of the women were from a rural area in central China near the village of Xichang in Sichuan Province. Dietary selenium intake of people

from this area averages 11 µg per day (15). Twenty of the women were from a rural area near the city of Beijing in northeast China. Selenium intakes by people in this area average 110 µg per day (15). Nineteen women were from eastern central China, in rural areas around the city of Enshi in Hubei Province. Dietary intakes of selenium are estimated to average 759 µg per day (15). Approximately one- half the women (11 from Xichang, 10 from Beijing, and 10 from Enshi) from each location provided samples within seven days of the birth of their infants (termed early samples). The other one-half of the subjects (10 from Xichang, 10 from Beijing, and 9 from Enshi) gave 2-18 mo postpartum samples (termed mature samples). The range of days postpartum over which mature milk samples were collected was from 44-306 for women from Xichang, from 84-171 for women from Beijing. and from 60-540 for women from Enshi.

All of the subjects were lifelong residents of these areas of China. They were selected based on similar lifestyles and living conditions. The dietary intakes of the subjects were approximated from the average nutrient intakes of a reference diet from each of the three areas. The data were obtained from the 1992 National Nutrition Survey of China (16). This study was approved by the Oregon State University Institutional Review Board and by the Chinese Academy of Preventive Medicine in Beijing.

Samples

Blood was collected from the women into VacutainerTM tubes containing 0.10 mL of Na, EDTA (1 g/L) from the antecubital arm vein by trained medical personnel. Plasma was prepared at the Chinese Academy of Preventive Medicine in Beijing. It was frozen and held at -20 °C. This sample was used for the measurement of selenium concentrations, Gpx activities and FAP.

Twenty-five milliliter breast milk samples were collected by the subjects by hand expression. The subjects were instructed to clean the skin before the milk was expressed. The samples were collected into a container and immediately sealed. They remained sealed until they were processed, as described below, at the Chinese Academy of Preventive Medicine in Beijing. Early milk samples were obtained within 1-7 days from parturition. Mature milk samples were obtained 2-18 months postpartum. The FAP of human mature breast milk remains constant over this interval of time (17). All of the milk sampLes were taken at the beginning of the nursing period and obtained on the same day as the blood samples. One fraction of the milk was frozen immediately, stored at - 20°C, and used for the determination of selenium concentration and Gpx activity. The other fraction was extracted for fatty acid measurement, as discussed below, and then frozen. The samples were transported on dry ice to Oregon State University by commercial airline where they were stored at -80 °C until analyses were done.

Analytical procedures

Selenium in plasma and milk was measured by a semi-automated fluorometric procedure (18). Gpx (EC 1.11.1.9) activity in plasma and milk was assayed by a coupled enzyme procedure (19) using 25 mmol/L t-butyl hydroperoxide as substrate. Protein concentrations were determined by the method described by Lowry et al. (20). Lipids were extracted with chloroform/methanol (21) from the breast milk and plasma samples. The FAP of the breast milk and plasma were measured by gas chromatography (GC) as described elsewhere (22). Because total milk fat concentrations change during nursing and from feeding to feeding, milk sampling conditions affect fatty acid concentrations; however, the relative percent contributions of major and minor fatty acids in

human milk are unaffected by changing milk fat concentration (22, 23). Therefore, relative weight percent data are used for comparisons of fatty acid values in the study.

Statistical analysis

The means and standard errors for all variables were calculated. A 2-way ANOVA was done to determine statistically significant effects with the two factors being time at which the samples were taken (early or mature milk) and geographic location (Xichang, a low selenium area; Beijing, an adequate selenium area; and Enshi, a high selenium area). If there were no interactions between time and location, significant differences produced by main effects were determined. Where a main effect was significant, comparisons between means were made using Fisher's protected least significant difference procedure (24). However, if interactions between time and location occurred, cell means were evaluated. A natural log transformation was performed on the selenium and Gpx activity values to obtain homogeneity of variance. Relationships between the appropriate variables were evaluated using Pearson correlation coefficients (25). A 1-way ANOVA was done to determine if the time of collection of the milk samples differed among the early samples and another to determine if it differed among the late samples. Data were analyzed using SAS 6.11 (SAS Institute Inc., Cary, NC. 1985) and were considered statistically different if P <_ 0.05.

RESULTS

Subjects

The dietary intake of the women from all three areas was obtained from published regional food habits (Table 1). The data suggest that the caloric intake was similar among all three areas; that the pattern of macronutrient intake of the women from Xichang differed from that of the women from Beijing or Enshi for they ate more carbohydrate but less fat; that the pattern of macronutrient intake in the women from Beijing and Enshi was similar;

Table 1. Dietary intakes of individuals from three geographic rural areas of China[1] .*(includes Xichang), **(includes Enshi)

	Sichuan Province*	Beijing	Hubei Province**
Energy (MJ per day)	10.2± 2.9	10.4± 3.0	10.0± 2.5
Protein (%KJ)	(9%)	(11%)	(11%)
(g per day)	57.3± 17.6	70.6± 21.4	62.5± 20.7
Fat(%KJ)	(18%)	(28%)	(24%)
(g per day)	47.6± 38.4	77.4± 45.4	62.9± 32.1

Carbohydrate (%KJ)	(72%)	(59%)	(65%)
(g per day)	441.8±125.6	366.6 ±105.5	386.0±116.2
α-Tocopherol			
(mg per day)	4.3 ± 3.1	1 3.5± 9.2	8.8± 5.7
Iron			
(mg per day)	23.1± 8.5	22.7± 8.4	21.5± 10.3
Vitamin C			
(mg per day)	107 ± 80	94 ± 67	113 ± 70
Food (g/d[2])			
Vegetable oil	6.7±12.0[3]	35.2± 32.5[4]	37.4± 24.6[5]
Animal fat[5]	16.7 ±22.8	4.7± 13.3	3.2± 10.3

[1]National Nutrition Survey of China (1992). Data are means ± SD.
[2]The intakes are g/d for a reference male.
[3]Primarily rapeseed oil, 54% oleic acid, 18% linoleic acid.
[4]Primarily peanut oil, 40% oleic acid, 38% linoleic acid.
[5]Primarily lard, 44% oleic acid, 8% linoleic acid, 43% saturated fatty acids.

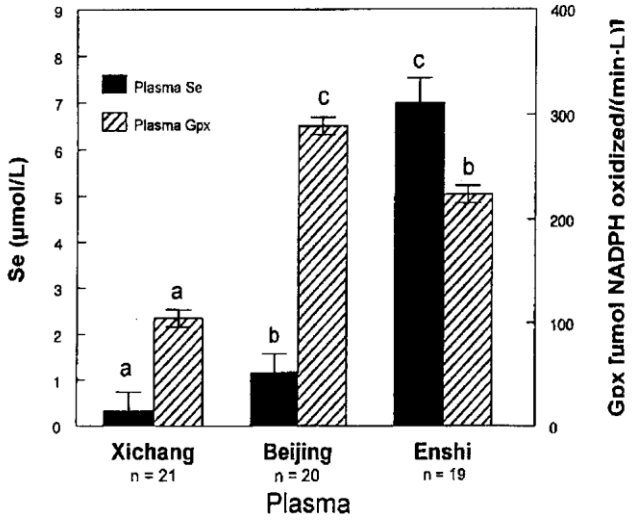

Figure 1. Plasma selenium concentration and glutathione peroxidase activity in women from three locations in China. Values represent mean ± SEM. All values were logarithmically transformed before statistical analysis because their variances were heterogenous, but untransformed data are shown. Values for a variable with the same letter above the bar indicate no significant diffrence, $p \leq 0.05$

and that the intakes of iron and vitamin C were similar among the three areas while that of o - tocopherol was lower in Xichang. There were no significant differences in the intakes of micronutrients such as retinol, thiamin, riboflavin and niacin, or elements such as potassium, sodium, phosphorus, manganese, magnesium, zinc or copper of the subjects living in the three regions of China (data not shown in Table 1.). From the recently published National Nutrition Survey of China of 1992, the approximate intake of fatty acids for people in these three geographic areas can be determined (16). Vegetable oils supply approximately 88% of the visible (also can be indicated as added or cooking) fat in the diet in rural Beijing and 92% in rural Enshi. Peanut oil is the most heavily used oil in Beijing. The typical peanut oil in China contains 40.4 g oleic acid and 37.9 g linoleic acid/100 g oil. Rapeseed oil is used more heavily in Enshi. It

provides 54 g oleic acid and 18.0 g linoleic acid/100 g oil. If for the first approximation of intakes, all the vegetable oil consumed in Beijing is assumed to be peanut oil and all that consumed in Enshi to be rapeseed oil, the intakes of oleic and linoleic acid in the two populations are similar. In Xichang, lard provides 71% of the visible fat and vegetable oil only 29%. Although the oleic acid content of Chinese pork is typically 44.3 g/100 g fat, the total intake of fat in Xichang is only 67% of that of the average total fat intake of Enshi and Beijing. As a consequence, even though the people in Xichang consume fat that contains large amounts of oleic acid, their intake of oleic acid will be much lower than that in the Beijing or Enshi population.

The average age of the women and collection time of the milk and plasma samples are given in Table 2. The women ranged in age from 21-30 years. The time of collection for the early milk samples ranged from 1-7 days postpartum. The average time of collection of the mature samples was similar although the collection times ranged from 2-18 months.

Selenium and protein concentrations and Gpx activities in plasma and breast milk

There were significant differences in the selenium concentration and Gpx activity in both plasma and breast milk. In the plasma, there were no interactions between time and location for selenium levels and Gpx activity. Consequently, the statistical model used to analyze the data included only the main effects of time and location. Plasma selenium did not change as a function of time but differences were highly significant among all three locations (Figure 1, $P <\_ 0.0001$). The plasma Gpx activity also varied as a function of geographic location ($P = 0.0001$) but not time (Figure 1). The activity measured in the samples from Xichang was significantly lower than that found in those from Beijing ($P = 0.0001$) and Enshi ($P = 0.0001$). Surprisingly, the activity of the samples obtained from Beijing was significantly higher than that of the samples obtained from Enshi ($P = 0.003$). The relationship between the activity of Gpx and the plasma selenium concentration from all the samples is given in a scatter plot (Figure 2). This plot shows that the activity of the samples obtained from Beijing is higher when compared to the other two locations.

In contrast to the selenium and Gpx activity in plasma, there was a significant interaction in breast milk between time and location ($P = 0.007$ and $P = 0.02$, respec-

Table 2. Age and collection time of plasma and milk samples from lactating women from three regions of China[1]

Region	Age (years[2])	Sample Collection (days postpartum)	
		Early	Mature
Xichang	23.5 ± 0.6^{a}	4.2 ± 0.3^{b}	152.2 ± 37.9
n	21	11	10
Beijing	28.2 ± 0.6^{b}	5.4 ± 0.3^{c}	121.0 ± 37.9
n	20	10	10
Enshi	26.6 ± 0.7^{b}	1.0 ± 0.3^{a}	253.3 ± 40.0
n	19	10	9

[1]Data represent means ± SEM for each group.
[2]When a column contains superscripts, values with different superscripts are significantly different from each other, $P<\_0.05$

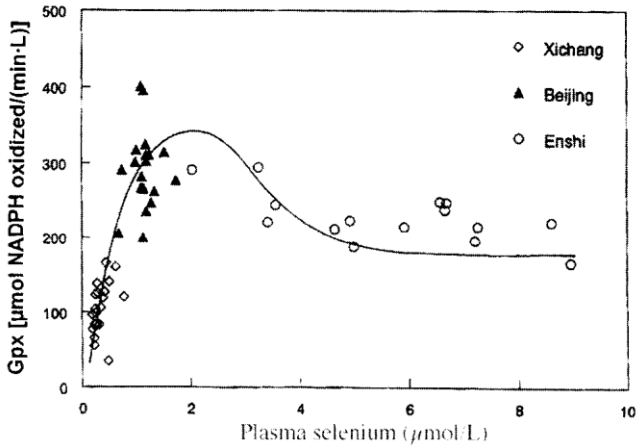

Figure 2. Scatter plot for plasma selenium and plasma glutathion peroxidase activity for women from Xichang, represented by a ---◇---, Beijing, represented by a ---▲---, and Enshi, represented by a ---○--- (n=60, with 19-21 per group)

tively). Consequently. the statistical model employed in evaluating these data used cell means rather than main effect means (Figure 3). Because the selenium concentration of the samples from Enshi was so much larger than the values measured in the other two locations, the interaction may be relatively unimportant to the interpretation of the data. Thus, the main effect of location was evaluated in addition to the comparison of cell means. When cell means were compared, in both the early and mature samples there were significantly higher concentrations of selenium in the samples from Enshi than those from Beijing or Xichang. However, when the main ef-

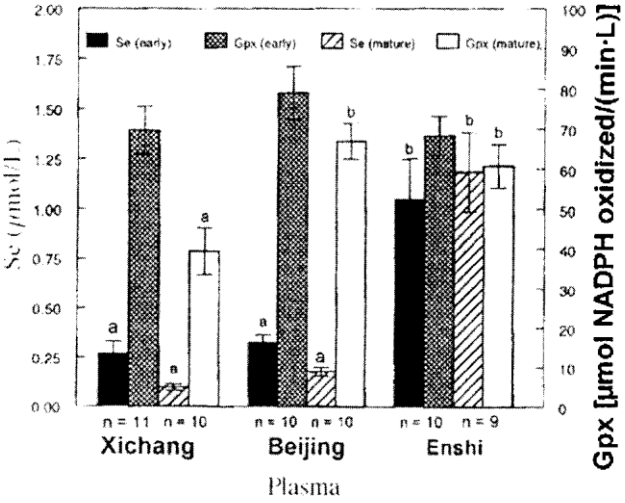

Figure 3. Breast milk selenium concentration and glutathione peroxidase activity in women from three locations in China. Values represent mean ± SEM. All values were logarithmically transformed before statistical analysis because their variances were heterogenous. but untransformed data are shown. Values for a variable with the same letter above the bar indicate no significant diffrence, $p \leq 0.05$

fect of location was evaluated, the selenium concentration in the breast milk samples from all three locations differed significantly.

There was a significant interaction (P = 0.02) between time and location in the activity of Gpx in the breast milk samples (Figure 3). Although the selenium concentrations differed markedly, there was no significant difference in the Gpx activity at the three locations in the early samples. In contrast, in the mature samples, the activity measured in the samples from Xichang was significantly lower than that in the samples from Beijing (P = 0.001) or Enshi (P = 0.01).

The protein concentration of the plasma samples obtained from Xichang (81.8±2.9 g/L) was significantly lower (P=0.0001) than that from either Beijing (113.0±3.0 g/L) or from Enshi (118.5±3.0 JL). There was an interaction between time and location (P= 0.0005) for the protein concentration of breast milk. This occurred because the protein concentration of the early samples from Enshi was significantly lower than that

Table 3. The fatty acid composition (g/100 g fatty acids) of plasma of lactating women from three regions of China' (number of subjects[1]

Fatty Acid	Xichang (21)	Beijing (20)	Enshi (19)
14:0	0.58± 0.06	0.55± 0.06	0.46± 0.06
16:0	22.72± 0.50[b]	21.19± 0.51[a]	20.40± 0.52[a]
18:0	6.75± 0.22[a]	6.62± 0.23[a]	7.83± 0.23[b]
20:0	0.20± 0.03[a]	0.36± 0.03[b]	0.31± 0.03[b]
ΣSFA[2]	31.07± 0.52	30.23± 0.54	29.90± 0.55
16:1(n-7)	2.74± 0.19[b]	1.60± 0.20[a]	1.97± 0.20[a]
18:1(n-9)c	23.13± 0.56[b]	15.79± 0.58[a]	21.99± 0.59[b]
18:1(n-9)t	0.03± 0.02[a]	0.10± 0.02[b]	0.02± 0.02[a]
ΣMUFA[3]	30.37± 0.71[b]	20.82± 0.73[a]	30.38± 0.75[b]
18:2(n-6)	24.08± 0.86[a]	32.94± 0.88[b]	26.05± 0.90[a]
20:4(n-6)	6.34± 0.24[b]	7.42± 0.29[c]	5.18± 0.30[a]
20:3(n-9)	0.11± 0.02[b]	0.00± 0.02[a]	0.07± 0.02[b]
18:3(n-3)	0.79± 0.06	0.80± 0.06	0.90± 0.07
20:5(n-3)	0.51± 0.06[a]	0.39± 0.06[a]	0.70 ± 0.06[b]
22:5(n-3)	0.46± 0.03[b]	0.29± 0.03[a]	0.60± 0.03[c]
22:6(n-3)	1.73± 0.08[b]	1.89± 0.08[b]	1.38± 0.09[a]
ΣPUFA[4]	35.78± 0.98[a]	45.52± 1.01[b]	36.90± 1.04[a]
(n-6)/(n-3)	9.21± 0.42[a]	12.56± 0.43[b]	9.56± 0.44[a]
PI[5]	60.61± 1.63[a]	72.23± 1.67[b]	59.23± 1.72[a]

[1]Values are means ± SEM. When a row contains superscripts. s values with different superscripts are significantly different from each other. P

ΣSFA=Sum of the saturated fatty acids = 13:0 + 14:0 + 15:0 + 16:0
+ 18:0 + 19:0 + 20:0 + 21:0 + 22:0 + 23:(l+24:0.

ΣMUFA= Sum of the monounsaturated fatty acids =16:1(n-7) + 18:1(n-9)cis +18:1(n-7) +18:101-9)trans + 20:101-9) + 22:101-9) + 24:1.

ΣPUFA = Sum of the polyunsaturated fatty acids = I8:2(n-6)trans + 18:20-6)cis + 18:3(1-3) + 18:4(n-3) + 20:2(n-6) + 20:3(n-6) +
20:3(n-3) + 20:4(n-6) + 20:5(n-3) + [2]2:5(n-3) + 22:6(n-3). 'P1=Peroxidizability index=)% dienoic fatty acids x I) + ((%r trienoic fatty acids x 2) + (°k tetraenoic fatty acids x 3) + ((4 pentaenoic fatty acids x 4) + (c4 hexaenoic fatty acids x 5).

Table 4. The fatty acid composition (g/100 g fatty acids) of early and mature breast milk from lactating women (number of subjects) from three regions of China[1]

Fatty Acid	Xichang		Beijing		Enshi	
	early (11)	mature (10)	early (10)	mature (10)	early (10)	mature (9)
10:0	0.28± 0.07[a]	0.98± 0.12	0.53± 0.14[a]	0.94± 0.13	1.16± 0.06[b]	1.01± 0.22
12:0	2.65± 0.70[a]	4.27± 0.63	3.91± 0.69[a]	4.46± 0.67	6.4± 0.76[b]	5.84± 1.01
14:0	4.10± 0.88[a]	4.18± 0.62[d]	5.52± 0.68[a,b]	4.38± 0.78[d,e]	7.75± 1.50[b]	6.76± 1.14[e]
16:0	20.23± 0.48[b]	20.97± 0.79	21.66± 0.75[b]	18.47± 0.91	18.62± 1.11[a]	19.68± 1.90
18:0	20.35± 3.73[b]	13.5 ± 1.36[c]	6.30± 0.20[a]	4.87± 0.20[d]	5.79± 0.50[a]	11.19± 2.32[e]
20:0	0.28± 0.02[a,b]	0.23± 0.02[d]	0.33± 0.01[b]	0.55± 0.09[c]	0.25± 0.03[a]	0.20± 0.04[d]
ΣSFA[2]	48.54± 2.97[b]	44.61± 1.91[e]	38.59± 1.35[a]	33.85± 1.17[d]	40.16± 2.83[b]	44.88± 2.83[d]
16:1(n-7)	1.87± 0.21[a]	2.82± 0.27[d]	1.65± 0.09[a]	1.81± 0.15[c]	2.58± 0.25[b]	2.53± 0.42[d,c]
18:1(n-9)c	28.60± 1.94[a]	32.75± 1.05	29.86± 0.61[a,b]	28.41± 1.17	33.68± 2.05[b]	31.09± 2.57
ΣMUFA[3]	35.83± 1.93[a]	40.08± 1.89[e]	35.89± 2.03[a]	34.36± 1.89[d]	44.86± 2.03[b]	39.68± 1.99[d,e]
18:2(n-6)	8.7 ± 0.80[a]	10.49± 0.81[d]	19.05± 0.70[b]	26.10± 1.38[c]	10.03± 0.58[a]	11.10± 0.99[d]
20:4(n-6)	0.86± 0.05[b]	0.52± 0.04[c]	1.18± 0.16[c]	0.63± 0.03[e]	0.45± 0.06[a]	0.35± 0.07[e]
18:3(n-3)	0.71± 0.12[a]	1.34± 0.13[d]	1.22± 0.06[a]	1.90± 0.08[e]	2.10± 0.38[b]	1.45± 0.18[d]
20:5(n-3)	0.13± 0.02[a]	0.12± 0.04	0.27± 0.02[b]	0.11± 0.02	0.21± 0.05[a,b]	0.10± 0.02
22:5(n-3)	0.25± 0.02	0.19± 0.03	0.22± 0.03	0.13± 0.02	0.20± 0.01	0.16±0.03
22:6(n-3)	0.50± 0.05[b]	0.22± 0.04[d,e]	0.55± 0.02[b]	0.28± 0.02[c]	0.20± 0.03[a]	0.15± 0.03[d]
ΣPUFA[4]	12.53± 0.84[a]	13.56± 1.20[d]	24.35± 0.88[b]	30.04± 1.20[e]	13.85± 0.88[a]	14.00± 1.27[d]
(n-6)/(n-3)	6.93± 0.44[b]	6.46± 0.56[d]	9.81± 0.46[c]	11.65± 0.56[c]	4.64± 0.46[a]	6.71± 0.59[d]
PI[5]	18.70± 1.11[a]	18.00± 1.44[d]	32.25± 1.16[b]	35.39± 1.44[e]	19.11± 1.16[a]	17.80± 1.52[d]

[1]Values are means ± SEM. When a row contains superscripts, values with different superscripts are significantly different from each other, P ≤0.05.
[2] ΣSFA=Sum of the saturated fatty acids = 13:0 + 14:0 + 15:0 + 16:0 + 18:0 + 19:0 + 20:0 + 21:0 + 22:0 + 23:0 + 24:0.
[3] ΣMUFA= Sum of the monounsaturated fatty acids = 16:1(n-7) + 18:1(n-9)cis + 18:1(n-7) + 18:1(n-9)trans + 20:1(n-9) + 22:1(n-9) + 24:1.
[4] ΣPUFA = Sum of the polyunsaturated fatty acids = 18:2(n-6)trans + 18:2(n-6)cis + 18:3(n-3) + 18:4(n-3) + 20:2(n-6) + 20:3(n-6) + 20:3(n-3) + 20:4(n-6) + 20:5(n-3) + 22:5(n-3) + 22:6(n-3).
[5]PI=Peroxidizability index=(% dienoic fatty acids x 1) + (% trienoic fatty acids x 2) + (% tetraenoic fatty acids x 3) + (% pentaenoic fatty acids x 4) + (% hexaenoic fatty acids x 5).

from Beijing (P = 0.0001) or Xichang (P = 0.0005) but the protein concentration in the mature samples from all three locations was statistically equivalent. When the plasma and milk Gpx activities were expressed relative to the protein concentration, the same trends were obtained as when expressed relative to volume (data not shown).

Fatty acid composition of plasma

Composites of three categories of fatty acids were compared to evaluate changes in the FAP due to sampling time and location. These are the sum of the saturated fatty acids (ΣSFA), the sum of the monounsaturated fatty acids (ΣMUFA), and the sum of the polyunsaturated fatty acids (ΣPUFA). In addition, individual fatty acids were ids (ΣMUFA), and the sum of the polyunsaturated fatty acids (ΣPUFA). In addition, individual fatty acids were also evaluated (Table 3).

There were significant differences in the concentration of /PUFA and ΣMUFA found in the plasma as a tion of ΣPUFA and ΣMUFA found in the plasma as a function of location (P = 0.0001). The women from Beijing had significantly higher concentrations of ΣPUFA and significantly lower concentrations of ΣMUFA than those from Xichang (P = 0.0001) or Enshi (P = 0.0001). There were no significant differences among the three lo cations in the SFA found in the plasma. The major contributor to the differences seen in the concentrations of ΣPUFA was linoleic acid [18:2(n-6)] (Table 3). The plasma from Beijing subjects had significantly higher concentrations of linoleic acid than those from Xichang (P=0.0001) or Enshi (P=0.0001). The concentration of arachidonic acid, 20:4 (n-6), was significantly lower in the samples from Enshi than the samples from Xichang (P = 0.006) or Beijing (P = 0.0001). Samples from Enshi had

significantly higher concentrations of eicosapentaenoic acid ΣEPA, 20:5(n-3)] than those from Beijing (P = 0.0006) and Xichang (P = 0.02), but significantly lower concentrations of docosahexaenoic acid [DHA, 22:6(n5)] than Beijing (P = 0.0001) and Xichang (P = 0.005). Oleic acid, 18:1(n-9), was the primary contributor to the ΣMUFA and its concentration was lower in the samples from the women from Beijing than those from women in Xichang (P=0.0001) and Enshi (P=0.0001).

Although there were no significant differences in the concentration of SFA among the three locations, the two major fatty acids from this group, palmitic acid, 16:0, and stearic acid, 18:0, differed (P = 0.007 and 0.0007, respectively). The concentration of palmitic acid was signifi-

Table 5. Pearson correlation coefficients (r) between select fatty acids from plasma and plasma glutathione peroxidase (Gpx) activity and select fatty acids from breast milk and both plasma and breast milk Gpx activity[1]

	Fatty acids in		
Fatty Acid	Plasma with plasma Gpx (r)	Breast milk with plasma Gpx (r)	milk Gpx (r)
ΣSFA[2]	-0.04	-0.40[b]	-0.02
18:1(n-9)	-0.54[c]	-0.02	-0.28[a]
ΣMUFA[3]	-0.54[c]	-0.11	-0.26[a]
18:2(n-6)	0.54[c]	0.61[c]	0.20
18:3(n-3)	0.00	0.19	0.02
20:4(n-6)	0.11	0.18	0.33[b]
20:3(n-9)	-0.44[c]	-0.12	0.02
20:5(n-3)	-0.11	0.27[a]	0.21
22:5(n-3)	-0.24	-0.15	0.18
22:6(n-3)	0.06	0.08	0.35[b]
ΣPUFA[4]	0.48[c]	0.62[c]	0.25[a]

[1]Superscripts indicate a significant correlation: a=P<_0.05: b=P<_0.01 : c=P<_0.001: n=60
[2]ΣSFA=Sum of the saturated fatty acids = 13:0 + 14:0 + 15:0 + 16:0'ΣSFA=Sum of the saturated fatty acids = 13:0 + 14:0 + 15:0 + 16:0
+18:0+19:0+20:0+21:0+22:0+23:0+24:0
ΣMUFA= Sum of the monounsaturated fatty acids = 16:1(n-7) + 18:1(n-9)cis + 18:1(n-7) + 18:1(n-9)trans + 20:1(n-9) + 22:1(n-9) + 24:1
ΣPUFA = Sum of the polyunsaturated fatty acids = 18:2(n-6)trans + 18:2(n-6)cis + 18:3(n-3) + 18:4(n-3) + 20:2(n-6) + 20:3(n-6) + 20:3(n-3) + 20:4(n-6) + 20:5(n-3) + 22:5(n-3) + 22:6(n-3)

cantly higher in the samples from Xichang compared to Beijing (P=0.04) and Enshi (P=0.001). The concentration of stearic acid was significantly higher in the samples from Enshi compared to those from Beijing (P = 0.0005) and Xichang (P=0.002).

Fatty acid profile of breast milk

Interactions were observed in the breast milk samples in the ΣPUFA analysis and many fatty acids that compose this fraction. Therefore, individual cell means were evalin the ΣPUFA analysis and many fatty acids that compose this fraction. Therefore, individual cell means were evaluated (Table 4). In both the early and mature milk samples, Beijing women had a significantly higher concentration of milk ΣPUFA than the women from Xichang (P = 0.0001) or from Enshi (P = 0.0001). Similar to plasma, breast milk linoleic acid was the major contributor to ΣPUFA. With respect to time, the concentration of linoleic acid increased in the samples from

all three locations but the Beijing women had significantly higher concentrations of linoleic acid in their breast milk than did the women from Xichang (P = 0.0001) or Enshi (P = 0.0001).

At all three locations, the arachidonic acid concentration decreased as a function of time. In the early milk samples, the concentration of the samples from Beijing was significantly higher than that measured in the samples from Xichang (P=0.03) or Enshi (P=0.0001) and the concentration in the samples from Xichang was significantly higher than that from Enshi (P=0.007). In the mature milk samples, however, the arachidonic acid concentration in samples from Enshi was significantly lower than samples from Xichang (P = 0.02) or from Beijing (P= 0.0003).

Linolenic acid, 18:3(n-3), concentration in the early milk samples from Enshi was significantly higher than that in the samples from Xichang (P=0.0001) or from Beijing (P=0.01). Linolenic acid in the mature milk samples from Beijing was significantly higher than in samples from Xichang (P=0.005) or Enshi (P=0.02). Both EPA and DHA concentrations decreased at all locations as a function of time, but the effect was more marked in Beijing samples. In early milk, the EPA concentration in samples from Beijing was significantly higher than in samples from Xichang (P=0.006). In the mature milk samples, however, the EPA concentration was similar at all three locations. The DHA concentration in early milk samples from Enshi was significantly lower than its concentration in samples from Xichang (P=0.0001) or Beijing (P= 0.0001). In mature milk samples, the DHA concentration in samples from Beijing was significantly higher than in samples from Enshi (P=0.008).

There was no interaction between time and location in the analysis of the ΣSFA and the ΣMUFA (Table 4) and the analysis of the ΣSFA and the ΣMUFA (Table 4) and most of the fatty acids that make up these composites. Milk concentration of SFA was significantly lower in the samples from Beijing than samples from Xichang (P=0.0001) or Enshi (P = 0.009). The largest contributors to the ΣSFA of breast milk were palmitic and stearic acids. There was an interaction between time and location in the concentration of stearic acid in breast milk (P=0.01). In the early milk samples the concentration of stearic acid was significantly higher in the samples from Xichang than in those from Beijing (P = 0.0002) or Enshi (P = 0.0001). However, in the mature milk samples the concentration of stearic acid in the samples from Beijing was significantly lower than that in the samples from Xichang (P = 0.0003) or Enshi (P = 0.0004). The milk from women in Enshi had significantly higher concentrations of ΣMUFA than from the women in Xichang (P = 0.03) or Beijing (P = 0.0007).

Pearson correlation coefficients between the selenium concentrations and Gpx activities in plasma and breast milk were calculated. The plasma and breast milk selenium concentrations were significantly correlated (r^2=0.84, P0.001) as was the Gpx activity from both matrices (r2=0.40, PS 0.001).

The correlation between plasma Gpx activity and concentration of selected fatty acids in the plasma was evaluated (Table 5). The concentrations of oleic acid, 18:1(n-9), and the ΣMUFA were negatively correlated evaluated (Table 5). The concentrations of oleic acid, 18:1(n-9), and the ΣMUFA were negatively correlated with the activity of Gpx whereas the concentrations of linoleic acid and ΣPUFA were positively correlated with this activity. Calculation of correlation coefficients for the concentration of breast milk fatty acids with both plasma and breast milk Gpx

activities (Table 5) revealed that the concentration of ΣSFA in breast milk was negatively correlated with plasma Gpx activity, but there was no correlation with breast milk Gpx activity. The concentrations of oleic acid and ΣMUFA were negatively correlated with breast milk Gpx activity but there was no correlation with plasma Gpx activity. The concentration of ΣPUFA was positively correlated with both plasma and breast milk Gpx activities. The concentrations of linoleic acid and EPA were positively correlated with plasma Gpx activity. Breast milk Gpx activity was positively correlated with arachidonic acid and DHA. Interestingly, when the early and mature samples were grouped separately, breast milk Gpx activity demonstrated a positive correlation with linoleic acid in the mature milk samples ($r^2=0.46$, $P = 0.01$) but not in the early samples (data not shown).

DISCUSSION

A unique aspect to this study was the naturally occurring, variable intakes of selenium over a very wide range. This allowed the effect of this nutrient to be evaluated after long-term intakes rather than short periods of supplementation. The maternal selenium intake is usually reflected in the concentration found in breast milk (1) and it is not surprising that the breast milk selenium concentrations were positively correlated with plasma selenium concentrations. The concentration of selenium in breast milk was higher relative to plasma concentrations than previously reported (14). Although others have shown breast milk selenium concentrations to be approximately 90% lower than plasma concentrations (14), breast milk samples in Xichang, Beijing and Enshi were approximately 45%, 78% and 84% lower, respectively, than plasma concentrations. The mature breast milk samples from women in Xichang had a selenium concentration (0.10 μmol/L) somewhat lower than the concentration of sele nium reported in breast milk from the United States (0.19 μmol/L, 10) and New Zealand (0.17 μmol/L, 25), but comparable to concentrations in milk from Finland (0.09 μmol/L, 26), where selenium intakes were also low.

The Gpx activities were consistent with other studies that demonstrate a positive correlation between plasma selenium levels and Gpx activity at low selenium intakes (28). Sunde et al. (29) showed that the regulation of Gpx and its mRNA levels are dependent on selenium concentrations in plasma where low levels of selenium lead to a decrease in the Gpx expression. Gpx activity reached a plateau in subjects with adequate plasma selenium levels, indicating saturation of the Gpx enzyme.

Rather surprisingly, the women's selenium status was not reflected in the activity of Gpx in breast milk. The enzymatic activity was adequate and statistically equivalent at all locations in the early milk samples, though breast milk selenium concentrations varied markedly. On the other hand, the activity of Gpx in the mature breast milk samples showed the same pattern as the plasma. These results suggest that Gpx plays a critical role in breast milk, particularly right after birth, and mechanisms exist to maintain its adequate activity even when selenium intakes are extremely low.

Calculated correlation coefficients (Table 5) suggest that the activity of Gpx in both plasma and breast milk plays a role in determining their respective FAP. The protective enzymatic action of Gpx could prevent extensive oxidation of unsaturated fatty acids. Linoleic acid is one of the most abundant polyunsaturated fatty acids in both plasma and breast milk and the activity of Gpx might be effective in maintaining high concentrations of this essential fatty acid. This hypothesis

is supported by the positive correlation between plasma Gpx activity and the plasma concentration of linoleic acid and between breast milk Gpx activity and linoleic acid in the mature milk samples.

We recognize that the intakes of fatty acids are obtained from dietary data on a representative subset of the population of an area, rather than directly from subjects on the study. Generalized dietary data was used to estimate the dietary intakes of the subjects because of the difficulty of obtaining these data from women in Xichang and Enshi, which are isolated areas at a great distance from Beijing. In addition, more accurate information could likely not have been obtained because of the limited number of fatty acid profiles present in the current Chinese food composition data base. It contains 18 oils/ fats compared to the USDA-based data base available in the United States which contains over 100 fats/oils (30).

However, there are several pieces of evidence that allow us to conclude that the concentration of linoleic acid in the breast milk of the women from Beijing is due to factors other than its intake. First, as shown in Table 1, the dietary patterns of the women living in Beijing and Enshi are similar when compared to those of the women living in Xichang, yet the FAP in the milk of women from Xichang and Enshi are more alike than that from the women living in Beijing. If dietary fatty acids were the major influence, then the FAP of the milk should reflect the dietary patterns. Second, a study conducted in women from New Zealand showed that selenium supplementation increased the concentration of PUFA in breast milk, especially linoleic acid (31). These women consumed similar diets, but one group received a selenium supplement while the other group did not. Third, the linoleic acid content of the breast milk in the samples from Beijing was high when compared to the amounts in samples from 24 European and African countries (23) yet consistent with the amounts measured from samples obtained from five different regions in China (32). The samples used in the Chulei study were not obtained from women with aberrant selenium intakes. From this, it can be concluded that the extremes in selenium intakes were associated with the low concentrations of linoleic acid. Finally, plasma concentrations of linoleic acid would also reflect dietary intakes. The plasma concentration of this fatty acid in samples from each of the three areas did not differ as markedly as in the breast milk and was similar to values previously reported in humans (33). This suggests that the differences in linoleic acid are not induced by the diet.

The role of Gpx in breast milk may be to protect the lipids from further peroxidation by lipid peroxides or to guard against the mechanisms they may trigger, i.e., activation of transcription factors such as nuclear factor kappa B and activator protein 1 (34). The fact that an increased concentration of linoleic acid was observed in plasma and breast milk of women with appropriate selenium nutriture suggests that lipid peroxidation may be less than that which occurs in women with compromised selenium status. Thus, the breast milk for their infants may be better protected from lipid oxidation. In conclusion, these data suggest that breast milk linoleic acid concentration is lower in populations where selenium intakes are either too low or excessive. Future studies under controlled dietary conditions are required to corroborate these findings.

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REFERENCES

1. JENSEN, R. & NEVILLE, M. (1985) Human Lactation: Milk Components and Methodology, Plenum Press, New York, NY. pp. 221-230.
2. BUTLER, J., WANDER, R., DU, S., & WHANGER, P. (1995) Effects of selenium supplementation on milk selenium and fatty acid levels in lactating women of low selenium status. FASEB J. 9: A159 (Abs)
3. DOTSON, K., PICCIANO, M., JERELL, J., & PERKINS, E. (1991) Maternal selenium nutrition and milk lipid profiles. FASEB J. 5: A917 (Abs)
4. CABRE, E., PERIAGO, J. L., MINGORANCE, M. D., FERNANDEZ-BANARES, F., ABAD, A., ESTEVE, M., GIL, A., LACHICA, M., GONZALES-HUIX, F., & GASSULL, M. A. (1992) Factors related to the plasma fatty acid profile in healthy subjects, with special reference to antioxidant micronutrient status: a multivariate analysis. Am. J. Clin. Nutr. 55: 831-837
5. MIETTINEN, T. A., ALFTHAN, G., & HUTTUNEN, J. K. (1983) Serum selenium concentration related to myocardial infarction and fatty acid content of serum lipids. Br. Med. J. 287: 517-519
6. KOK, F. J., DE BRUIJN, A. M., & VERMEEREN, R. (1987) Serum selenium, vitamin antioxidants, and cardiovascular mortality: a 9-year follow-up study in the Netherlands. Am. J. Clin. Nutr. 45: 462-468
7. ALAEJOS, M. & ROMERO, C. (1995) Selenium in human lactation. Nutr. Rev. 53: 159-166
8. LEE, A., HUEL, G., GODIN, J., HELLIER, G., SAHUQUILLO, J., MOREAU, T., & BLOT, P. (1995) Interindividual variation of selenium in maternal plasma, cord plasma and placenta. Sci. Total Environ. 159: 119-127
9. LEVANDER, O. (1987) A global view of human selenium nutrition. Ann. Rev. Nutr. 7: 227-250
10. LEVANDER, 0., MOSER, P., & MORRIS, V. (1987) Dietary selenium intake and selenium concentrations of plasma, erythrocytes. and breast milk in pregnant and postpartum lactating and nonlactating women. Am. J. Clin. Nutr. 46: 694-698
11. PERRONE, L., DIPALMA, L., DITORO, R., GIALANELLA, G., & MORO, R. (1994) Interaction of trace elements in a longitudinal study of human milk from full-term and pre-term mothers. Biol. Trace Elem. Res. 41(3): 321-330
12. ZACHARA, B.. WASOWICZ, W., & SKLODOWSKA, M. (1986) Glutathione peroxidase activity, selenium, and lipid peroxide concentration in blood from a healthy Polish population. Biol. Trace Elem. Res. 10: 175-187
13. BUTLER, J. & WHANGER, P. (1992) Metabolism of selenium by pregnant women. J. Trace Elem. 5: 175-188
14. MANNAN, S. & PICCIANO, M. (1987) Influence of maternal selenium status on human milk selenium concentration and glutathione peroxidase activity. Am. J. Clin. Nutr. 46: 95-100

15. XIA.Y., ZHAO. X., ZHU, L., & WHANGER, P. (1992) Distribution of selenium in erythrocytes, plasma, and urine of Chinese men of different selenium status. J. Nutr. Bioch. 3: 211215
16. KEYOU, G. (1996) The dietary and nutritional status of Chinese population (1992 National Nutrition Survey), vol. 1. People's Medical Publishing House, Beijing, China
17. JENSEN. R. (1989) The Lipids of Human Milk. pp. 93-152. CRC Press. Boca Raton, FL
18. BROWN, M. & WATKINSON, J. (1977) An automated fluorometric method for the determination of nanogram quantities of selenium. Ana. Chimica. Acta. 89: 29-35
19. PAGLIA. D. E. & VALENTINE. W. N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase . J. Lab. Clin. Med. 70: 158-169
20. LOWRY, O., ROSENBROUGH, N.. FARR, A., & RANDALL, R. (1951) Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265-275
21. FOLCH, J.. LEES, M., & SLOANE-STANLEY, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509
22. SONG, J. & WANDER, R. C. (1991) Effects of dietary selenium and fish oil (MaxEPA) on arachidonic acid metabolism and hemostatic function in rats. J. Nutr. 121: 284-292
23. KOLETZKO, B., THEIL, I., & ABIODUN, P. (1992) The fatty acid composition of human milk in Europe and Africa. J. Pediatr. 120: S62-70
24. PETERSON, R. G. (1985) Design and Analysis of Experiments. pp. 76-77. Marcel Dekker, New York, NY.
25. SNEDECOR, G. W. & COCHRAN, W. G. (1989) Statistical Methods, Eighth Edition. pp.192-193. Iowa State Univ. Press, Ames, IA.
26. DOLAMORE, B. A., BROWN, H., DARLOW, B. A., GEORGE, P. M., SLUIS, K. B.. & WINTERBOURN, C. C. (1992) Selenium status of Christchurch infants and the effect of diet. New Zealand Med. J. 105: 139-142
27. KUMPULAINEN, J., SALMENPERA, L., STIMES, M., KOIVISTOINEN, P., & PERHEENTUPA, J. (1985) Selenium status of exclusively breast-fed infants as influenced by maternal organic or inorganic selenium supplementation. Am. J. Clin. Nutr. 42: 829-835
28. WHANGER, P., BEILSTEIN, M.. THOMSON, C., ROBIN- SON, M., & HOWE, M. (198 8) Blood selenium and glutathione peroxidase activity of populations in New Zealand, Oregon, and South Dakota. FASEB J. 2: 2996-3002
29. SUNDE, R. A., SAEDI, M. S., KNIGHT, S. A. B., SMITH, C. G., & EVENSON, J. K. (1988) Regulation of expression of glutathione peroxidase by selenium. In: Selenium in Biology and Medicine, Fourth International Symposium on Selenium (Wendel, A. ed.). pp. 8 - 13. Springer-Verlag, Berlin, Germany
30. REEVES III, J. B. & WEIHRAUCH. J. L. (1979) Composition of Foods, Fats and Oils, Raw, Processed, Prepared. Ag. Handbook No. 8-4. Science & Ed. Admin. USDA. Supervisor of Documents, U. S. Govt. Printing Office, Washington. D.C.
31. DODGE, M. L., WANDER R. C.. BUTLER, J. A., DU, S.-H., THOMSON, C. D., & WHANGER, P. W. (1999) Selenium supplementation increases the polyunsaturated fatty acid content of human breast milk. J. Trace Elements Exp. Med. 12.3744
32. CHULEI, R., XIAOFANG, L., HONGSHENG, M., XIULAN, M., GUIZHENG, L.. GIANHONG, D., DEFRANCESCO, C., & CONNER, W. (1995) Milk composition in

women from five different regions of China: The great diversity of milk fatty acids. J. Nutr. 125: 2993-2998
33. WANDER, R. C., DU, S-H., KETCHUM. S. 0., & ROWE, K. E. (1996) ot-Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. J. Nutr. 126: 643-652
34. WINYARD, P. G., MORRIS, C. J., WINROW, V. R., ZAIDI, M., & BLAKE. D. R. (1994) Free radical pathways in the inflammatory response. In: Free Radical Damage and its Control. (Rice-Evans, C. A. and Burdon, R. H., eds.). pp. 361-379. Elsevier Science BV, Elsevier, Amsterdam, Netherlands
35. BUTLER, J. A., WANDER, R. C., DODGE, M. L., DU, S-H.. THOMSON, C. D., XIA, Y., & WHANGER, P. D. (1996) Effects of selenium supplementation on milk fatty acids in women of low selenium status. proceedings: Ninth International Symposium Trace Elements in Man and Animals 9:33 (abs)
36. DODGE, M. L.. WANDER, R. C., WHANGER, P. D.. BUT- LER, J., & XIA, Y. (1997) The effect of selenium on the fatty acid profiles of human breast milk in Chinese women. The FASEB J. I I: A359 (abs)