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Determination of Selenium Concentration and Glutathione Peroxidase Activity in Plasma and Erythrocytes

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We determined selenium concentrations and activities of the selenoenzyme, glutathione peroxidase (EC 1.11.1.9), in the plasma and erythrocytes of 38 apparently healthy women. We determined selenium concentrations directly by polarized Zeeman-effect flameless atomic absorption spectroscopy. Within-run precision studies for the assays gave CVs of 5.6% for a mean erythrocyte selenium concentration of 149.9 (SD 8.3) μg/L (n = 10) and 6.4% for a mean plasma selenium concentration of 97.3 (SD 6.2) μg/L (n = 12). For the women, mean selenium concentrations were 141.4 (SD 14.3) μg/L of erythrocytes [0.49 (SD 0.07) μg/g of hemoglobin] and 96.3 (SD 14.2) μg/L of plasma. Glutathione peroxidase activities were measured by a modification of the method of Paglia and Valentine (*J. Lab. Clin. Med.* **70:** 158–169, 1967). Within-run precision studies for the glutathione peroxidase assays gave CVs of 12.8% for mean erythrocyte glutathione peroxidase activity of 77.2 (SD 9.9) U/g of hemoglobin (n = 13), and 8.1% for mean plasma activity of 312.5 (SD 25.2) U/L (n = 11). Mean enzyme activity was 78.7 (SD 12.9) U/g of hemoglobin for erythrocytes and 424 (SD 40) U/L for plasma. Erythrocyte selenium concentrations and glutathione peroxidase activities were positively, but poorly, correlated ($r = 0.41$, $p < 0.01$).

Additional Keyphrases: *atomic absorption spectroscopy · enzyme activity · pediatric chemistry · selenium nutritional status · peak area vs peak height measurements*

In humans, selenium deficiency may contribute to a form of hemolytic anemia in premature infants (*1*, *2*) and a cardiomyopathy syndrome (*3*, *4*). The only well-characterized mammalian selenoprotein is glutathione peroxidase (EC 1.11.1.9; glutathione:hydrogen-peroxide oxidoreductase), an enzyme known to metabolize both hydrogen peroxide and lipid hydroperoxides and found in most human tissues (*5–7*). Decreased activity of this enzyme in erythrocytes has been linked to hemolytic anemia in premature infants (*2*). Decreased erythrocyte glutathione peroxidase activity has also been reported in a child with a selenium-responsive cardiomyopathy (*3*). Several investigators have suggested that plasma and (or) erythrocyte glutathione peroxidase activity may serve as a useful index of selenium nutritional status (*8*, *9*), but conflicting data have been reported. Behne and Wolters (*10*) found no relationship between glutathione peroxidase activity and selenium concentration in either blood fraction. Rudolph and Wong (*11*), however, found a high correlation between erythrocyte and plasma activities and their respective selenium concentrations. Thomson and Robinson reported a correlation with erythrocyte glutathione peroxidase activity when selenium concentrations were less than 150 μg/L (*12*). Lane et al. (*13*) reported correlation with erythrocyte enzyme activity when selenium concentrations were less than 1.2 μg/g of hemoglobin (Hb), approximately 400 μg/L of erythrocytes.

Vitamin supplementation (*14*, *15*), iron deficiency (*16*), heavy metal toxicity (*17*), hormonal status (*18*), and ethnic background (*19*) have all been reported to affect glutathione peroxidase activity. We have determined erythrocyte and plasma selenium concentrations for 38 apparently healthy women and investigated possible correlation with glutathione peroxidase activities. We investigated the effects of age, smoking history, vitamin supplementation, caffeinated beverages, and oral contraceptives on the mean glutathione peroxidase activity and selenium concentration in these women. In addition, plasma iron and erythrocyte lead concentrations were also determined, to see whether these metals might affect enzyme activity or selenium concentrations and thus obscure a relationship between them. Selenium concentrations were determined by polarized Zeeman-effect flameless atomic absorption spectroscopy (PZAA) (*20*), a sensitive, highly specific technique in which selenium is quantitated directly in an undigested dilution of specimen.

Materials and Methods

Equipment

For the metals analyses we used a polarized Zeeman-effect flameless atomic absorption spectrometer (Model 180-70), equipped with a fast-response, dual-pen recorder (Model 056); tube-type cuvets, pyrolytic or standard graphite, were used (all from N.S.A. Hitachi, Ltd., Mountainview, CA 94043). Corrected atomic absorbances and background absorbance, sampled every 10 ms, were stored in the spectrometer's microprocessor; after atomization, the signals were fed to the recorder. This allowed simultaneous monitoring of the analyte peak shape (and area) and background absorption. The microprocessor also printed out the peak area and maximum background absorbance for each atomization, so we could check that the background absorbance remained below the correctable value, 1.7 *A* (manufacturer's instruction manual).

For fluorometric selenium analyses we used a scanning fluorescence spectrophotometer (Model 204), equipped with a xenon power supply (Model 150; both from Perkin-Elmer Corp., Norwalk, CT 06856).

To determine enzyme activities and hemoglobin concentrations, we used a Cary 219 UV-VIS Scanning Spectrophotometer (Varian Associates, Sunnyvale, CA 94086), keeping the temperature of the cell compartment at 37 °C (for the enzyme analyses) with a circulating water bath (EX 100; Neslab, Portsmouth, NH 03801), continuously monitored with a thermistor accessory (Varian Associates).

All polypropylene labware (Falcon, Cockeysville, MD 21030) was acid-washed with 6 mol/L nitric acid, reagent grade (Fisher Scientific Co., Pittsburgh, PA 15219) and soaked in de-ionized water before use (*21*). No detectable selenium, iron, or lead could be leached from the labware after this procedure.

Venous whole blood was collected in Trace Element Vacutainer Tubes containing 143 USP units of heparin per tube or 1.5 mg of tripotassium ethylenediaminetetraacetate

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