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Received 7 June 1990.
Accepted 27 July 1990.
Associate Editor: Pollock.

AN 2460

WHOLE BLOOD SELENIUM LEVELS AND GLUTATHIONE PEROXIDASE ACTIVITY IN ERYTHROCYTES OF BLACK-TAILED DEER

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Abstract: I determined the relationship between whole blood selenium (Se) and erythrocyte glutathione peroxidase (GSH-Px) activity in black-tailed deer (*Odocoileus hemionus columbianus*). Whole blood Se levels were linearly correlated ($r^2 = 0.91$) to GSH-Px activities over a wide range of blood Se concentrations. The response of whole blood Se levels and GSH-Px activities to Se supplementation indicated that the Se status of indigenous deer may be established conveniently by whole blood analysis. Such monitoring may be necessary depending on known Se levels in soils and plants and the level of anthropogenic influences on Se bioavailability or requirements.

J. WILDL. MANAGE. 55(1):26–31

Selenium (Se) has an important protective function in mammals as part of the enzyme glutathione peroxidase (GSH-Px; glutathione : H_2O_2 oxidoreductase, EC 1.11.1.9) (Keen and Graham 1989). Many areas of California, and particularly mountain ranges in the eastern portion, provide very little Se to herbivores (Kubota et al. 1967). Based on whole blood Se levels of 1,695 deer, Se deficiency (by livestock standards) appeared to exist in most deer herds and regions of California (Ros-McGauran 1989), and the reproductive success of 1 herd was shown to respond to Se supplementation (Flueck et al. 1989).

Frequently, the Se status in black-tailed deer and other cervids has been evaluated by measuring the total Se concentration in whole blood and making comparisons to livestock standards (Brady et al. 1978, Ros-McGauran 1989). This requires the assumption that whole blood Se is a valid substitute variable of the bioactive form (GSH-Px) and that Se compartmentalization is similar to that in livestock species. I tested the first assumption by examining the relationship between whole blood Se concentration and erythrocyte GSH-Px activity in black-tailed deer. I also tested for a relationship between whole blood Se and plasma GSH-Px activity.

I thank N. K. Jacobsen for permission to collect blood samples from captive animals and for reviewing earlier drafts, and the California Department of Fish and Game for the capture of free-ranging deer. I am also thankful to B. B. Norman for blood analysis and review of the manuscript, and to J. M. Smith-Flueck, M. N. Oliver, T. J. DeLiberto, and an anonymous referee for commenting on this manuscript.

METHODS

Blood Collection.—Blood samples were collected from 72 females (includes 5 sampled twice) and 4 males, ≥1.5 years old. Wild deer were captured in late March or May 1984–86 ($n = 48$) with drive nets along a migration route in Shasta County, California (40°34'N, 121°55'W) (Flueck 1989), and whole blood samples were collected by jugular venipuncture. In November 1984–85, 14 samples were obtained from wild deer by cardiocentesis immediately after they

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were shot in the head with a rifle. These animals had been marked and supplemented with Se during the spring capture. Captive animals ($n = 14$) were chemically restrained (with 4 mg of ketamine hydrochloride and 0.8 mg of xylazine per kg body mass) as part of an ongoing project at the University of California, Davis, and whole blood samples were collected by jugular venipuncture.

Sample Preparation and Analysis for Blood Se Levels.—Blood samples for determining Se in whole blood were collected with 3.8-cm × 16-gauge disposable needles into evacuated EDTA tubes and stored immediately at 4 C until testing. The Se concentration was determined within a few weeks by the fluorometric procedure of Whetter and Ullrey (1978) through the Veterinary Extension Unit of the University of California, Davis. Tissue digestion was modified by using a Technicon BD-40 heating unit and a block digester, and upon completion of the digestion and addition of hydrochloric acid, the samples were reheated for 5 minutes at 150 C. During an interlaboratory study of blood Se determinations, results from this laboratory coincided with the mean of the 51 participating laboratories (Koh 1987). The low margin of sensitivity of the procedure is 0.005 ppm. However, results lower than 0.010 ppm were routinely reported as <0.010 ppm. These data points were replaced with 0.009 ppm for the statistical analysis.

Sample Preparation and Analysis for Hgb GSH-Px Activity.—Blood samples for GSH-Px assays were collected in heparinized tubes from the same individual at the same time as samples for whole blood Se determination. Differing storage and preparation conditions could have affected results for these samples. Seventeen samples collected in May and 5 in November 1984 were stored immediately in liquid nitrogen. These samples from wild deer were assayed in December 1986 after being thawed, centrifuged, and after the supernatant had been removed. Six samples were collected in May 1985 from wild deer and stored immediately in liquid nitrogen. They were assayed in December 1985 after being thawed, centrifuged, and after the supernatant had been removed. In November 1985, 9 samples were obtained from wild deer and 14 samples from captive deer. In all these samples, erythrocytes were washed immediately (twice) in physiological saline and then kept frozen at −20 C or colder until the assays were performed in December 1985. In late March 1986, 25 samples were obtained from wild deer. Erythrocytes were washed immediately (twice) in physiological saline and then frozen in liquid nitrogen. The enzyme assays were performed in April 1986.

I determined erythrocyte GSH-Px activities by a modified procedure of Paglia and Valentine (1967) at the Department of Agricultural Chemistry, Oregon State University (Whanger et al. 1977). The disappearance of nicotinamid adenine dinucleotide phosphate (NADPH) was measured in the coupled reaction between the oxidation of reduced glutathione in the presence of erythrocyte GSH-Px and the substrate hydrogen peroxide. Sodium azide was used to inhibit catalase, and Drabkin's solution was added to stabilize hemoglobin (Hgb). The erythrocyte GSH-Px activity was measured at 30 C and was expressed in international units per gram (U/g) of Hgb. One international unit is equivalent to 1 micromole of NADPH oxidized per minute. Samples were assayed in duplicates and the means reported.

In addition to erythrocyte GSH-Px activity, some of the whole blood GSH-Px activity could be contributed by plasma. Accordingly, in 10 blood samples the plasma GSH-Px activity was also measured and the means of duplicates expressed as U/L.

Se Supplementation.—The wild deer collected in autumn ($n = 14$) had been caught and blood sampled in the previous spring. They were subsequently supplemented with Se administered orally as 2 iron-selenium alloy boluses, a design adapted specificially for ruminants (Permasel Sheep Pellets, ICI Aust. Ltd., now called Copper Animal Health, Melbourne, Aust.) (Handreck and Godwin 1970). Pellets will remain in the reticulum or rumen by gravity and their size (10 g). They consist of elemental iron and selenium (95:5 by mass), will result in an increase of GSH-Px activity in erythrocytes for many months, and may sometimes maintain activity levels for several years by releasing about 0.5–1.3 mg of Se per day (Handreck and Godwin 1970). Five of these animals also provided GSH-Px blood samples in spring before they received the Se supplementation.

Statistics

I used linear regression analysis to examine the relationship between whole blood Se and GSH-Px activity. Pre- and postsupplementation

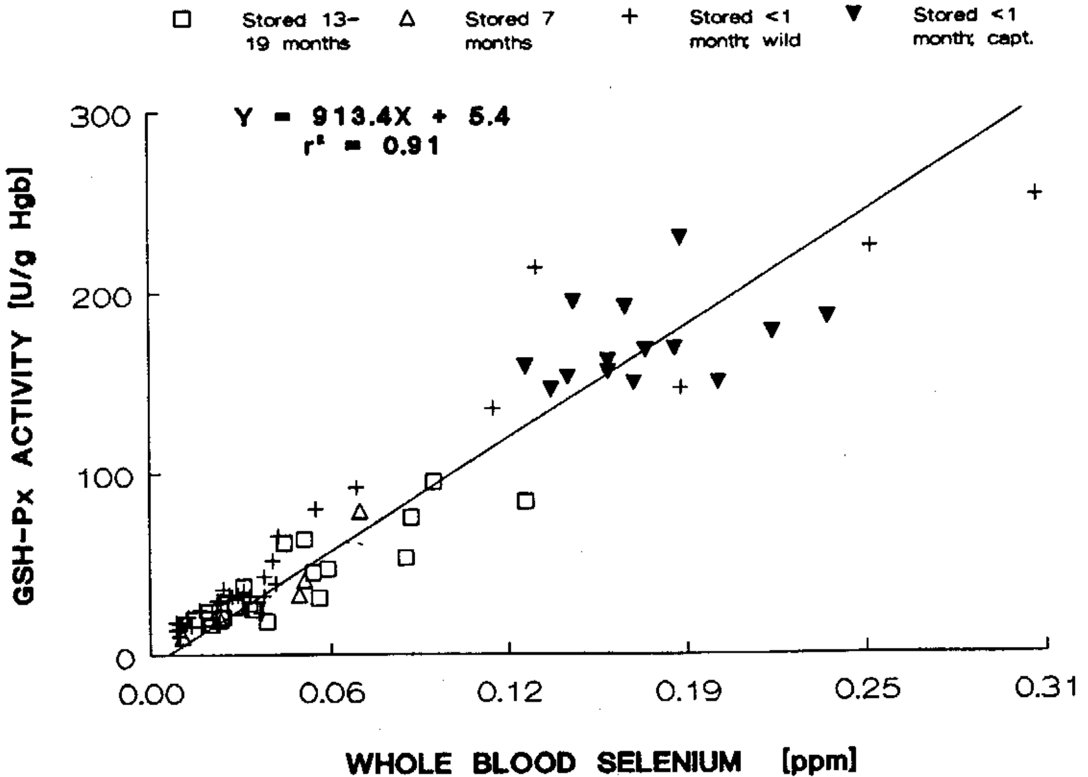

Fig. 1. Relationship between whole blood selenium levels and erythrocyte glutathione peroxidase activity of black-tailed deer, Shasta County, California, 1984–86. Storage times relate to GSH-Px analysis; capt. refers to blood sample from captive animals.

blood Se levels were compared with a 2-sample t-test. Statistical significance was accepted at $P < 0.05$.

RESULTS

Whole blood Se predicted GSH-Px activity in a linear relationship (Fig. 1). Storage conditions of samples did not appear to affect the relationship (Sheppard and Millar 1981).

The nutritional history of the 3 sample groups is reflected in the means and ranges of whole blood Se levels and Hgb GSH-Px activities (Table 1). The mean Se level prior to supplementation with Se rumen pellets was 0.047 ± 0.009 (SE) ppm and differed significantly ($P = 0.04$) from the postsupplementation level (Table 1).

Seasonal changes in whole blood Se levels of unsupplemented deer did not appear to have occurred between 1984 and 1987. Spring ($n = 101$) and autumn control ($n = 34$) Se concentrations did not differ ($P = 0.66$), whereas supplemented deer ($n = 42$) had autumn Se levels significantly different from these controls ($P < 0.0001$) (Flueck 1989).

In a subsample ($n = 10$) there was a significant linear correlation between erythrocyte GSH-Px and plasma GSH-Px (U/L) activity as the independent variable, and between whole blood Se and erythrocyte GSH-Px activity (Table 2). However, I found no correlation between plasma GSH-Px activity and whole blood Se levels (Table 2).

DISCUSSION

The wide range of Se levels in wild unsupplemented deer was reflected in the range of GSH-Px activities. Apparently, some animals either live in pockets of habitat that supply substantially more Se through plants, or they may exhibit different feeding behavior. Variations in Se levels were also reported in wild mountain goats (*Oreamnos americanus*) (Robbins et al. 1985).

The high blood Se levels measured in wild deer collected in autumn appeared to be due to Se rumen pellets administered the previous spring. The response of both whole blood Se levels and GSH-Px activities in these supple-

Table 1. Whole blood Se concentration and erythrocyte GSH-Px activity in black-tailed deer, Shasta County, California, 1984–86.

History	Whole blood Se (ppm)			GSH-Px (U/g Hgb)		
	$\bar{x}$	SE	Range	$\bar{x}$	SE	Range
Supplemented with Se	0.101	0.024	0.020–0.307	102.3	20.50	23.9–253
Wild unsupplemented	0.034	0.004	0.009–0.119	32.6	3.26	10.0–135
Captive unsupplemented	0.171	0.008	0.130–0.235	171.0	6.23	146–230

mented deer supports the concept that whole blood Se can be used to predict the concentration of the bioactive form of Se and hence the Se status of the deer.

Contribution of plasma GSH-Px activity to the erythrocyte GSH-Px activity was negligible. The ranges of published plasma volume and Hgb for *O. hemionus* have been summarized by Anderson (1981) as 41.8–69.4% and 9.8–20.5 g/dL, respectively. Assuming an average plasma volume of 56% and 16.4 g Hgb/dL blood in black-tailed deer (n = 17) (W. T. Flueck, unpubl. data), the calculated GSH-Px activity contributed by plasma amounted to 0.002–0.007% of whole blood activity. In bovids and ovids the plasma GSH-Px contribution also has been shown to be minimal.

Robbins et al. (1985) hypothesized that wild animals like mountain goats may be adapted to low Se environments because blood GSH-Px activity per unit Se is about double that published for the domestic cow and horse. This may allow some species to be successful in low Se habitats, although they may be more susceptible to changes in the bioavailability of Se and/or to changes in requirements for the active form, in particular GSH-Px, because the initial availability of Se is already minimal. However, domestic sheep have 38–122% higher GSH-Px activity per unit Se as compared to mountain goats and black-tailed deer (Anderson et al. 1978, Sheppard and Millar 1981). Following Robbins' et al. (1985) hypothesis, sheep may thus be expected to be very resistant to low Se availability,

Table 2. Erythrocyte (Hgb) GSH-Px and plasma GSH-Px activity as a function of whole blood Se, and Hgb GSH-Px as a function of plasma GSH-Px (N = 10) in black-tailed deer, Shasta County, California, 1984–86.

Relationship	Slope	P	r^2
Se vs. Hgb GSH-Px	947	0.00003	0.87
Hgb GSH-Px vs. plasma GSH-Px	0.05	0.01	0.53
Se vs. plasma GSH-Px	7,252	0.13	0.23

yet they are quite susceptible to Se depletion and disease; they require about 0.11 ppm in whole blood for health (Oldfield et al. 1963, Wheatley and Beck 1988).

The absolute Se concentration in blood does not consistently reflect the actual physiologically active Se among different mammal species. Moreover, other substances interact with Se metabolism. Heavy metals, sulfur, fatty acid concentration, and vitamin E levels all need to be considered. For instance, increased exposure to heavy metals may lower GSH-Px activity per unit Se by forming metal selenides (Frost 1987). Furthermore, non-Se dependent GSH-Px occurs in varying degrees in different species and affects the overall requirement for Se. Therefore, GSH-Px activity per unit whole blood Se probably is not a valid measure of environmental adaptability. Rather, the wide range of normal whole blood Se levels and Se/GSH-Px slopes reported for ruminants implies different strategies of Se compartmentalization, levels of exposure to interacting substances, and distributions of non-Se dependent enzyme systems.

MANAGEMENT IMPLICATIONS

GSH-Px is recognized as the major biologically active form of Se in mammals (Keen and Graham 1989). However, blood samples for whole blood Se determination can be collected and stored more easily than those for GSH-Px determination; and whole blood Se concentrations adequately predict the erythrocyte GSH-Px activity of free-ranging black-tailed deer.

Both whole blood Se levels and erythrocyte GSH-Px activities reflect chronic Se exposure that can vary if animals have recently migrated to a different habitat. The lifespan of erythrocytes is approximately 120 days, and thus, erythrocyte GSH-Px activities can reflect past nutritional history. Whole blood Se, however, responds more rapidly to changes in dietary concentration of Se than does erythrocyte GSH-Px. Furthermore, samples should be taken during

the same season of different years to allow more accurate between-year comparisons.

Contrary to Fielder's (1986) suggestion that the influence of Se deficiency on wild game production would have been present for thousands of years, there is growing evidence that anthropogenic manipulation of ecosystems can rapidly alter Se cycling and bioavailability or requirements of free-ranging herbivores. A continuous evaluation of the Se status of indigenous herbivores may be necessary depending on known Se levels of soils or plants and the degree to which a particular habitat is subjected to anthropogenic influences (reviewed in Flueck and Smith-Flueck 1989). Major impact on Se availability might result from acidification of soils (Geering et al. 1968) in areas exposed to continuous acid precipitation (Frost 1972, Mushak 1985). Factors that may also affect Se availability are eutrophication of terrestrial systems through immissions (i.e., aerial deposition and absorption of sulfur and nitrogen) (Gissel-Nielsen 1977, Aber et al. 1989), increased exposure of soils and animals to heavy metals (Frost 1987), and accelerated removal of biomass (Swaine 1978, also see Federer et al. 1989).

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Received 9 January 1990.
Accepted 7 September 1990.
Associate Editor: DeYoung.