

BLOOD SELENIUM CONCENTRATIONS AND ENZYME ACTIVITIES RELATED TO
GLUTATHIONE METABOLISM IN WILD EMPEROR GEESE

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Abstract—In 1998, we collected blood samples from 63 emperor geese (*Chen canagica*) on their breeding grounds on the Yukon-Kuskokwim Delta (YKD) in western Alaska, USA. We studied the relationship between selenium concentrations in whole blood and the activities of glutathione peroxidase and glutathione reductase in plasma. Experimental studies have shown that plasma activities of these enzymes are useful biomarkers of selenium-induced oxidative stress, but little information is available on their relationship to selenium in the blood of wild birds. Adult female emperor geese incubating their eggs in mid-June had a higher mean concentration of selenium in their blood and a greater activity of glutathione peroxidase in their plasma than adult geese or goslings that were sampled during the adult flight feather-molting period in late July and early August. Glutathione peroxidase activity was positively correlated with the concentration of selenium in the blood of emperor geese, and the rate of increase relative to selenium was greater in goslings than in adults. The activity of glutathione reductase was greatest in the plasma of goslings and was greater in molting adults than incubating females but was not significantly correlated with selenium in the blood of adults or goslings. Incubating female emperor geese had high selenium concentrations in their blood, accompanied by increased glutathione peroxidase activity consistent with early oxidative stress. These findings indicate that further study of the effects of selenium exposure, particularly on reproductive success, is warranted in this species.

Keywords—Emperor goose Selenium Glutathione metabolism Biomarker

INTRODUCTION

The emperor goose (*Chen canagica*) is restricted to arctic and subarctic regions of the Bering Sea. Small numbers of emperor geese occur along coastal Russia, but most breed and winter in Alaska on the Yukon-Kuskokwim Delta and the Aleutian Islands, respectively [1]. The Alaskan population of emperor geese declined by an estimated 60% between 1964 and 1994 [2]. Concern over the status of this species has stimulated investigations to identify potential factors, including exposure to contaminants, that may be associated with the population decline. Reports of lead and selenium exposure in spectacled eiders (*Somateria fischeri*) nesting on the YKD prompted a survey of lead, selenium, and mercury in blood collected from emperor geese on their breeding grounds in 1996 and 1997 [3–5]. Concentrations of mercury in blood samples were low (mean = 0.05 ppm wet wt), and lead was detected in only 18% of the samples tested, but female emperor geese had high concentrations of selenium (mean = 5.6 ppm wet wt) in their blood during the incubation period [5].

Although selenium is an essential trace element, it bioaccumulates in food chains, and excess exposure in birds can result in embryonic deformities and death, emaciation and death of adults, and a variety of gross and histopathologic lesions [6]. Sublethal effects of selenium, including immunosuppression and histopathological effects, also have been reported in birds [7,8]. Excess selenium exposure may cause oxidative stress, a disturbance of the prooxidant-antioxidant balance in favor of the former leading to pathophysiological effects in cells with adverse effects on immunocompetence,

inflammation, and a variety of other defense systems [9]. Studies of the resultant antioxidant response and hepatotoxicity have led to the identification of biomarkers in birds based on changes in the activities of certain enzymes, including glutathione peroxidase and glutathione reductase [10]. Elevated glutathione peroxidase activity in plasma is an early indicator of oxidative stress associated with selenium exposure and has been reported in mallard (*Anas platyrhynchos*) ducklings receiving as little as 2 ppm dietary selenium [8,10]. Glutathione reductase is apparently less sensitive to selenium exposure, but mallards fed 40 ppm selenium exhibited increased plasma activity [10]. In the field, the toxic and reproductive effects of selenium in birds have been most frequently described in freshwater species in association with contaminated agricultural drain water [11]. Less is known about selenium toxicity in marine birds, which often have concentrations of selenium in their tissues that are reported to cause adverse effects in other species [12]. In the present study, we sampled emperor geese to examine the relationship between selenium concentrations in whole blood and activities of two enzymes related to glutathione metabolism.

MATERIALS AND METHODS

Sample collection

In 1998, we collected blood samples from 63 emperor geese on their breeding grounds near the Kashunuk River (61°21'N, 165°30'W) on the YKD. Adult females ($n = 18$) were captured on their nests during late incubation (mid-June) using bow traps [13]. In late July and early August, during the molt of adult flight feathers, we captured adults ($n = 30$) and goslings ($n = 15$) using corral traps [14]. Goslings were approximately

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five to six weeks of age at the time of sampling. About 3 ml of whole blood were collected by jugular venipuncture with heparinized Monovettes® (Sarstedt, Newton, NC, USA) equipped with 21-gauge needles. On our return to the camp site each day, 1 ml of whole blood from each sample was transferred to a cryovial. The remaining whole blood was centrifuged for 10 min, and the plasma was harvested and placed in a second cryovial. Whole blood and plasma samples were frozen in a liquid nitrogen vapor shipper in the field. On return to the laboratory, whole blood was stored at -20°C and plasma at -80°C until analysis for selenium and enzyme activities, respectively.

Analysis of enzymes in plasma and selenium in whole blood

We measured the activities of glutathione peroxidase (GSH peroxidase, EC 1.11.1.9) and glutathione reductase (GSSG reductase, EC 1.6.4.2) in plasma on a centrifugal analyzer (Centrifchem® 500, Baker Instruments, Allentown, PA, USA) using micromethods [15]. Whole blood was analyzed for selenium at the Wisconsin Animal Health Laboratory, Wisconsin Department of Agriculture, Trade, and Consumer Protection (Madison, WI, USA). Selenium was quantified on a Perkin-Elmer (Norwalk, CT, USA) atomic absorption spectrometer (Model 5100PC), equipped with a graphite furnace with Zeeman background correction (Model 5100ZL) and autosampler (Model AS-71). The electrodeless discharge lamp wavelength and slit width were 196.0 and 2.0 nm, respectively. Blood samples were diluted 1:2 with 0.1% Triton X-100 (alkylaryl polyether alcohol, J.T. Baker, Phillipsburg, NJ, USA) in deionized water. Ten microliters of diluted sample, 10 μl of diluent, and 5 μl of a matrix modifier (0.17% Pd + 0.1% $\text{Mg}[\text{NO}_3]_2$ in deionized water) were injected into the furnace and atomized at $1,900^{\circ}\text{C}$. The lower limit of detection was 0.01 ppm (wet wt), and the recovery from spiked samples was $111 \pm 2.5\%$. Selenium residues are reported in ppm wet weight, uncorrected for percentage recovery.

Statistics

The Statistical Analysis System [16] was used for all statistical evaluations. We developed analysis of covariance (ANCOVA) models to test whether GSH peroxidase and GSSG reductase activities in plasma were related to selenium concentrations in whole blood and whether the strength of this relationship differed among sexes, ages (adults vs goslings), or seasonal periods (incubation vs wing molt). The most complex ANCOVA model for each enzyme had 10 parameters: mean enzyme activity for each of five groups (adult females during incubation, adult females during molt, adult males during molt, gosling females during molt, and gosling males during molt) and five slope parameters describing the relationship between selenium and enzyme activity for each group. Models with fewer parameters also were applied to the data, and inference was made by comparing the relative fit of models to data [17]. For example, an explicit test of whether selenium's effect on GSH peroxidase differed among sexes is to compare the most complex model described previously to a model with only three slope parameters: one pertaining to adults during incubation, one pertaining to adults during molt, and one pertaining to goslings during molt, that is, models with no distinction relative to sex. We judged the best model to be that with the lowest value for the Akaike information criterion [17]. We used the sample size-adjusted Akaike information criterion

Table 1. Selenium concentrations (ppm wet wt) in the blood of emperor geese (*Chen canagica*) captured in western Alaska, USA, during incubation (mid-June 1998) and wing molt (late July to early August 1998)

Sampling period	Mean (standard error)	Min-max	<i>n</i>
Incubation	4.98 (0.83)	0.10–11.3	18
Wing molt			
Adults	1.60 (0.18)	0.06–3.37	30
Goslings	0.03 (0.005)	0.01–0.08	15

(AIC_c), which was calculated as $\text{AIC} + [2p(p + 1)]/(n - p - 1)$, where *n* is the sample size and *p* is the number of estimated parameters. To provide a more intuitive means of assessing the quantitative rankings of the various models, we also calculated an AIC_c weight for each model [18]. The sum of AIC_c weights for a set of models equals 1.0, and the model with the lowest AIC_c always has the greatest weight. The AIC_c weights provide a way to evaluate the collective strength of evidence for various factors included in the models. For instance, the sum of AIC_c weights for all models including blood selenium concentration as a covariate affecting GSH activity provides a probabilistic interpretation of whether selenium is an important factor governing GSH activity that is independent of uncertainties about which model is best. We chose to use AIC model selection techniques in lieu of the more traditional probability values calculated from statistical null hypothesis tests. Use of AIC to statistically evaluate fit of data to linear models, such as ANCOVA and analysis of variance (ANOVA), is more reliable, replicable, and informative than null hypothesis testing [18].

Some data were not normally distributed. We therefore repeated the previous analyses using bootstrap resampling methods [19]. Using these methods, we obtained similar parameter estimates and identical model selection results as we did without resampling, which confirms prior knowledge that ANOVA and ANCOVA models are relatively robust to departures from normally distributed data [20]. We therefore present results from the analyses in which resampling was not used.

RESULTS

Selenium concentrations in the blood of incubating females were much higher than in adults at molt or goslings (Table 1). Sex did not affect the activities of GSH peroxidase or GSSG reductase or the effect of selenium on GSH peroxidase or GSSG reductase activities (Table 2, models 2 and 7). Blood selenium concentration was an important factor influencing GSH peroxidase activity, demonstrated by the fact that the sum of AIC_c weights for the models that included selenium effects was 1.0 (Table 2, models 1–5, 7, 9, and 11). The best model for GSH peroxidase indicated that incubating females had a higher mean activity of this enzyme than adult females or adult males during molt (Table 2, model 1; Fig. 1A). The best model also indicated that GSH peroxidase activity was positively related with the selenium concentration in the blood of all adults, but no significant difference was observed between incubating females and adults (both males and females) during molt in the slope describing this relationship (24.7 IU/L, standard error [SE] = 6.8). The GSH peroxidase activity in the plasma of goslings also was positively correlated with selenium in their blood, although the enzyme activity was much lower in goslings. The rate of increase in GSH perox-

Table 2. ΔAIC_c (sample size-adjusted Akaike information criterion) values and AIC_c weights (values providing a quantitative strength of evidence for each model and whose sum = 1.0) for analysis of covariance models relating concentrations of Se in blood to activities of glutathione peroxidase (GSH peroxidase) and glutathione reductase (GSSG reductase) in plasma of emperor geese in western Alaska. Models are based on the hypothesis that the groups or Se effects listed within the model differ from one another. The $\Delta AIC_c = (AIC_c \text{ for model} - AIC_c \text{ for model of best fit})$; hence, $\Delta AIC_c = 0$ for the best model; AFI = adult females during incubation; AFM = adult females during molt; AMM = adult males during molt; YFM = young females during molt; YMM = young males during molt; AM = adults during molt; YM = young during molt; A = all adults; Y = all young

Model no.	Model description	GSH peroxidase		GSSG reductase	
		ΔAIC_c	AIC_c weight	ΔAIC_c	AIC_c weight
1	3 groups (AFI, AM, YM), 2 Se effects (A, Y)	0.0	0.53	2.7	0.16
2	3 groups (AFI, AM, YM), 3 Se effects (AFI, AM, YM)	2.0	0.20	5.2	0.05
3	2 groups (A, Y), 2 Se effects (A, Y)	3.2	0.11	41.7	0.00
4	3 groups (AFI, AM, YM), 2 Se effects (incubation, molt)	3.3	0.10	4.2	0.07
5	2 groups (A, Y), 1 Se effect	4.3	0.06	40.2	0.00
6	3 groups (AFI, AM, YM)	11.4	0.00	0.0	0.60
7	5 groups (AFI, AFM, AMM, YFM, YMM), 5 Se effects (AFI, AFM, AMM, YFM, YMM)	11.5	0.00	13.8	0.00
8	5 groups (AFI, AFM, AMM, YFM, YMM)	15.2	0.00	3.2	0.12
9	2 groups (incubation, molt), 2 Se effects (incubation, molt)	28.0	0.00	50.4	0.00
10	2 groups (A, Y)	30.8	0.00	48.7	0.00
11	1 group, 1 Se effect	45.9	0.00	86.8	0.00
12	2 groups (incubation, molt)	59.1	0.00	68.4	0.00
13	1 group	85.4	0.00	110.4	0.00

idase activity relative to selenium concentration was much greater for goslings than adults, although the high value for this slope estimate (2,767 IU/L, SE = 1,459) was driven by just two data points (Fig. 1B). For GSSG reductase, the sum of the AIC_c weights of models containing selenium effects was 0.28 (Table 2, models 1–5, 7, 9, and 11). Thus, the selenium concentration in the blood was a much less important factor governing GSSG reductase activity than it was for GSH peroxidase activity. The best model for GSSG reductase indicated that the activity of this enzyme was greater in plasma of goslings than adults and greater in molting adults than in incubating females, but GSSG reductase was not significantly correlated with blood selenium in adults or goslings (Table 2, model 6; Fig. 2A, 2B).

DISCUSSION

Selenium concentrations in blood

Selenium concentrations in tissues of marine animals, including birds, are generally higher than in freshwater species [21]. For example, mean selenium concentrations of about 3 to 9 ppm wet weight have been reported in the blood of wild marine birds, compared with 0.4 ppm or less in blood of free-ranging freshwater birds and control birds in experimental studies [22–25]. Our findings of high selenium concentrations in the blood of incubating adult female emperor geese and lower concentrations several weeks later in adults at molt and in goslings agree with an earlier report from the same area [5]. The authors suggested that, because of exposure to selenium on their wintering and staging areas, emperor geese arrive on the breeding grounds with high selenium concentrations in their blood. Over the next several weeks, blood selenium levels decrease, probably because selenium exposure is lower on the breeding grounds. Our finding of much lower concentrations of selenium in the blood of five- to six-week-old goslings than in adults supports the hypothesis of low selenium exposure on the breeding grounds because goslings and adults have been observed to feed on the same plants [26].

Although few experimental studies have been done relating dietary selenium to concentrations of selenium in the blood

of birds, the reports in the literature suggest that this relationship differs among groups of birds. Over the course of an 11-week study, American kestrels (*Falco sparverius*) fed 12 ppm selenium had a maximum mean blood selenium concentration of 12 ppm dry weight (or about 2.6 ppm wet wt, based on the reported moisture content of 78.7%) [27]. In one study with mallards, blood selenium concentrations of ducks fed 10 ppm selenium peaked at 4.5 ppm wet weight [23]. In another mallard experiment with 10 ppm selenium in the feed, the mean concentration of selenium in blood reached a maximum of 8.4 ppm wet weight and declined to about 1.5 ppm wet weight after 10 weeks on feed without added selenium [28]. Mean concentrations of selenium in blood of adult emperor geese were 4.98 ppm wet weight about four to six weeks after their arrival on the breeding grounds and declined to 1.60 ppm by early August, about 10 to 12 weeks after their arrival. If the relationship between dietary selenium and selenium in the blood is similar for emperor geese as in mallards and if little selenium exposure occurs on the breeding grounds, it is possible that emperor geese could be ingesting as much as about 10 ppm selenium on wintering and staging areas. Based on studies with mallards fed selenomethionine, an organic form of selenium, adverse reproductive effects can be expected at dietary concentrations of 7 to 8 ppm selenium [29,30]. Emperor geese may be exposed to less toxic forms of selenium, and they may accumulate more selenium in their blood relative to dietary concentrations than mallards. Marine birds, including emperor geese, may also transfer less selenium to their eggs than freshwater birds. For example, eggs of white-winged scoters (*Melanitta fusca*) contained considerably less selenium than what was predicted from selenium concentrations in their livers, based on liver-to-egg relationships in freshwater birds, and selenium concentrations in eggs of eiders were several times less than the selenium levels in blood samples [24,31,32]. However, the fact that incubating female emperor geese had a mean selenium concentration of approximately 5 ppm wet weight in their blood points to the need to study the effect on selenium exposure on reproductive success in this species.

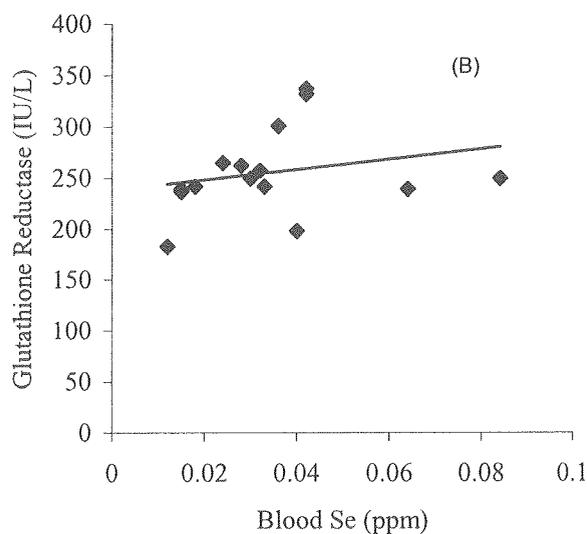
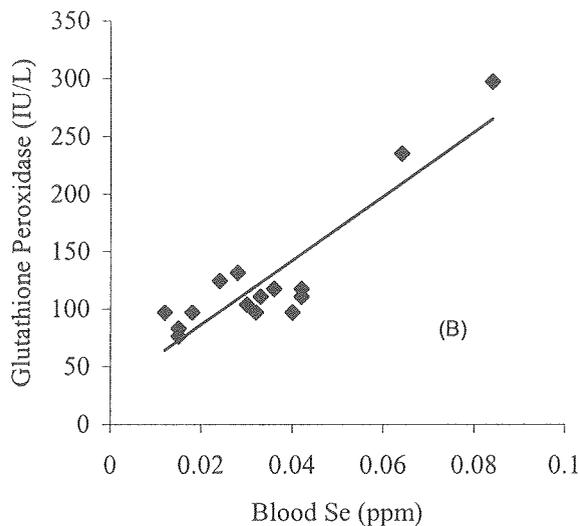
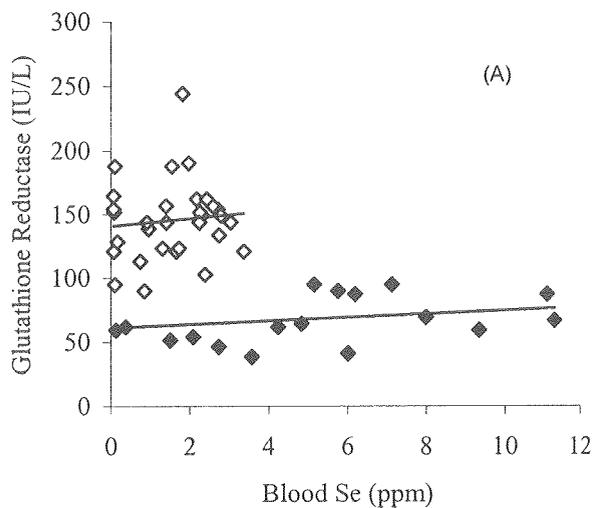
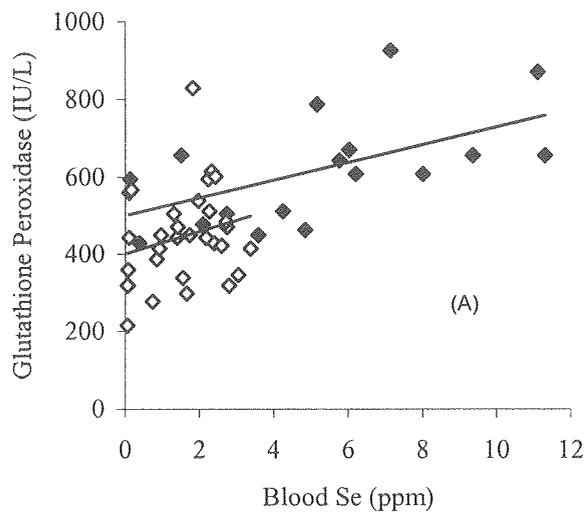


Fig. 1. (A) Relationship between the activity of glutathione peroxidase in plasma and concentrations of selenium in the blood of incubating adult female emperor geese (\blacklozenge , top line) and molting adults (\diamond , lower line). (B) Glutathione peroxidase activity and selenium concentrations in the blood of goslings. $r^2 = 0.773$ for the analysis of covariance model incorporating (A) and (B).

Fig. 2. (A) Glutathione reductase and selenium concentrations in blood of molting adults (\diamond , top line) and incubating adult females (\blacklozenge , lower line). (B) Glutathione reductase activity and selenium concentrations in the blood of goslings. Glutathione reductase activity was not significantly correlated with selenium in the blood of adults or goslings.

GSH peroxidase and GSSG reductase

Plasma GSH peroxidase activity increased with selenium concentration in the blood within each of the three groups of emperor geese (incubating females, adults at molt, and goslings) that we sampled. Incubating females, which had the greatest mean concentration of selenium in their blood, had the highest activity of plasma GSH peroxidase. We also found that the increase of plasma GSH peroxidase activity relative to blood selenium concentration was much greater for emperor goose goslings than for adults. These findings agree with experimental studies in mallards reporting that dietary selenium causes increased GSH peroxidase activity in plasma and that the relative increase is greater in ducklings than in adults [8,33]. Although adult emperor geese had high concentrations

of selenium in their blood shortly after arriving on the YKD, the blood selenium concentrations in goslings were very low, suggesting that little selenium exists in the diet of emperor geese on the breeding grounds. When selenium exposure reaches the point of causing physiological effects, increases in GSH peroxidase activity reflect the onset of oxidative stress. However, because GSH peroxidase is a selenium-dependent enzyme, its activity also increases as dietary selenium levels rise within the nutritionally suboptimal range, reaching a plateau when selenium levels attain the nutritional requirement. Thus, the steeper slope of the GSH peroxidase: blood selenium relationship in emperor goose goslings may reflect the fact that, on diets with suboptimal selenium, increases in plasma GSH peroxidase activity vary directly with additions of dietary

selenium, as reported in ducklings [34]. In contrast, the high blood selenium concentration of adult emperor geese, paired with the positive slope for the relationship between selenium and GSH peroxidase, implies that adults have recently, perhaps on their wintering and staging areas, experienced selenium exposure in excess of the optimum.

We cannot compare the relationship that we found between selenium in the blood and GSH peroxidase in plasma of emperor geese with controlled experimental studies in mallards because concentrations of selenium in blood were not reported in the mallard studies. However, the activities of GSH peroxidase were lower in emperor geese than in mallards exposed to selenium, while GSSG reductase activities were higher in emperor geese. Our findings regarding GSSG reductase agree with earlier experimental studies because we found no significant relationship between the activity of the enzyme and the selenium concentration in the blood within the three groups of geese. The activity of GSSG reductase in plasma of emperor goose goslings was higher than GSH peroxidase activity, which is the reverse of what has been reported in mallard ducklings [33]. Furthermore, the GSSG reductase activity in plasma of adult emperor geese was considerably greater than the activity in adult mallards fed selenium [8]. Species-related differences in tissue activities of GSH peroxidase and GSSG reductase have previously been reported in mallard ducklings and Canada goose (*Branta canadensis*) goslings [35].

CONCLUSIONS

The positive relationship that we found between plasma GSH peroxidase activity and selenium concentrations in whole blood of emperor geese indicates that GSH peroxidase activity is a useful biomarker for early selenium-induced effects in this wild population and suggests that individuals with the highest selenium levels are experiencing oxidative stress. The temporal differences that we found in blood selenium concentrations agree with an earlier study suggesting that emperor geese are exposed to higher levels of selenium on their wintering and staging areas in the marine environment than on their breeding grounds. Although marine birds may tolerate greater selenium exposure than freshwater birds, the finding of high selenium concentrations in the blood of incubating female emperor geese suggests the need to evaluate additional parameters of selenium-induced oxidative stress and to study the effect of selenium exposure on reproductive success in this reduced population.

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