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BODY CONDITION EFFECTS IN AMERICAN KESTRELS FED SELENOMETHIONINE

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ABSTRACT: Body composition was measured in male American kestrels (*Falco sparverius*) beginning after a 77-day exposure to 0, 6, or 12 ppm (dry wt.) selenium as seleno-L-methionine in their diet. Total body mass, lean body mass, and body fat were compared among groups to identify potential wasting effects of selenium, as had been reported for wild waterfowl from a selenium-contaminated site. On the last day of selenium treatment, selenium concentrations in the blood of kestrels was significantly negatively correlated with lean mass. Kestrels that had been exposed to 12 ppm selenium in the diet exhibited relatively higher lean mass (relative to total body mass) and lower normalized body fat than kestrels fed 0 or 6 ppm dietary selenium. These differences persisted throughout the 6 mo study period. The effect observed on body condition of kestrels at environmentally relevant exposure levels has implications for wild birds with respect to both overwinter survival and reproductive success.

Key words: American kestrel, blood selenium, body condition, dietary selenium, *Falco sparverius*, Kesterson Reservoir, selenium, total body electrical conductivity

INTRODUCTION

One effect of high selenium exposure in birds and mammals is body mass loss and wasting. At Kesterson Reservoir (Merced County, California, USA), American coots (*Fulica americana*) exposed to high environmental levels of selenium had body masses that averaged 25% less than those collected from a reference site (Ohlendorf et al., 1988; 1990). These birds were in poor condition, exhibiting marked breast-muscle atrophy and lacked subcutaneous fat deposits. In captive birds, elevated dietary levels of selenium (i.e., 20–30 ppm) have been shown to cause significant body mass loss in adult birds (Smith et al., 1988; Heinz and Fitzgerald, 1993a; Wiemeyer and Hoffman, 1996). Exposure of birds to selenium at Kesterson Reservoir has been reduced since the mid-80's, primarily by conversion of aquatic habitat to upland habitat. Biological monitoring efforts, since conversion of Kesterson to upland habitat, have not identified reproductive impairment or other health problems in terrestrial birds currently utilizing this site (Ohlendorf and Santolo, 1994).

While body mass is frequently used as a general indicator of condition in animals,

this parameter can vary greatly with size, reproductive condition, sex, feeding status, and other factors. Alternatively, measurement of body composition can provide a more sensitive and direct assessment of an animal's health status. Even small changes in fat and muscle can represent significant changes in the energy content of the body and can influence both reproductive success as well as survival (Gessaman, 1987). Measurement of total body electrical conductivity (TOBEC) is a noninvasive method for estimation of fat and lean mass in living animals. This approach has been previously used for determination of body composition in a variety of captive and free-living birds and mammals, including rodents (Walsberg, 1988), shorebirds (Castro et al., 1990; Roby, 1991; Lyons and Haig, 1995), and raptors (Harden, 1993). In this study we investigated the effect of dietary selenium concentrations similar to those expected for wild birds living at Kesterson on body mass and body composition in post-breeding American kestrels (*Falco sparverius*).

METHODS

Animals and treatments

Captive-bred adult male and female American kestrels were obtained from McGill Uni-

versity (Montreal, Quebec, Canada) and housed communally at the University of California (UCD; Davis, California, USA) in large outdoor flight pens. Kestrels were cared for according to animal care protocols approved by the Office of the Campus Veterinarian at UCD.

Prior to the breeding period (early March), male and female kestrels were paired and randomly assigned to treatment groups. Individual pairs were housed in pens (approximately 2 m × 2 m × 1.75 m) which were maintained at ambient temperature and lighting in a large, covered building with screen siding. Each pen was equipped with a shelf and a rope perch, and a wooden nest-box.

All treatment groups were fed a commercial raptor chow (Nebraska Bird of Prey Diet, Central Nebraska Packing, Inc., North Platte, Nebraska, USA) with seleno-L-methionine (Sigma, St. Louis, Missouri, USA) added at concentrations of 0 ($n = 10$ pairs; Control group), 6 ($n = 15$ pairs; Low group) or 12 ($n = 15$ pairs; High group) ppm selenium (dry wt.). Seleno-L-methionine dissolved in triple-distilled water and a vitamin supplement (Vionate, ARC Laboratories, Atlanta, Georgia, USA) were blended into the diet using an industrial mixer. Moisture added to the diet by the seleno-L-methionine solution was less than 1%. The Control diet was prepared in a similar fashion, using triple-distilled water instead of seleno-L-methionine solution. Over the course of the study, three batches of Control diet, seven batches of the Low-Se diet, and nine batches of the High-Se diet were prepared, stored at -20°C , and thawed for use one day prior to feeding. Dietary treatments were initiated upon introduction to the breeding pens. Each pair was provided with about 100 g of fresh food daily, and tap water was provided *ad libitum*. Birds were fed treatment diets until the end of egg laying for a total of 11 wk, after which they were changed over to the Control diet. During the treatment period, food consumption was measured to the nearest gram on a weekly basis for each pair. Thereafter, daily food consumption for each pair was monitored qualitatively by recording in the morning whether some or all of the food had been consumed the previous day. Samples of diet were collected each time a Control, Low, or High treatment diet was mixed. Diet samples were stored at -70°C , then shipped to a commercial laboratory (Laboratory and Environmental Testing, Inc., Columbia, Missouri) on dry ice for total selenium analysis. Sample preparation consisted of lyophilization, homogenization, and acid digestion (nitric acid followed by hydrochloric acid reduction). Total selenium concentrations were determined using hydride

generation atomic absorption spectroscopy. Quality control included at least 10% duplicates, 10% spikes, 5% blanks, and 5% reference samples. Lyophilization data were used to calculate percent moisture in all samples. Selenium concentrations (dry weight) in diet samples ranged from 0.60 to 0.70 ppm in the Control diet ($n = 3$, $\bar{x} = 0.63$), 5.9 to 6.7 ppm in the Low-Se diet ($n = 7$, $\bar{x} = 6.3$), and 11 to 14 ppm in the High-Se diet ($n = 9$, $\bar{x} = 12$).

TOBEC measurements

TOBEC measurements of male kestrels were initiated on the day selenium treatment ended (day 77 of treatment), after the reproductive period, in order to avoid disruption of breeding activities. Measurements were conducted over the following 6 months. Because female kestrels exhibited highly variable body masses during the reproductive and post reproductive periods, they were not included in this study. TOBEC was measured using a commercially available instrument (EM-SCAN SA-3000 model, Springfield, Illinois, USA), which uses an electro-magnetic coil for measuring the difference in conductivity between lipids and other body constituents. Measurements were conducted on April 28 (final day of selenium treatment), June 16, July 19, and November 11 (sample days 1, 49, 82, and 196, respectively). Whole blood samples were collected from the wing vein of kestrels on sample Day 1 for selenium analyses; samples were stored and analyzed as described above for diet samples. Numbers of birds sampled for each treatment group and for each of the four sample dates varied among sampling periods. Birds were not handled when ambient temperature was high enough to induce heat stress or affect the TOBEC readings (generally above 27°C).

Prior to TOBEC measurement, kestrels were weighed to the nearest 0.1 g, then restrained by hooding and wrapping bandaging tape around their legs. After a warm-up period of at least 1 hr for the instrument, a reference reading of the empty chamber was taken. Restrained birds were placed on their backs on a carrier tray, inserted into the chamber head first, and moved through the chamber until the peak electromagnetic intensity was reached and then slowly removed from the chamber. Between three and five readings were taken of each bird at each sampling time in order to get a reading with a coefficient of variation (CV) $\leq 3\%$.

Lean body mass was calculated using the equation developed for kestrels by Harden (1993): $\text{LM} = (\text{E} + 229.554)/3.552$; where LM is lean mass and E is the index value for lean

TABLE 1. Mean (\pm SE) body, lean, and fat mass (g) of male American kestrels after exposure to Control, Low, and High concentrations of selenium in diets for 77 days. Dietary selenium treatments had ended on 28 April 1996 (Day 1). Different letters within rows indicate significant differences within groups over time using Fisher's PLSD test for multiple means comparisons ($P = 0.05$).

Treatment	April 28 (Day 1)			June 16 (Day 49)			July 19 (Day 82)			November 11 (Day 196)		
	\bar{x}	\pm SE	<i>n</i>	\bar{x}	\pm SE	<i>n</i>	\bar{x}	\pm SE	<i>n</i>	\bar{x}	\pm SE	<i>n</i>
Body Mass												
Control	112.5	3.0	10	114.1	3.0	10	111.5	2.8	9	118.7	2.5	10
Low-Se	109.2 A	2.1	15	107.3 A	2.3	14	105.3 A	2.2	15	121.3 B	1.4	14
High Se	105.1 A	1.6	14	103.7 A	1.6	13	102.7 A	1.8	14	114.1 B	1.4	10
Lean Mass												
Control	94.3 A	0.8	10	93.9 A	0.8	10	96.9 B	1.2	9	96.7 B ^a	0.7	10
Low-Se	92.8 A	0.5	15	91.9 A	0.7	14	91.7 B	0.6	15	97.6 B	0.7	14
High Se	89.7 A	0.5	14	91.9 B	0.4	13	92.9 B	0.5	14	94.7 C	0.6	10
Fat Mass												
Control	18.2 AB	2.3	10	20.2 A	2.5	10	14.6 B ^b	1.8	9	22.0 A	2.2	10
Low-Se	16.4 A	1.7	15	15.4 A	1.7	14	13.6 A	1.8	15	23.7 B	1.0	14
High Se	13.7 A	1.3	14	11.8 AB	1.4	13	9.8 B ^c	1.7	14	19.4 C	1.2	10

^a November is nearly significantly higher than April ($P = 0.07$).

^b July is nearly significantly lower than June ($P = 0.09$).

^c July is nearly significantly lower than April ($P = 0.06$).

mass determined by the EM-SCAN instrument. The equation for lean mass was not corrected for our instrument but any estimation error was assumed to be consistent within our study. Calculated lean mass was multiplied by a normalization constant of 0.937. Body fat mass was determined by taking the difference of body mass and normalized calculated lean mass.

Statistical analyses

Body composition data for control and treated kestrels were found to be normally distributed (Shapiro-Wilk *W* test for normality, $P = 0.05$). Comparisons of absolute body mass, lean mass, and fat mass within treatment groups over time were analyzed using repeated measures analysis of variance and Fisher's Protective Least Significant Difference (PLSD) test for multiple means comparisons (SAS Institute, 1998). Comparisons of normalized values for body mass (body mass/initial [April] body mass), lean mass (lean mass/body mass), and fat mass (fat mass/body mass) among treatment groups were analyzed using one-way analysis of variance (ANOVA), and the Mann-Whitney *U* test (SAS Institute, 1998). Significance for analyses was inferred at the $P = 0.05$ level. The relations between selenium concentrations in blood and body mass, fat, and lean mass on Day 1 were analyzed with linear regression and ANOVA.

RESULTS

Food intake

For the overall selenium treatment period (11 wk), food consumption was higher by 9% in the High-Se and 8% in the Low-Se groups than it was in Controls. Qualitative monitoring of daily food intake thereafter indicated no differences among Control, Low-Se, or High-Se kestrels in food consumption.

Total body mass

There were no significant differences in normalized body mass among Control and selenium treatment groups for any of the sample days. Body mass changed significantly during the treatment period (April–November; $F_{3,17,1}$, $P < 0.001$). In both Low-Se and High-Se groups, Day 196 mean body masses were significantly higher than on Days 1, 49, or 82 ($P < 0.01$ for all; Table 1). Mean body mass of Control kestrels did not change significantly during the study period (Table 1).

Lean mass

Normalized lean mass of Controls was significantly lower than that of High-Se

kestrels on Day 49 ($P = 0.013$) and tended to be lower on Days 1 ($P = 0.113$), 82 ($P = 0.089$), and 196 ($P = 0.173$; see Fig. 1). Normalized lean mass of Low-Se kestrels was significantly lower than High-Se birds on Day 196 ($P = 0.035$). No differences in normalized lean mass were observed between Controls and Low-Se birds on any sample days.

Lean mass changed significantly during the treatment period (April–November; $F_{3,19.9}$, $P < 0.001$), and significantly changed during the treatment period within treatment groups ($F_{6,4.4}$, $P = 0.001$; Table 1). The mean lean mass of Control kestrels exhibited a significant increase by Day 82 compared with Day 1 ($P = 0.05$) and 49 ($P = 0.03$) and this difference was still observed by Day 196 ($P = 0.03$). Lean mass in Low-Se kestrels was significantly higher on Day 196 than on all previous sample days ($P < 0.01$ for all), but no other significant differences among sample days were observed for this group. For High-Se kestrels, lean mass was significantly higher on Days 49, 82, and 196 than on Day 1 ($P < 0.01$). Lean mass increased at each sample day, relative to the previous sample day and only Days 49 and 82 were not significantly different (i.e., lean mass on Day 1 < Day 49 [$P < 0.01$] < Day 82 [$P = 0.16$] < Day 196 [$P = 0.02$]).

Fat mass

Normalized fat mass was significantly lower in the High-Se group than in Controls on Day 49 ($P = 0.013$), and tended to be lower than Controls on Days 1 ($P = 0.113$), 82 ($P = 0.089$), and Day 196 ($P = 0.173$; see Fig. 1). Normalized fat mass of Low-Se kestrels was significantly lower than High-Se birds on Day 196 ($P = 0.035$). No differences in normalized fat mass were observed between Controls and Low-Se birds on any sample days.

Fat mass changed significantly during the treatment period (April–November; $F_{3,14.3}$, $P < 0.001$). Fat mass was significantly greater by Day 196 compared with Day 82 for Controls ($P = 0.03$), and com-

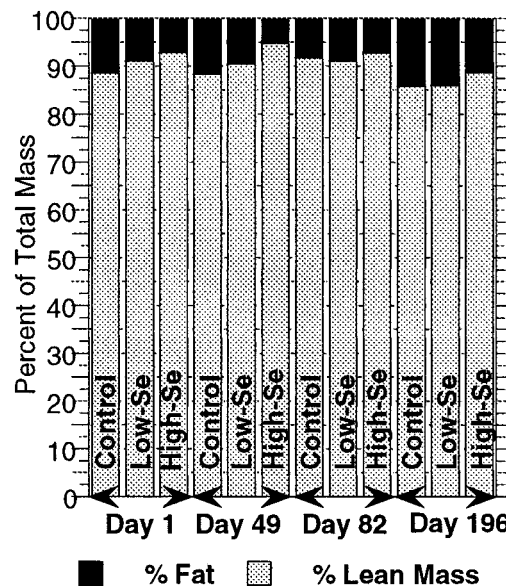


FIGURE 1. Relative contribution of fat and lean mass to total body mass of male kestrels over time after exposure to Control, Low, and High concentrations of selenium in diets for 77 days. Dietary selenium treatments had ended on Day 1.

pared with all other days for Low-Se and High-Se kestrels (i.e., $P < 0.01$ for all comparisons for Low-Se and $P = 0.01$ for Day 1 and $P < 0.01$ for other sample days for High-Se; Table 1).

Selenium concentrations in blood

Negative relationships were observed between selenium concentrations in blood measured on Day 1 and kestrel body mass (Fig. 2A; $r^2 = 0.244$, $n = 37$, $P = 0.002$), fat mass (Fig. 2B; $r^2 = 0.114$, $n = 37$, $P = 0.041$) and lean mass (Fig. 2C; $r^2 = 0.496$, $n = 37$, $P < 0.001$) measured on that day. The strongest relationship was observed between lean mass and the concentration of selenium in blood of kestrels in Control, Low- and High-Se groups (Fig. 2C).

DISCUSSION

In this study, exposure to elevated dietary selenium during reproduction caused significant changes in fat and lean mass, but not body mass, in male kestrels. These data are in agreement with previous find-

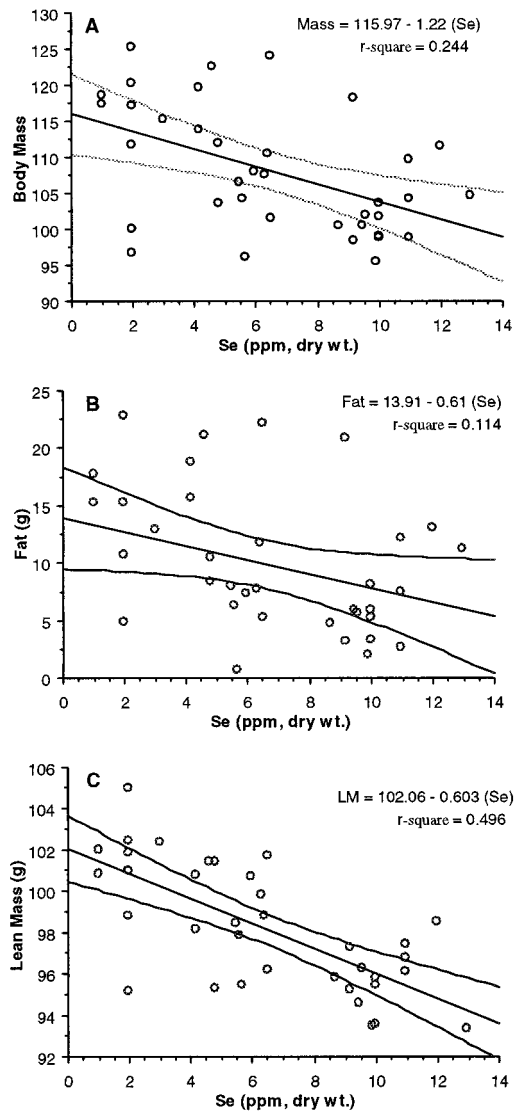


FIGURE 2. Selenium concentration in blood of male kestrels (circles represent individual birds) after exposure to Control, Low, and High concentrations of selenium in diets for 77 days. (Day 1) is plotted against body mass (A), fat mass (B), and lean mass (C) calculated from total body electrical conductivity on Day 1 ($n = 37$). Outer lines show 95% confidence intervals.

ings that univariate metrics (e.g., body mass) are inadequate measures of overall body size or condition in birds (Rising and Somers, 1989; Freeman and Jackson, 1990; Bortolotti and Iko, 1992; Wiebe and Bortolotti, 1996). That lean mass was negatively correlated with selenium concen-

tration in blood at the end of the selenium treatment period (i.e., the beginning of the TOBEC measurement period) suggests that lean mass was reduced in a dose-dependent manner and that blood is a useful tissue for indication of such an effect. Furthermore, the continuous gain in lean mass observed only in the High-Se (12 ppm) group throughout the study may reflect a compensatory response to recover critical muscle mass lost due to the 77-day period of selenium exposure. This is corroborated by the failure of High-Se kestrels to increase their pre-winter body fat to the degree exhibited by Control and Low-Se groups.

Prior studies have reported body mass effects in adult birds exposed to different forms of selenium, including selenomethionine, with anorexia most frequently cited as the probable cause in the black-crowned night heron (Smith et al., 1988), mallard (Heinz and Fitzgerald, 1993a,b; Albers et al., 1996; Green and Albers, 1997), and eastern screech-owl (Wiemeyer and Hoffman, 1996). In these studies, moderate to severe body mass reductions have been observed at dietary selenium concentrations higher than those used in this study (e.g., 20–30 ppm versus 12 ppm) and found to occur in concert with other signs of selenium poisoning, including mortality. Food avoidance (as well as other signs of toxicity) was not observed in the High-Se group of the present study, so it is likely that another mechanism was responsible for the observed effects on body condition.

Gessaman (1979) reported that body fat of male kestrels decreased from about 8% of total body mass in April to 4% in early September, then increased sharply to nearly 13% in late September. This is a similar pattern to that found in this study and a typical seasonal pattern observed in migratory birds, in which lowest fat levels tend to occur after the reproductive season, followed by rapid deposition prior to the fall migration (King and Farner, 1965). Recovery of lean mass and percent body

fat was a relatively prolonged process in kestrels exposed to dietary selenium during the breeding period, and attainment of fat reserves comparable to those of controls prior to fall was not observed within the time span of the study. Extrapolation of effects observed in the High-Se group to wild kestrels, which are presumably faced with considerably greater energetic stresses than captive birds, suggests potential for significant disruption of the normal cycle of fat deposition.

While reduced body condition of wild birds may not have immediately lethal results, such alterations can nevertheless have important long-term consequences. For instance, in some bird species, winter body condition or fat storage is an indicator of whether breeding will be successful (Newton, 1979; Alisauskas and Ankney, 1985; Wiebe and Bortolotti, 1995). American kestrels subjected to moderate to significant reductions in body fat and lean mass during the pre-laying and laying periods produce smaller eggs with lower hatchability (Wiebe and Bortolotti, 1995, 1996) and females may delay egg formation until their condition improves (Meijer et al., 1989; Aparicio, 1998). Furthermore, Iko (1991) found that parent American kestrels in good physical condition were more likely to deliver more food to offspring than parents in poor condition. Based on these observations, birds exposed to elevated dietary selenium during the winter may not be in adequate condition for surviving migration to the breeding location and/or successful reproduction because of persistent reductions in muscle mass and body fat.

Previous efforts to monitor and assess selenium effects on birds at Kesterson Reservoir and other selenium-contaminated sites have focused primarily on potential effects on reproduction (Ohlendorf and Santolo, 1994). However, the results of this study suggest that the body condition of kestrels, and perhaps other birds that utilize high selenium areas, may be impaired at concentrations below or com-

parable to those known to directly affect reproduction (e.g., 8 ppm in the diet of mallards; Heinz et al., 1989). Severe wasting was observed in waterfowl when the aquatic food chain was initially contaminated with selenium (Ohlendorf et al., 1988, 1990), but it is not clear whether this effect was due to anorexia induced by acute selenium poisoning, such as that observed in some captive bird studies, or to another mechanism. To date, neither wasting nor direct embryotoxicity have been observed in terrestrial birds at Kesterson. In particular, subtle wasting effects, such as those reported in this study, would be difficult to observe in free-living birds because of natural variability and difficulties associated with making quantitative and sequential measurements of fat and lean mass in the field. However, because of the demonstrated negative effects on survival and reproduction previously shown for reduced body condition in birds, further characterization of this apparent effect of selenium is warranted.

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