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Abstract To evaluate the potential toxicity of copper (Cu) in raptors that may consume Cu bullets, shotgun pellets containing Cu, or Cu fragments as they feed on wildlife carcasses, we studied the effects of metallic Cu exposure in a surrogate, the American kestrel (*Falco sparverius*). Sixteen kestrels were orally administered 5 mg Cu/g body mass in the form of Cu pellets (1.18–2.00 mm in diameter) nine times during 38 days and 10 controls were sham gavaged on the same schedule. With one exception, all birds retained the pellets for at least 1 h, but most (69%) regurgitated pellets during a 12-h monitoring period. Hepatic Cu concentrations were greater in kestrels administered Cu than in controls, but there was no difference in Cu concentrations in the blood between treated and control birds. Concentration of the metal-binding protein metallothionein was greater in male birds that received Cu than in controls, whereas concentrations in female birds that received Cu were similar to control female birds. Hepatic Cu and metallothionein concentrations in kestrels were significantly correlated. Histopathologic alterations were noted in the pancreas of four treated kestrels and two controls, but these changes were not associated with hepatic or renal Cu concentrations, and no lesions were seen in other tissues. No clinical signs were observed, and there was no treatment effect on body mass; concentrations of Cu, hemoglobin, or methemoglobin in the blood; or Cu concentrations in kidney, plasma biochemistries, or hematocrit. Based on the parameters we measured, ingested Cu pellets pose little threat to American kestrels (and presumably phylogenetically related species), although the retention time of pellets in the stomach was of relatively short duration. Birds expected to regurgitate Cu fragments with a frequency similar to kestrels are not likely to be adversely affected by Cu ingestion, but the results of our study do not completely rule out the potential for toxicity in species that might retain Cu fragments for a longer time.

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Lead poisoning from ingested lead shotgun pellets was first reported in birds in the late 1800s (Friend et al. 2009). Although documented in waterfowl for many years, the ingestion of lead and/or poisoning, based on lead residues in tissues, has also been reported in at least 58 species of free-ranging terrestrial birds from throughout the world, including 28 raptors (Pain et al. 2009). A nationwide ban on the use of lead shotgun pellets for hunting waterfowl and American coots (*Fulica americana*) was instituted in the United States in 1991, primarily because of lead poisoning in waterfowl and in eagles that fed on waterfowl carcasses containing ingested or embedded lead shot (Friend et al. 2009). Although some states have implemented additional restrictions, lead ammunition is often still used for hunting upland game, large game animals, and animals that are considered pests, potentially exposing scavengers that feed on unretrieved carcasses or offal left in the field to lead shotgun pellets or lead rifle bullets and their fragments (Rattner et al. 2008). In a study with deer killed with lead-based bullets, radiography showed averages of 160 and 551 metal fragments, respectively, in offal piles and entire carcasses, with clusters of fragments radiating from an average of 7 cm to a maximum of 15 cm

from wound channels (Hunt et al. 2006). In another study of deer shot with lead ammunition, radiography showed an average of 180 metal fragments in the viscera and 356 fragments in carcasses (Knott et al. 2010). Lead exposure and poisoning associated specifically with ingested rifle bullet fragments has been reported in free-ranging raptors, including the white-tailed sea eagle (*Haliaeetus albicilla*), Steller's sea eagle (*H. pelagicus*), bald eagle (*H. leucocephalus*), golden eagle (*Aquila chrysaetos*), and California condor (*Gymnogyps californianus*) (Wiemeyer *et al*. 1988; Craig et al. 1990; Gill and Langelier 1994; Iwata et al. 2000; Helander et al. 2009). Furthermore, evidence of greater lead exposure during hunting versus nonhunting seasons has been noted in several species of predatory and scavenging birds, including the California condor (Pain et al. 1997; Hunt et al. 2007; Craighead and Bedrosian 2008; Neumann 2009).

Andean condors (*Vultur gryphus*), an experimental surrogate for California condors, were found to be quite sensitive to lead poisoning, exhibiting retention of administered lead shot and rapid dissolution and absorption of lead, leading to quick onset of poisoning (Pattee et al. 2006). Lead poisoning from ingested lead ammunition was first reported in the California condor in the 1980s (Wiemeyer et al. 1988). In 2005, the Arizona Game and Fish Department began offering nonlead ammunition to big-game hunters in an effort to decrease lead exposure in California condors, and in 2008 regulations were instituted in California restricting the use of lead ammunition in the range of the California condor in the central and southern part of the state (Arizona Game and Fish Department 2009; Avery and Watson 2009). Copper (Cu) has been reported to be a comparable alternative to lead for rifle bullets, and Cu bullets tend not to fragment as lead bullets do (Hunt et al. 2006; Knott et al. 2009). Steel is generally the preferred nontoxic alternative to lead for shotgun pellets (Oltrogge 2009). Of the 11 other alternatives approved by 2009, only two contain Cu, one with 44% Cu and one with 9 to 16% Cu, although Cu coatings on approved nontoxic shot types are also allowed (United States Fish and Wildlife Service 2009).

At least one experimental study of Cu exposure has been conducted with raptors, in which turkey vultures (*Cathartes aura*) were fed Cu pellets in food items (Risebrough et al. 2001). During the 8.5-week study, birds were radiographed weekly and administered additional pellets to sustain the original dosage of 2.3 gm Cu/kg body mass. At the end of the study, Cu concentrations were somewhat greater in livers of treated birds versus controls, but concentrations in blood were similar, and no effects were noted on body mass, hematology, plasma biochemistries, or histopathology (Risebrough et al. 2001). Previous studies of Cu pellet exposure in mallards (*Anas platyrhynchos*) and Pekin ducks have shown no toxic effects (Bellrose 1965; Irby et al. 1967; Locke et al. 1967; Krone et al. 2009). Thomas and McGill (2008) studied the dissolution of Cu from tungsten–bronze pellets containing 44% Cu in a simulated avian gizzard and concluded that the amount released would not pose a toxic risk if wild birds ingested the pellets.

In contrast to the negative results of experimental studies of metallic Cu in mallards and vultures, mortality has been reported in captive Canada geese (*Branta canadensis*), which began to die within hours after a pond was treated with Cu sulfate used as an algicide (Henderson and Winterfield 1975). The deaths of several free-ranging mute swans (*Cygnus olor*) were attributed to Cu poisoning of undetermined source (Kobayashi et al. 1992). Some groups of animals, particularly ruminants, are more sensitive to increased dietary Cu exposure than others and may develop clinical pathologies, such as enzymatic indications of liver damage, hemoglobinemia, and methemoglobinemia, sometimes resulting in death (National Research Council 1977). Domestic poultry are more resistant to Cu toxicity than mammals, but studies have shown decreased weight gain, weakness, anorexia, lethargy, and anemia in chickens (*Gallus domesticus*) exposed to Cu salts (National Research Council 1977). Metallothionein synthesis can be induced by various metals, including Cu, and significant correlations between metallothionein and Cu concentrations in liver have been reported in some species of wild birds (Dunn et al. 1987; Kojadinovic et al. 2007).

Because most types of nontoxic shot approved for waterfowl hunting do not contain Cu, exposure of avian predators or scavengers to metallic Cu when feeding on wildlife carcasses or offal is more likely to result from ingesting Cu bullets made entirely of Cu than shotgun pellets containing some fraction of Cu. Because published information on Cu toxicity in avian wildlife, particularly raptors, is limited, we evaluated metallic Cu exposure in American kestrels (*Falco sparverius*), a surrogate often used as a model for other raptors, particularly in toxicology studies (Eisler 2000; Bardo and Bird 2009). Using orally administered Cu pellets, we studied the effects of Cu exposure on survival, clinical signs, body mass, Cu concentrations in tissues, biochemical markers in tissues, and histopathology in kestrels.

Materials and Methods

Birds, Husbandry, and Cu Administration

Twenty-six captive-bred, 1-year-old American kestrels were housed individually in outdoor flight pens (6 × 2.4 × 2.4 m) at the Patuxent Wildlife Research

Center in Laurel, MD. Each pen included a covered perch box, exposed rope perch, water bowl, and covered feeding tray. Birds were fed 50 g of a bird of prey diet (3.3 mg/kg Cu as fed; Nebraska Brand, North Platte, NE) once daily and two mice (*Mus musculus*; Charles River Laboratories, Frederick, MD) approximately once weekly. Uneaten food was removed before the next feeding.

Kestrels were administered Cu pellets (99.9% Cu; Fisher Scientific, Pittsburgh, PA) nine times during the study at the rate of 5 mg Cu/g body mass by way of oral gavage tube. The pellets were spherical in shape, and the size range included those that passed through a no. 10 (2.00-mm mesh) soil sieve but not through a no. 16 (1.18-mm mesh) sieve. Thus, the approximate diameter of Cu pellets used in the study ranged from >1.18 to <2.00 mm, with a surface area >4.37 mm^2 but <12.6 mm^2. Kestrels were fed mice several days after dosing to minimize the effect that regurgitation of fur and bones might have on Cu pellet retention (Ford 2010). The dose exceeded the amount calculated based on a 1.2-kg red-tailed hawk (*Buteo jamaicensis*) consuming a .22-caliber solid Cu bullet (e.g., Barnes Bullets, Mona, UT) and a 8.5-kg California condor consuming a .270-caliber solid Cu bullet, resulting in total estimated Cu exposures of 4.1 and 1.14 mg/g body mass, respectively. The use of small Cu pellets allowed for accurate dosing and resulted in a relatively large combined surface area, thus providing for greater erosion in the stomach and subsequent absorption of Cu compared with a single pellet of the same mass. Clear plastic flexible tubing, 5 mm in diameter with a 3-mm internal diameter, was passed distal to the crop; Cu pellets were delivered through a funnel; and a metal stylet was passed to the end of the tube to expel any pellets remaining in the tubing into the upper gastrointestinal tract. Control birds were sham gavaged. Each day before being dosed, kestrels were weighed for dose calculation. After being fasted overnight, birds typically received approximately 17–22 pellets/day on days 0, 12, 14, 16, 29, 31, 33, 35, and 37. Immediately after dosing, each kestrel was placed in a stainless steel cage (23 × 33 × 38 cm) for 1 h for observation and to detect regurgitation of Cu pellets and were fed the bird of prey diet 1 h after release into the large pen. The only exception was that on day 12 birds were held in the small cages overnight (12 h) to detect regurgitation and were fed after release the next morning. In addition to being weighed on the day before dosing, all kestrels were weighed at weekly intervals. Each bird was monitored by visual inspection for a minimum of 10 min twice daily for the occurrence of clinical signs, such as lethargy, ataxia, or anorexia. Once during the study, each dosed kestrel was radiographed 48 h after Cu administration. In addition, three dosed birds were radiographed 24 h after dosing.

Blood Collection, Hematology, and Plasma Biochemistries

Blood samples were collected from all kestrels by way of jugular venipuncture on days 1, 8, 17, 27, and at the end of the study, when controls were bled on day 37 and dosed birds were bled on day 38. Blood was placed in heparinized tubes, and the hematocrit was measured on each sampling day. Methemoglobin was measured on days 1, 17, and at the end of the study, and hemoglobin concentration was measured at the end of the study. A portion of the heparinized blood sample collected at the end of the study was centrifuged and the plasma collected and stored in a liquid nitrogen vapor shipper at −150°C, transported to the laboratory, and held at −80°C for biochemistry analysis. A portion of each whole blood sample collected on days 1, 17, and at the end of the study were placed in the vapor shipper and transferred to the laboratory, where they were stored at −20°C for subsequent Cu analysis.

The hemoglobin concentration in fresh whole blood was determined as cyanomethemoglobin using a commercially available Drabkin reagent and a hemoglobin reference standard (Pointe Scientific, Canton, MI). Duplicate American kestrel whole-blood samples (10 µl) were each vortex mixed in 2 ml Drabkin reagent and incubated at 4°C until analysis. All samples were analyzed on the same day, 6 days after blood was collected from controls and 5 days after blood was collected from dosed birds. A hemoglobin standard was prepared in distilled water and mixed with Drabkin reagent to achieve final concentrations of 2.88, 5.75, 11.50, 17.25, and 23.0 g/dl. Absorbance of samples and standards was determined at 540 nm with a Beckman DU640 spectrophotometer (Beckman Instruments, Fullerton, CA). A standard curve was generated by linear regression, and kestrel whole-blood hemoglobin concentration was interpolated from the curve. Methemoglobin concentration in fresh whole blood was determined as the change in absorbance after the addition of cyanide, which converts methemoglobin to cyanomethhemoglobin (Fairbanks and Klee 1987). Briefly, 100 µl whole blood was added to 3.9 ml distilled water and vortex mixed, then an equal volume of 0.15 M potassium phosphate buffer (pH 6.6) was added to the hemolysate. The absorbance difference between the blank cuvette (hemolysate only) and a sample cuvette (hemolysate plus potassium ferrocyanide) was determined at 630 nm. Potassium cyanide was then added to both cuvettes, and the absorbance was again determined at 630 nm. Methemoglobin was calculated as the ratio of these absorbance differences and expressed as percent total hemoglobin. Analysis of biochemistries, including glucose, aspartate aminotransferase, alkaline phosphatase, creatine kinase, cholesterol, triglycerides, total protein, albumin, calcium, uric acid, nonesterified

fatty acids, and beta-hydroxybutyrate, was performed by Marshfield Labs Veterinary Services (Marshfield, WI) in plasma collected at the end of the study.

Necropsy, Tissue Collection, and Histopathology

Control and dosed kestrels were euthanized on days 37 and 38, respectively, with an intravenous injection of a pentobarbital sodium and phenytoin sodium solution (Beuthanasia-D Special; Intervet Schering-Plough Animal Health, Boxmeer, The Netherlands), and necropsies were completed within 30 min. Pieces of liver, kidney with gonad and adrenal, spleen, stomach, duodenum with pancreas, junction of the small and large intestine and cecae, and bursa of Fabricius were fixed in 10% buffered formalin, sectioned at 4 μm, and stained with hematoxylin–eosin (HE) for histopathologic examination with light microscopy. Liver for Cu and metallothionein analysis, and kidney for Cu analysis, were frozen at −20°C.

Cu and Metallothionein Analysis

Liver and kidney samples (approximately 0.75 g) were dried overnight at 105°C and combined with 5 ml nitric acid. Whole-blood samples (approximately 0.3 g) were combined with 2 ml nitric acid. Samples were prepared for analysis by pressure-controlled microwave digestion (MDS 2000; CEM, Matthews, NC). Cu analysis was performed by flame atomic absorption spectroscopy (liver and kidney) or graphite furnace atomic absorption spectroscopy (blood) (Thermo Elemental M6 Solaar; Thermo, Franklin, MA) at a wavelength of 324.8 nm with Zeeman background correction. The lower limit of detection was 0.25 μg/g dry weight (dw) for liver and kidney and 0.02 μg/g wet weight (ww) for blood. Average recovery from spiked samples and standard reference materials (DOLT 3; National Research Council Canada) was 102%. Results are expressed as μg/g dw for liver and kidney and μg/g ww for blood. Moisture content of liver and kidney tissue was 68.5 and 75.6%, respectively. Metallothionein concentration of liver was measured according to the silver saturation assay of Scheuhammer and Cherian (1991), with the exception that samples were subjected to an additional hemolysate, heating, and centrifugation step. Metallothionein concentrations are given in μg/g ww.

Statistics

For each parameter that was measured multiple times during the course of the experiment (body mass, PCV, MetHb, and blood Cu), we performed repeated measures analysis of variance (ANOVA) on rank-transformed data and evaluated the time × treatment interaction term for significance to determine if responses of the sham-dosed controls and Cu-dosed kestrels differed through time. For the parameters measured only at the end of the experiment (plasma biochemistries, liver metallothionein, hemoglobin, and Cu concentration in liver and kidney), factorial analysis of variance on rank-transformed data was used to evaluate treatment and sex effects and sex × treatment interaction. Among Cu concentrations in liver tissue of control kestrels, there was one outlier (58.9 μg/g dw). We excluded this value from the study based on Dixon's test, where $Q_{\text{calculated}}$ (0.885) > Q_{table} (0.568) at the 99% confidence level (Rorabacher 1991). We evaluated the correlation between metallothionein and Cu in the liver with the Spearman rank correlation procedure. The Wilcoxon two-sample test was used to compare Cu concentrations in liver and kidney of kestrels that exhibited microscopic pancreatic changes with those that did not. SAS software (SAS, Cary, NC) was used for all statistical analysis, and $\alpha = 0.05$ was considered significant.

Results

Pellet Retention and Body Mass

During the 12-h monitoring period after Cu pellet administration on day 12, 11 of 16 dosed kestrels regurgitated stomach contents containing an average of 18 pellets. Eight of ten control birds regurgitated stomach contents during the same period. On each of the other days that Cu pellets were administered (i.e., days 0, 14, 16, 29, 31, 33, 35, and 37), all birds retained the pellets for at least 1 h, except one bird on day 0. The three dosed kestrels that were radiographed at 24 h after dosing had 1, 3, and 4 Cu pellets in the proventricular/ventricular region. Three of the 16 dosed kestrels that were radiographed at 48 h after dosing had retained 1–2 Cu pellets in the proventricular/ventricular area. No treatment effect was noted on body mass during the study, and no behavioral changes or clinical signs suggestive of toxicity were observed.

Hematology, Blood Cu, Plasma Biochemistries, Necropsy, and Histopathology

In treated birds and controls, mean hematocrit during the five sampling times varied from 40–42% and 39–42%, respectively, and mean hemoglobin concentrations at the end of the study were 10.3 and 9.9 g/dl, respectively; there was no treatment effect on either variable. Cu exposure did not affect Cu or methemoglobin concentrations in blood (Table 1), and there was no treatment effect on any of the plasma biochemistries (Table 2). At necropsy, both the Cu-treated and control birds were in good body condition with adequate fat reserves. Nine of the 16 treated kestrels,

Table 1 Mean (SE) Cu concentration (μg/g ww) and methemoglobin (as % of total hemoglobin) in the blood of Cu-dosed and control kestrels

Cu and methemoglobin	Day 1	Day 17	End of study[a]
Cu[b]			
Control	0.28 (0.02)	0.28 (0.02)	0.31 (0.02)
Cu-treated	0.31 (0.01)	0.34 (0.04)	0.30 (0.01)
Methemoglobin[c]			
Control	7.8 (0.55)	9.1 (1.26)	5.4 (0.89)
Cu-treated	7.2 (0.68)	8.4 (0.81)	6.1 (0.59)

[a] Day 37 for controls, day 38 for Cu-treated kestrels

[b] $n = 16$ for Cu-treated kestrels; $n = 10$ for control kestrels

[c] $n = 13$ on day 1, $n = 14$ on day 17, and $n = 16$ on Day 38 for Cu-treated kestrels; $n = 10$ each day for control kestrels

which had been administered Cu 24 h before they were euthanized, had Cu pellets (average 5 [range 1–11]) in the ventriculus. One bird had 1 Cu pellet in the proximal duodenum. The lining of the ventriculus ranged from light yellow to green in all treated and control birds except for 1 Cu-treated bird that had a light pink ventricular lining. No gross lesions indicative of toxicity were noted. Microscopic evaluation of tissues showed apoptosis (cell death without inflammation) and vacuolation of acinar cells (exocrine gland cells) in the pancreas (Fig. 1) of 4 of 16 kestrels that received Cu and 2 of 10 controls, 1 of which was the individual with the outlying hepatic Cu concentration of 58.9 μg/g dw. Mean Cu concentrations in liver (26.6 μg/g dw) and kidney (12.4 μg/g dw) of kestrels with pancreatic changes were not significantly different ($p = 0.810$ for liver; $p = 0.952$ for kidney) from those without pancreatic changes (liver = 22.4 μg/g dw; kidney = 12.2 μg/g dw). No histopathologic lesions were noted in other tissues examined.

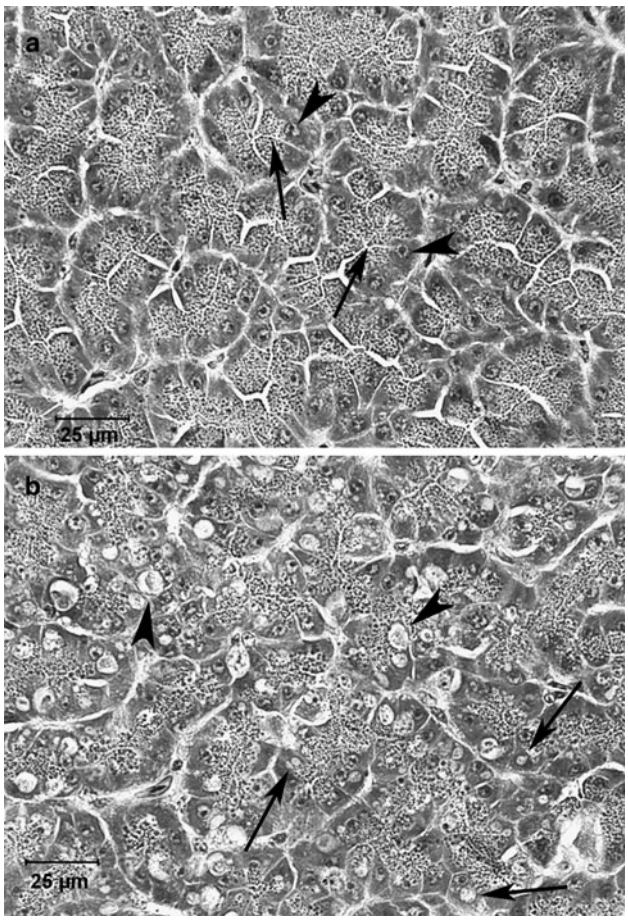

Fig. 1 Histologic sections of pancreas from 1-year-old female kestrels, trimmed 4-μm thick, and stained with HE. **a** Normal pancreas from kestrel not exposed to Cu pellets with liver Cu residue of 17.0 μg/g dw. Note the distinct nuclei of acinar cells (*arrowheads*) and abundant zymogen granules in the cytoplasm (*arrows*). **b** Abnormal pancreas from kestrel administered Cu pellets with liver Cu residue of 20.7 μg/g dw. Cells have vacuolated cytoplasm (*arrows*) and apoptosis; cell death without inflammation (*arrowheads*)

Table 2 Mean (SE) of biochemistries in plasma of Cu-treated and control kestrels

Biochemistry	Control ($n = 10$)	Cu-treated ($n = 16$)
Glucose (mg/dl)	398 (10)	368 (8.4)
Aspartate aminotransferase (IU/l)	62 (3.7)	69 (4.8)
Alkaline phosphatase (IU/l)	202 (13)	180 (8.9)
Creatine kinase (IU/l)	855 (42)	1098 (88)
Cholesterol (mg/dl)	151 (9.1)	166 (4.4)
Triglycerides (mg/dl)	81 (6.7)	89 (5.7)
Total protein (g/dl)	2.17 (0.1)	2.20 (0.1)
Albumin (g/dl)	0.85 (0.03)	0.84 (0.03)
Calcium (mg/dl)	8.03 (0.12)	7.96 (0.11)
Uric acid (mg/dl)	9.8 (1.5)	8.8 (1.0)
Nonesterified fatty acids (meq/l)	0.29 (0.04)	0.27 (0.02)
Beta-hydroxybutyrate (mg/dl)	13.2 (0.83)	14.8 (1.0)

Cu and Metallothionein Concentrations in Liver and Cu Concentrations in Kidney

A significant treatment effect, driven primarily by greater levels in male birds, and sex effect occurred in hepatic Cu concentrations of kestrels (Table 3). The mean concentration of Cu in livers of birds administered Cu was 26.9 μg/g dw in male and 20.7 μg/g dw in female birds, whereas control birds had hepatic concentrations <20 μg/g dw (Table 3). Metallothionein concentrations in liver differed by treatment, but not sex, and there was a significant sex × treatment interaction (Table 3). Thus, the metallothionein concentration in treated male birds was nearly four times the concentration in control male birds, but in female birds the concentrations in livers of treated and control birds were similar (Table 3). Metallothionein and Cu concentrations in liver tissue were significantly correlated (Spearman $r = 0.50$, $p = 0.010$). Cu concentrations in kidney tissue did not differ by treatment or sex (Table 3).

Discussion

Although Cu concentrations in livers of American kestrels differed by treatment, the magnitude of the difference was small, and there was no difference in Cu concentrations in the blood between kestrels administered Cu and control birds. Furthermore, renal Cu concentration, body mass, and blood biochemistries were not affected by Cu exposure, and we observed no behavioral changes or clinical signs of toxicity. Similarly, toxicity was not observed in a study with turkey vultures administered Cu pellets (Risebrough et al. 2001). The Cu introduced into the stomach of kestrels (5 mg/g body weight) was approximately twice that (2.3 g/kg) used in the vulture study, and the pellets were administered to kestrels nine times during the course of the 37-day experiment, whereas vultures received pellets weekly during the 8.5-week study (Risebrough et al. 2001). Thus, both the mass of Cu (per unit of body mass) and the frequency of pellet administration were greater in our study, but the kestrels probably retained Cu for a shorter time than turkey vultures. During a 12-h monitoring period, 69% of kestrels regurgitated pellets and when radiographed 48 h after Cu administration only 19% of kestrels retained one or more pellets. Cu pellet retention in the vulture experiment was described as highly variable, but in a preliminary trial with six individuals, some pellets were still in the intestines of one or more vultures after 3 weeks (Risebrough et al. 2001).

Reports in mallards and other avian species also indicate that birds are quite tolerant to metallic Cu. In an early experimental study, mallards administered Cu pellets ≤13 g exhibited transient weight loss but no mortality (Bellrose 1965). Another study reported that 1 of 24 mallards receiving 8 no. 6 Cu pellets died, but the death could not be attributed to Cu toxicity, and no histopathological lesions were found in tissues of 4 ducks receiving Cu pellets (Irby et al. 1967; Locke et al. 1967). In a 24-day test with mallards that received 2–8 Cu-plated steel shot, none of the shot were voided by the ducks; no changes in body mass or hematocrit were noted; and histologic examination of tissues showed no lesions of Cu toxicosis (United States Fish and Wildlife Service, unpublished data). In a recent study, Pekin ducks were administered 6 Cu pellets, each approximately 3 mm in diameter and weighing 148 mg, and the birds were killed 4 weeks later (Krone et al. 2009). No ducks died; Cu did not affect body weight; and no treatment-related lesions were noted in organs (Krone et al. 2009). Cu concentrations in liver and kidney tissue of Pekin ducks were approximately 100 and 10 μg/g ww, respectively (Krone et al. 2009). Calculated on a ww basis, Cu concentrations in liver and kidney of kestrels in our study were much lower at 7.5 and 2.9 μg/g, respectively. Thomas and McGill (2008) studied the release of Cu, tin, and iron from tungsten–bronze pellets (44.4% Cu by mass) in vitro in a simulated avian gizzard at pH 2.0. Cu was released at the rate of 43.17 mg/d from 8 tungsten–bronze pellets, and the investigators concluded that although this level exceeded daily Cu requirements of domestic birds, it was lower than levels known to cause toxicity. Pigeons (*Columba livia*) that were dosed daily for 14 days with soil from a small-arms firing range containing lead and Cu had increased lead, but not Cu, concentrations in tissues (Bannon et al. 2011). However, the maximum daily dosage of Cu used in pigeons (approximately 0.002 mg/g body mass) was much less than the amount of Cu administered to kestrels (5 mg/g) each of 9 times during our 38-day study.

Table 3 Mean (SE) Cu concentration in liver and kidney (μg/g dw) and metallothionein concentration (μg/g ww) in liver of Cu-treated and control kestrels

Cu and metallothionein	Control ($n = 10$)	Cu-treated ($n = 16$)
Liver Cu[a]		
Male	19.2 (0.8)	26.9 (1.7)
Female	18.0[b] (0.4)	20.7 (1.4)
Liver metallothionein[c]		
Male	11.6 (1.6)	42.1 (8.4)
Female	30.6 (10.4)	31.4 (5.5)
Kidney Cu	12.6 (0.4)	12.0 (0.2)

[a] Significant treatment effect ($p \leq 0.001$) and sex effect ($p = 0.007$) in factorial ANOVA

[b] One outlier, 58.9 μg/g dw, not included in this mean

[c] Significant treatment effect ($p = 0.008$) and sex × treatment interaction ($p = 0.015$) in factorial ANOVA

Cu is an essential trace element, and concentrations of Cu in tissues of wild birds vary considerably within and among species and species groups; differences are not necessarily related to dietary intake but may be related to differences in excretion (Davis and Mertz 1987; Eisler 2000). A comparison of Cu concentrations in tissues of wild California condors with concentrations in other Accipitriformes and Falconiformes suggests that condors accumulate comparatively high levels of Cu. The mean Cu concentration in livers of eight nestling California condors and 30 older, flighted birds was 255 and 70 mg/kg ww, respectively (B. Rideout, personal communication). Another study reported a mean hepatic Cu concentration in four flighted California condors of 41 μg/g ww (Wiemeyer et al. 1986). However, the mean liver Cu concentration in turkey vultures collected by shooting within California condor range was 7.1 μg/g ww, and the mean in turkey vultures dosed with Cu pellets was 20.5 μg/g ww (Wiemeyer et al. 1986; Risebrough et al. 2001). In other studies, the mean hepatic Cu concentration was approximately 20 μg/g dw in peregrine falcons (*F. peregrinus*) from Sweden, 4.2 μg/g ww (approximately 16 μg/g dw based on stated moisture content of 74.4%) in white-tailed eagles in Poland, and 17 μg/g and 43 μg/g dw in northern goshawks (*Accipiter gentiles*) and Japanese sparrowhawks (*A. gularis*), respectively, from Japan (Ek et al. 2004; Kalisińska et al. 2006; Horai et al. 2007). In Italy, the mean Cu concentration in livers of Eurasian kestrels (*F. tinnunculus*) was approximately 25 μg/g dw and ranged from approximately 15–22 μg/g dw in four species of hawks (Zaccaroni et al. 2008). Thus, reported Cu concentrations in livers of several wild hawks and falcons are similar to the levels we found in American kestrels dosed with Cu pellets.

In contrast to the aforementioned studies in raptors, some species of waterfowl have been reported to have high liver Cu concentrations, with evidence that male birds accumulate more than female birds. For example, mean concentrations of Cu in livers of common eiders (*Somateria mollissima*) collected in unpolluted areas of western Alaska were 607 μg/g dw in male birds compared with 80 μg/g dw in female birds, and common eider male birds in the Baltic Sea had mean hepatic Cu concentrations as high as 1381 μg/g dw, whereas the mean concentration in female birds was 43 μg/g dw (Franson et al. 2000; Stout *et al*. 2002). Another species of sea duck collected in Alaska, the Barrow's goldeneye (*Bucephala islandica*), had a mean of approximately 42 μg/g dw Cu in liver tissue, but there was no difference in concentrations between male and female birds (Franson et al. 1995). Perhaps the highest levels of Cu reported in birds have been in mute swans. Kobayashi et al. (1992) diagnosed Cu poisoning in several mute swans with a mean Cu concentration in liver tissue of 2150 mg/kg dw compared with 200 mg/kg dw in three swans that died at other locations. Molnar (1983) reported a mean hepatic Cu concentration of 3957 μg/g dw in three mute swans found dead or dying in New York, suggesting that the source of Cu was antifouling paint. Two captive mute swans used as controls had 64 μg/g and 121 μg/g dw Cu in their livers (Molnar 1983). Mute swans sampled from an area in Denmark reported to be polluted with Cu had approximately 1096 mg/kg ww of Cu in their livers, more than twice as much as in swans from other areas of Denmark (Clausen and Wolstrup 1978).

Captive Canada geese diagnosed with Cu poisoning died within several hours of Cu sulfate application to the pond on which they were kept, exhibited necrosis of the proventricular and gizzard mucosa, and had hepatic Cu concentrations of ≤97 μg/g ww (Henderson and Winterfield 1975). Necrotic lesions similar to those seen in the dead Canada geese have also been reported in commercial poultry poisoned by Cu sulfate (Gilbert et al. 1996). When Cu salts are ingested in high concentrations during a short period of time, they function as protein coagulants, causing severe irritation of the alimentary mucosa, intravascular hemolysis, shock, and death (Blood and Henderson 1974). Frequent ingestion of small amounts of Cu salts typically causes no immediate toxic effects but results in an accumulation of Cu in the liver; if maximum hepatic concentrations are reached, Cu is released into the blood, and the animal dies from intravascular hemolysis (Blood and Henderson 1974). Our study as well as others with birds have not shown acute toxic effects of ingested metallic Cu, and the fact that hepatic Cu concentrations increased little after repeated Cu exposure suggests that the physiological effects of metallic Cu are considerably different than those of Cu salts.

Metallothioneins are metal-binding proteins induced by various metals, including Cu (Cousins 1985). We hypothesized that metallothionein concentrations would be greater in American kestrels that received Cu than in controls, which was the case in male but not female birds. This may have been due in part to the fact that the magnitude of the difference in mean hepatic Cu concentrations between treated and control birds was greater in male than female birds, resulting in a correspondingly greater difference in metallothionein concentrations in male birds. A significant correlation between metallothionein and Cu concentrations in the liver, as we noted in kestrels, has previously been reported in other species of birds (Kojadinovic et al. 2007).

Apoptosis and acinar cell vacuolation of the pancreas are nonspecific histologic alterations that have been reported in experimental zinc exposure in Pekin ducklings and in cases of zinc toxicosis in captive and free-ranging wild birds (Kazacos and Van Vleet 1989; Droual et al. 1991; Sileo et al. 2004). The fact that these changes occurred in 25% of kestrels that received Cu suggests the

possibility that they may be associated with Cu exposure. However, pancreatic changes also occurred in 20% of controls, and Cu concentrations in liver and kidney did not differ between birds with normal pancreas and those with apoptosis and vacuolation. When chickens were fed ≤4000 mg Cu/kg of diet, the pancreas was unaffected, although the birds exhibited anorexia and gizzard lesions (Wight et al. 1986). Thus, the significance of the findings in kestrels is unclear. However, the absence of effects of Cu exposure on body mass, hematocrit, hemoglobin, methemoglobin, and plasma biochemistries indicates that under our study design, metallic Cu was not toxic to American kestrels. Retention time of pellets in the stomach of kestrels was of relatively short duration, and birds expected to regurgitate Cu fragments with a frequency similar to kestrels are not likely to be adversely affected by Cu ingestion. Although the findings in kestrels do not rule out the potential for Cu toxicity in species that might retain Cu fragments for a longer time, our results agree with previous experimental studies, adding to the evidence to date suggesting that Cu ammunition poses little threat of toxicity to birds. However, because the hepatic Cu concentrations in American kestrels administered Cu pellets were considerably less than concentrations found in wild California condors, kestrels may not be a good surrogate for condors, specifically, as Risebrough et al. (2001) suggested for turkey vultures.

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