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Bioaccumulation of Cd by a European lacertid lizard after chronic exposure to Cd-contaminated food

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Abstract

Apart from analyses for elemental contaminants in field collected specimens, very little is known about the assimilation, accumulation and toxic effects of inorganic contaminants in reptiles. This study examined the chronic accumulation of Cd in a European lacertid lizard (*Podarcis carbonelli*) following dietary provision of an environmentally realistic concentration of Cd for 21 weeks. Lizards were provided with Cd that had either been biologically incorporated into crickets, or as $Cd(NO_3)_2$ added superficially to crickets just prior to feeding. Among both treatment groups Cd accumulated in tissues in the following order of concentration: gut > liver > kidney > carcass. The majority of the Cd was retained within the gut, and transfer to internal organs was low. Morphological indices, brain and plasma cholinesterase activities, gut and liver metallothionein content, and standard metabolic rate were measured as biomarkers of exposure and effect; however, no differences between control lizards and Cd-treated lizards were observed.

Keywords: Lacertidae; Wall lizard; Metallothionein; Cd-*S*-conjugate; Cholinesterase; Trophic transfer; Metal bioavailability; Cadmium toxicity; Biomarker; Standard metabolic rate

1. Introduction

In Europe, cadmium (Cd) is a priority contaminant (Council Directive 76/464/EEC) as a consequence of widespread contamination of agricultural lands. Although Cd occurs naturally in soils and waters at low concentrations, deposition within the biosphere has increased dramatically over the last century. The main anthropogenic sources of Cd are atmospheric emissions originating from non-ferrous-metal smelting and burning of fossil-fuels (Beak, 2002), and the amendment of agricultural soils with cadmium-contaminated bio-solids, rock-phosphate fertilizers and industrial bi-products (McLaughlin et al., 1996; Westfall et al., 2005). Concern arises because unlike many other toxic metals, Cd has a propensity to accumulate in plants, and thereby enter the food chain. Although there is a prevailing view that Cd is not biomagnified along food chains, biomagnification of Cd has recently been demonstrated in an aquatic system (Croteau et al., 2005). Within terrestrial food chains, as in aquatic systems, the occurrence of biomagnification, and the extent to which it occurs depends on who (or what) is being eaten, and by whom (Dallinger and Kautzky, 1985; Hopkin and Martin, 1985; Merrington et al., 1997; Maryański et al., 2002; Scheifler et al., 2002; Hendrickx et al., 2003; Scheifler et al., 2003). These differences occur as a consequence of variation in trophic interactions among organisms and the biological mechanisms available to different species for handling different elements (e.g. detoxification and excretion systems).

Within some terrestrial food chains, snakes and lizards are important middle to top order predators and numerous authors have advocated an increased research effort to investigate the toxic effects of pollutants in reptiles

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(e.g. Hall and Henry, 1992; Hopkins, 2000; Pauli and Money, 2000; Lambert, 2004). However, ecotoxicologists have been slow to meet this challenge, in stark contrast to the recent prominence of amphibian toxicological studies (Mann, 2005), and despite the fact that several species of reptiles, including lacertids, present themselves as valuable models for ecotoxicological studies (Lambert, 2004; Mann et al., 2006; Selcer, 2006).

The majority of existing reptile studies are restricted to examinations of metal residues in whole organisms or in the organs where metals are known to accumulate (i.e. liver, kidney) in animals sampled from polluted sites (Hopkins, 2006). Although a substantial number of these studies describe accumulation of Cd (among other elements) by squamates (for reviews see Campbell and Campbell, 2000; Linder and Grillitsch, 2000; Campbell and Campbell, 2001; and more recently, Burger et al., 2004; Campbell et al., 2005), there is very little experimental work to evaluate the kinetics and subsequent effects of metal accumulation in reptiles in a contaminated environment. A recent exception is a study that examined the movement of trace elements (including Cd) derived from coal-ash between a reptile (snake) and its prey (fish) (Hopkins et al., 2002; Rania Ganser et al., 2003). In that study, snakes accumulated several elements in a dose dependent manner following consumption of a contaminated diet over a 2-year period. Manipulative studies like these (see also Hopkins et al., 2005) provide an understanding of the mechanisms and kinetics of metal uptake, tissue distribution and excretion, and are necessary to establish the path of metal transfer through the food-webs to which reptiles belong.

In this study (and a related study, Mann et al., 2006) we examine the uptake of Cd by a lacertid lizard (*Podarcis carbonelli*; Pérez-Mellado, 1981) from a Cd-contaminated diet. Tissue distribution of Cd as well as physiological (standard metabolic rates (SMR)) and biochemical (metallothionein (MT) levels and cholinesterase (ChE) activities) biomarkers of metal exposure were examined after long-term dietary exposure.

Tissue–Cd concentrations seem to be dependent on the metal exposure time. In reptiles, acute exposure to Cd results in a preferential accumulation in the liver (with respect to kidney); however, long-term exposure to this metal results in a shift in Cd distribution to other tissues, in particular, the kidney (Linder and Grillitsch, 2000).

A measure of SMR provides an estimate of basal energy demand. An increase in SMR in animals exposed to a pollutant may indicate metabolic trade-offs at the expense of other physiological demands such as reproduction or growth (Rowe et al., 2001). Increases in SMR have been demonstrated in snakes and amphibians exposed to elemental contaminants associated with coal-ash (Hopkins et al., 1998; Hopkins et al., 1999).

Cadmium has a high affinity for thiol groups (Nieboer and Richardson, 1980) and absorption of Cd is likely to result in the induction of thiol pathways and alterations in the levels of sulfur rich proteins (i.e. MT). Metallothioneins are involved in the transport, storage and homeostasis of essential metals such as Zn or Cu and in the detoxification of non-essential heavy metals such as Cd, Pb or Hg, and have been identified as potential biomarkers of metal exposure. The induction of these low molecular weight and cysteine-rich proteins has frequently been measured in the gut, liver, hepatopancreas or kidney of a broad range of metal-exposed organisms (Laflamme et al., 2000; Amiard et al., 2006).

Although cholinesterases (ChE) are biomarkers normally associated with pesticide (i.e., organophosphorus and carbamate) exposure (Sánchez-Hernández, 2001; Venturino and Pechen de D'Angelo, 2005), the use of Acetylcholinesterase (AChE) as a specific biomarker for pesticides has been questioned (Guilhermino et al., 1998). Increases in brain AChE activity have been described in fish exposed to waterborne Cd (Gill et al., 1991) and decreases in brain AChE activity have been described in crayfish exposed to either waterborne or dietary Cd (Devi and Fingerman, 1995; Devi et al., 1996) and fish exposed to acid mine drainage-affected lake (Castro et al., 2004).

2. Materials and methods

2.1. Lizards

Adult individuals of the species *P. carbonelli* were collected from the coastal dune systems of São Jacinto and Torreira on the north-central coast of Portugal between the months of February and August 2004. The lizards were collected under a permit issued by the Instituto da Conservação da Natureza. The animals were either caught by hand or by use of pit-traps. Hand capture tended to bias the capture toward the larger males. The animals were transported to, and maintained in a climate control room within the Department of Biology at the University of Aveiro. They were held under conditions that simulated the prevailing season, and that subsequently entrained them to autumn and winter conditions before feeding trials were initiated under simulated summer conditions. All animals were housed in individual glass aquaria ($40 \times 20 \times 25$ cm). Each aquarium was fitted with a nylon mesh lid, a terracotta building block with numerous cavities, a 50 cm length of polyethylene foam tubing, and a 25 W incandescent globe connected to a variostat (dimmer switch) and a 7-day timer-switch.

The climatic conditions under which the animals were maintained varied depending on the season. The climate room was fitted with fluorescent lighting, high pressure sodium lamps (400 W, 7000–12 000 Lx), and natural sunlight. During the summer months, the animals were provided with 12 h of fluorescent lighting and within this interval, 10 h of sodium lighting. This was progressively reduced during the autumn and limited to 10 and 8 h in early winter. The ambient room temperature was maintained at 22 ± 1 °C during summer and progressively reduced during autumn to 10 ± 1 °C during the winter.

This temperature range reflected the range of temperatures recorded within lizard burrows at the collection sites in summer and winter. Heating during the day was provided by the incandescent globes and the radiant energy from basking lamps was varied to limit body temperature elevation by behavioural thermoregulation to levels expected for each season. During the summer months, 8 h of heating was provided to a level of ~35 °C at basking locations in one corner of each tank. This was progressively reduced during autumn down to a level of 6 h of heating to a level of ~25 °C at basking locations during early winter and early spring. While the heating lamps were on, the temperature in the opposite corner of each tank remained at ambient room temperature. During January the sodium lighting was turned off completely and the heating lights reduced to ~20 °C for 2 h. All climate parameters were increased during February.

No ultraviolet light was provided, although it is known that ultraviolet light is essential for the long-term well being of lacertid lizards primarily because of its role in vitamin D production which in turn is required for calcium metabolism (Mantel, 1994). Therefore, vitamin D3 supplements (Dagravit®8; Viatris Farmacêutica, Lisbon, Portugal and Vigantoletten®1000; Merck, Darmstadt, Germany) were added to deionised water (16000 IU l^{-1}) and supplied in shallow petri-dishes. A vitamin A supplement (Dagravit®8) was also provided in the water (18000 IU l^{-1}) (http://www.podarcis.nl/info/halsbandhaguk.php3). Water was renewed every 2–3 days.

The animals were maintained for a minimum of 2 months before the commencement of an over-wintering period and 4 months prior to the feeding study. During this acclimation period the animals were provided with mealworms and crickets. The crickets were wood crickets (*Nemobius sylvestris*) collected from the São Jacinto foredunes. Mealworms (*Tenebrio molitor*) were raised in-house on wheat-germ. Prior to feeding the nutritional quality of the mealworms was improved by feeding them (gut-loading) for 24 h with a vegetable (e.g. carrot, potato).

2.2. Experimental treatments

Directly following the over-wintering period, the heat output of heating lamps was increased substantially (~30 °C) to encourage feeding. Dietary treatment commenced one week later. Lighting and heating were raised over a 2-week period to pre-winter levels. For the remainder of the feeding study, the ambient room temperature was maintained at 17.5 ± 1 °C.

Cadmium was provided in two different forms to each of two treatment groups. One group of lizards was provided with Cd that had been biologically incorporated within the food item (cricket), while the other group was given Cd as a simple salt in conjunction with a food item. Data for both groups are presented in this paper; however, the rationale for the two treatment groups and the data pertaining to Cd assimilation efficiency with reference to Cd speciation are presented in Mann et al. (2006). Also, the Cd contaminant included a radiotracer (^{109}Cd) which provided an estimation of Cd-burden without destruction of the tissue, and therefore allowed for the preservation of tissues for biomarker analysis.

Three dietary treatment groups were established:

1. Biologically contaminated crickets (BCC).
2. Superficially contaminated crickets (SCC).
3. Non-contaminated (control) crickets (CON).

Each of the three treatment groups included 15 lizards with similar numbers of males and females (eight males and seven females). Males and females were randomly allocated to each of the three treatment groups by random number allocation.

Field collected crickets were used for all treatment groups. When adult crickets (~70 mg) became difficult to find in sufficient numbers (i.e. in April), they were supplemented with juvenile crickets (~15 mg). All crickets were maintained at 25 ± 1 °C.

2.3. Biologically contaminated crickets

Crickets were provided for 4 days with lettuce contaminated with Cd (0.4 mg g^{-1} [3.6 μmol g^{-1}]) followed by uncontaminated lettuce for a further 24 h to eliminate unassimilated Cd from the gut. The Cd solution used to contaminate the lettuce was 360 μM $Cd(NO_3)_2$ spiked with 1.2 μCi ml^{-1} ^{109}Cd (PerkinElmer, Boston, MA). The concentration of the nominally 360 μM Cd contamination-solution was verified by inductively coupled plasma mass spectrometry (ICP-MS) in a X series ICP-MS with a Poly-Con nebuliser. The level of contamination in individual crickets was determined on the fifth day by radiospectrometry. Whole live crickets were counted in a 1470 Wallac WIZARD automated gamma counter (PerkinElmer, Wellesley, MA, USA) for 2 min (i.e. long enough to maintain counting errors below 1%). Cd concentrations determined by radiospectrometry have previously been demonstrated to be comparable to those obtained by ICP (Mann et al., 2005). Specific activity was assessed by comparing gamma counts and ICP-MS analysis of the exposure solution. The crickets were fed to the lizards the following morning. Uneaten cricket parts (i.e. the hind legs) were returned to the counting tube and counted along with any faecal material deposited within the tubes to measure the amount of uneaten Cd.

2.4. Superficially contaminated crickets

Immediately prior to feeding, the crickets were contaminated by applying 1 μl of a Cd solution between the wings. The contamination solution was 8.9 mM $Cd(NO_3)_2$ spiked with 26 μCi ml^{-1} ^{109}Cd. Specific activity was assessed by comparing gamma counts and ICP-MS analysis of the exposure solution. These crickets were not

counted prior to feeding because there was a risk of loss of Cd from the crickets if they were not eaten immediately.

2.5. Lizard feeding

In the BCC group, lizards were provided with one or more live, contaminated crickets selected according to their Cd load (previously determined by radiospectrometry) and resulted in consumption of (mean ± standard deviation(SD)) 0.49 ± 0.2 μg Cd per week. Weekly Cd consumption in the BCC group varied slightly as a consequence of cricket Cd burden and availability of adequate numbers of crickets (Fig. 1). In the SCC group, to ensure that the amount of Cd consumed could be reliably estimated, the crickets were hand-fed directly to the lizard and resulted in consumption of (mean ± SD) 0.91 ± 0.09 μg Cd per week. The variation in this consumption was due to the reproducibility inherent in the pipette. The CON group was provided with uncontaminated crickets. All lizards were provided with extra uncontaminated supplemental food as crickets or mealworms according to their weight, twice per week. This usually amounted to 1–3 crickets and 1–2 mealworms per week. This feeding regime was maintained for 20 weeks (and a further week after the final meal). The field collected crickets were analysed for various elements by ICP-MS. Individual adult crickets or pooled samples of 4–6 juveniles (~70 mg) were digested in 1 ml of 2 M HNO_3 (GR for analysis, Merck, Darmstadt, Germany) for seven days at 70 °C, diluted to a volume of 10 ml, and centrifuged at 3.2*g* to obtain supernatants for analysis. The crickets contained the following elements on a wet-weight basis ($mean_{n=10}$ ± SE μg/g): Ca, 584 ± 41; Fe, 170 ± 11; Zn, 47 ± 3; Cu, 11 ± 0.5; Pb, 0.5 ± 0.3; Cd, 0.04 ± 0.01. No Cd was detected in juvenile crickets (detection limit ~0.015 μg/g). The level of Cd contamination achieved by the procedures described above are comparable to the range reported for invertebrates sampled from sites contaminated by mining activities (i.e. up to ~40 μg/g dry wt in isopods, gastropods and coleopterans, Avery et al., 1983; Milton et al., 2004).

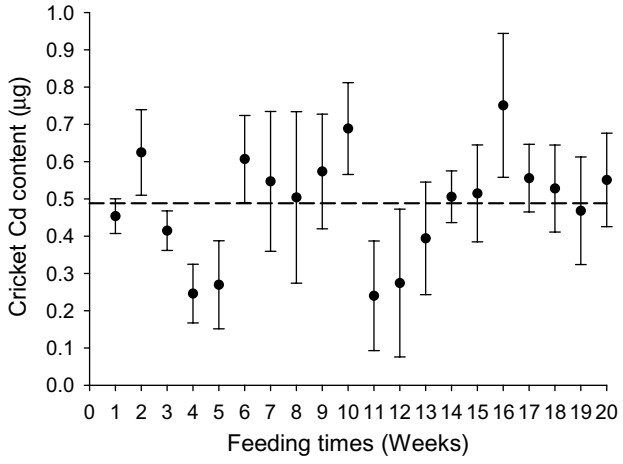

Fig. 1. Amount of Cd provided to lizards of the BCC treatment group in each week of the study. Error bars represent SD ($n = 15$). Dashed line indicates mean weekly Cd consumption.

2.6. Standard metabolic rate (SMR)

Five and six days after their final meal, SMR was measured in two sets of post-absorptive lizards. The first set comprised 12 females representing the three treatment groups (i.e. 4 × controls, 4 × BCC, 4 × SCC), followed the next day by 12 males, similarly representing the three treatment groups. The lizards were placed in covered perspex tubes (~230 cm^3) connected to 12 channels of a respirometer (Analytical Developments Co., Ltd, ADC-225-MK3, Hoddesdon, England) housed in a constant temperature room (20 ± 1 °C). An open-flow system was employed with a flow rate of 200 ml min^{-1}. Flow rate and CO_2 production was recorded at 30-min intervals, and CO_2 production was calculated for each 30-min interval. Twenty sequential measurements of CO_2 production representing 10 h in a resting state (at night when the animals were least active) were averaged and used to calculate the molar quantity of oxygen usage by each individual lizard (assuming a respiration quotient of 1:1). The data were plotted as mean Log_{10}(O_2 consumption) against Log_{10}(animal mass) (Dorcas et al., 2004). Regression analyses were used to fit lines to combined (males and females) data.

To examine the effect of dietary treatment and body mass on SMR, we used ANCOVA with Log_{10}(animal mass) as the covariate in a univariate linear model (SPSS 12). To linearize their relationship, body mass and O_2 consumption data were log_{10}-transformed prior to ANCOVA and regression analysis. Statistical significance was assessed at $\alpha = 0.05$.

2.7. Animal sacrifice and analysis for Cd

After 21 weeks, and 8 days after their final meal, all animals were weighed. They were then sacrificed by a blow to the head followed by decapitation. A 10 μl blood-plasma sample was centrifuging at 1000*g* for 10 min at 4 °C to isolate plasma. The brain, gut, liver, and kidneys were removed, weighed, and along with the plasma sample, frozen in liquid nitrogen for subsequent cadmium and biomarker analyses. The remaining carcass was also weighed and then dried at 60 °C prior to Cd analysis by radiospectrometry. The dried carcasses of control animals were ground in a mortar and pestle and 100 mg analysed for Cd by ICP-MS (as above) to provide an estimate of background Cd concentration. With the exception of the blood-plasma samples, the frozen samples of tissue were analysed for Cd by radiospectrometry immediately prior to biomarker analyses. These Cd analyses were performed using a Genesis Gamma-1 bench-top gamma counter (Laboratory Technologies, IL, USA). Animal mass and tissue mass measurements were used to generate estimates of liver somatic index ($LSI = mass_{liver}/mass_{body}$) and kidney somatic index ($KSI = mass_{kid}/mass_{body}$).

2.8. Biomarker assays

Cholinesterases. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were determined in the brain and plasma, respectively, according to the Ellman method (Ellman et al., 1961). Thawed plasma samples were used without further processing, whereas brain samples were homogenised in 0.1% Triton X-100 in 25 mM Tris–HCl (pH 8.0). Brain homogenates were centrifuged at 10000*g* for 10 min at 4 °C, and AChE activity was assayed immediately. All ChE assays were conducted at ambient temperature (~25 °C). Plasma or brain homogenates were pre-incubated for 2 min with DTNB (final concentration (FC) of 3×10^{-4} M) in 25 mM Tris–HCl, 1 mM $CaCl_2$ (pH 7.6) before the substrate (acetylthiocholine for AChE and butyrylthiocholine for BChE) was added (FC of 2×10^{-3} M). The variations in optical density were recorded at 412 nm for 2 min. Non-enzymatic hydrolysis of the substrates was periodically checked in absence of the sample in the reaction medium. Plasma BChE activity was expressed as micromoles substrate hydrolysed per minute per millilitre, and brain AChE activity was expressed as micromoles per minute per milligram of total protein, using a molar absorption coefficient of 14.15 times 10^3 M^{-1} cm^{-1} (Eyer et al., 2003). Protein concentrations were determined by the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as a standard.

Metallothioneins (MT). The concentration of MT was determined according to the spectrophotometric method by Viarengo et al. (1997). Whole gut or liver was homogenised (1:3 w/v) in a buffer (pH 8.6) containing 20 mM Tris–HCl, 0.5 M sucrose, 0.5 mM phenylmethylsulphonylfluoride, 6 μM leupeptin and 0.1% mercaptoethanol. The homogenate was then centrifuged for 20 min at 30000*g* and supernatants were frozen in liquid nitrogen and then stored at −80 °C. Aliquots of 1 ml supernatant were vortexed with 1.05 ml of cold ethanol and 80 μl of chloroform, and then centrifuged at 6000*g* for 10 min. The resultant supernatant is combined with ribonucleic acid and 37% HCl, and subsequently with cold ethanol (1:3 w/v). The samples were then kept at −20 °C for 1 h and centrifuged at 6000*g* for 10 min. The pellet containing the MT was resuspended in a solution containing 0.25 M NaCl, and 1 N HCl with 4 mM EDTA. Aliquots of 100 μl of the sample were added to a solution of 2 M NaCl and 0.43 mM DTNB buffered to pH 8.0 with 0.2 M sodium phosphate. Samples were finally centrifuged at 3000*g* for 5 min and read at 412 nm. The MT concentration was calculated using GSH as external standard and expressed as micrograms of MT per gram of fresh tissue (Viarengo et al., 1997).

3. Results

3.1. Animal condition

One lizard from the SCC treatment group died during the course of the experiment. There was no indication that its death was related to the dietary treatment. The remainder of the animals appeared to be in good physical condition at the end of the experiment. Most of the animals lost weight during the trial (Fig. 2a). This was an expected artefact of the need to ensure that all animals were hungry

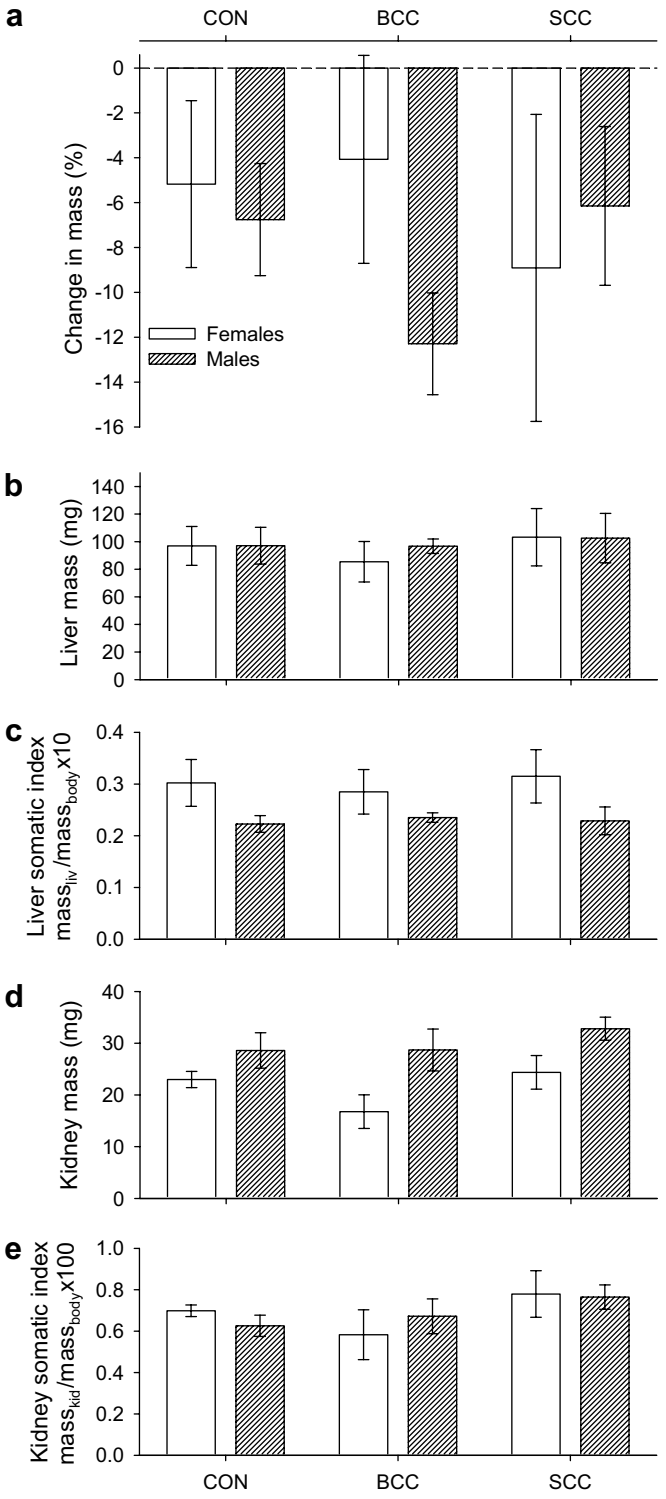

Fig. 2. Indices of whole-body mass and tissue mass. (a) percentage weight loss; (b) liver mass; (c) liver somatic index; (d) kidney mass; (e) kidney somatic index. Error bars represent SE ($n = 13$–15).

enough to eat their Cd-contaminated food ration. There were no statistical differences (*t*-tests, $P > 0.05$) in tissue mass between controls and individual treatment groups (Fig. 2b and d), and similarly, no statistical differences (*t*-tests, $P > 0.05$) in LSI and KSI (Fig. 2c and e).

3.2. Cd burden

The carcasses of control lizards contained (mean ± standard error (SE)) 6.0 ± 1.0 ng Cd g^{-1}. Lizards in both treatment groups accumulated Cd. Lizards provided with biologically incorporated Cd (BCC) consumed a total (mean ± SE) of 8.85 ± 0.13 μg Cd and accumulated 0.37 ± 0.05 μg Cd (Fig. 3a and c). Those lizards provided with superficially amended Cd (SCC) consumed 16.90 ± 0.25 μg Cd and accumulated a total of 1.03 ± 0.13 μg Cd (Fig. 3b and d) (For a fuller discussion of assimilation efficiency, see Mann et al., 2006). The Cd was preferentially accumulated in the gut up to a concentration of (mean ± SE) 2.85 ± 0.56 (BCC) and 6.04 ± 0.70 (SCC) μg g^{-1}, although it was also detected in liver, kidney, and other undifferentiated compartments within the carcass (Fig. 3). No Cd was detected in the brain or moulted skins, and only trace amounts were detected in the testes, plasma and the developing ova.

3.3. Biomarkers

No significant variations were observed in the biomarker responses between the treated and control groups after 21-week Cd exposure (Wilks $\lambda = 0.82$, $F_{10,64} = 0.63$, $P = 0.78$). AChE activities were (mean ± SE) 39.7 ± 3.3, 41.9 ± 3.1 and 35.9 ± 2.6 nmol min^{-1} mg^{-1} protein for CON, BCC and SCC groups, respectively (Fig. 4). BChE activities were (mean ± SE) 6.25 ± 0.65, 6.18 ± 0.46, 6.24 ± 0.39 μmol min^{-1} ml^{-1} plasma for CON, BCC and SCC treatment groups, respectively (Fig. 4).

A slight increase in MT concentrations was observed in the guts of treated groups (BCC = 163.02 μg g^{-1}, and SCC = 153.2 μg g^{-1} wet wt) with respect to the controls

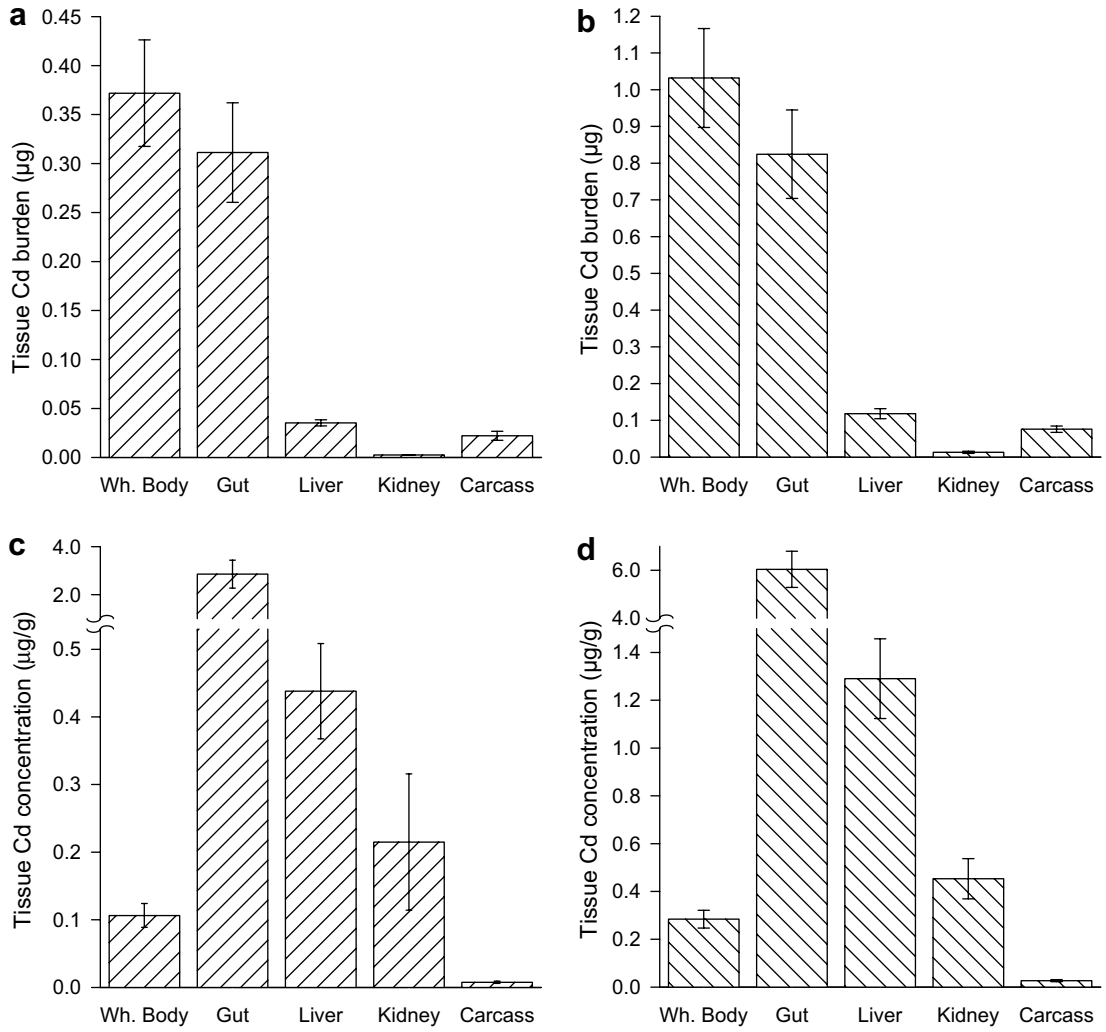

Fig. 3. Tissue-Cd burden following 21 weeks of accumulation for BCC ((a) and (c)) and SCC ((b) and (d)) treatment groups, expressed as total quantity of Cd in whole tissues ((a) and (c)) and concentration of Cd per unit mass ((c) and (d)). Note that the *y*-axis in (c) and (d) is split into two ranges. Error bars represent SE ($n_{gut} = 12$–14, $n_{liv} = 14$, $n_{kid} = 11$, $n_{carc} = 14$–15).

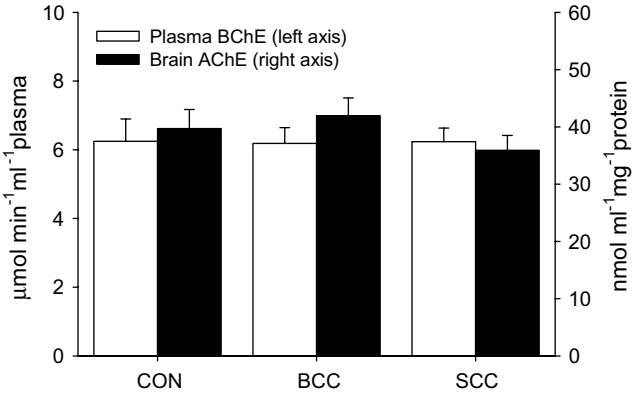

Fig. 4. BChE activity in plasma and AChE activity in brain. Error bars represent SE ($n = 13$–15).

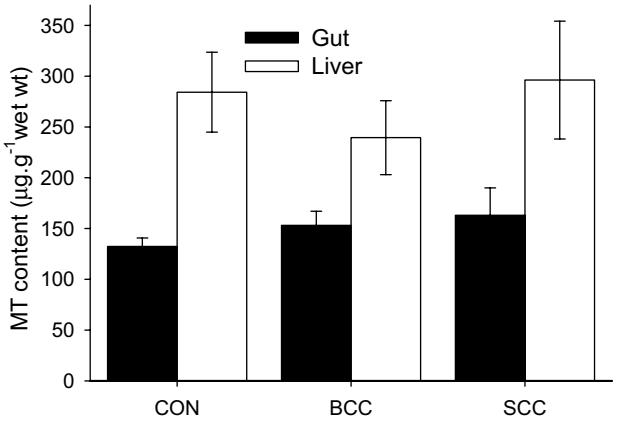

Fig. 5. MT in the gut and liver. Error bars represent SE ($n = 14$).

(132.4 µg g^{-1} wet wt), although it was not statistically significant (Fig. 5). MT concentrations were twofold higher in the liver than in the gut ($P < 0.001$, Mann–Whitney U test). Mean (±SE) MT concentrations in the liver were 284.3 ± 39.3, 239.5 ± 36.4 and 296.2 ± 58.0 µg g^{-1} wet wt for CON, BCC and SCC treatment groups, respectively (Fig. 5).

3.4. SMR

Oxygen usage ranged between 0.67 and 1.33 µmol h^{-1} g^{-1}. Oxygen usage by lizards increased with size (Fig. 6). There was a significant effect of mass on O_2 consumption, but no significant differences between treatment groups (ANCOVA; $\log_{10}$mass: $F_1 = 68.277$, $P < 0.001$; Treatment: $F_2 = 0.746$, $P = 0.487$).

4. Discussion

4.1. Cadmium accumulation

From the outset it must be remembered that all the animals in this study already had an incipient and increasing Cd burden as indicated by the ICP-MS analyses of control carcasses and crickets. These Cd burdens were not

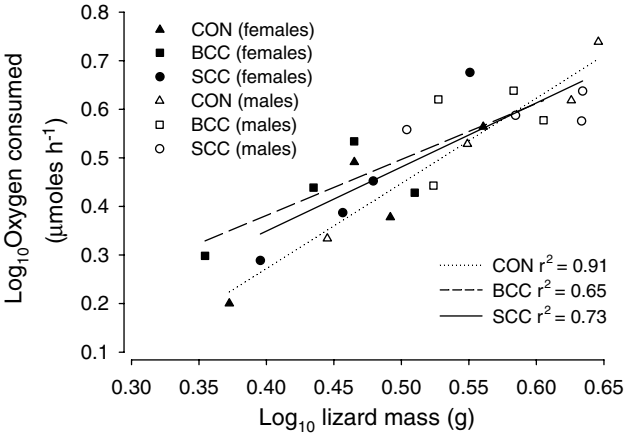

Fig. 6. $\log_{10}$ oxygen consumption (µmol h^{-1}) plotted against $\log_{10}$ animal mass (g). Data represent four males and four females from each treatment group (CON, BCC, SCC) obtained in two trials at 20 °C in post-absorptive lizards. Each data point represents the mean of 20 individual measurements over a 10-hour period. Error bars represent SE ($n = 20$). Lines represent least square regressions for pooled (males and females) data ($P < 0.05$).

accounted for by radiospectrometry. Indeed the carcass [Cd] accumulated by the BCC group (8 ng g^{-1}, determined by radiospectrometry and calculated with reference to the exposure solutions used to contaminate the crickets/lettuce) is very similar to the ICP-MS concentration of Cd in the control carcasses (6 ng g^{-1}), indicating that the carcass-Cd-burden was only twofold higher in the BCC group than in control animals.

The main target organs for Cd accumulation in mammals and other vertebrates, including reptiles, are the liver, kidney and the intestinal mucosa (Gutleb and Gutleb, 1991; Cooke and Johnson, 1996; Furness, 1996; Linder and Grillitsch, 2000). This general pattern of Cd tissue distribution was also supported by our results in the lizard *P. carbonelli* (see also Mann et al., 2006). In both treatment groups Cd accumulated in tissues in the following decreasing order of Cd-burden – gut > liver > kidney > carcass. Similarly, the concentration of Cd within the gut ($[Cd]_{gut}$) > $[Cd]_{liv}$ > $[Cd]_{kid}$. However, Cd is known to remobilise (possibly through the induction of MT) and preferentially accumulate in the kidney (WHO, 1992a,b; Zalups and Ahmad, 2003). Therefore, it is reasonable to assume that with a longer duration of exposure, the proportional distribution of Cd to the kidney and liver (i.e. the ratio of $[Cd]_{kid}$:$[Cd]_{liv}$) will change, resulting in $[Cd]_{liv} < [Cd]_{kid}$ as was reported for lacertid lizards sampled from an ancient lead mine (Avery et al., 1983), and among amphibians following water-born Cd exposure (Vogiatzis and Loumbourdis, 1998; Sura et al., 2006), mammals and trout following dietary Cd exposure (Chan and Hale, 2000; Franklin et al., 2005) and in wild-caught turtles (Maffucci et al., 2005), mammals (Gutleb and Gutleb, 1991; Shore et al., 1995; Cooke and Johnson, 1996) and birds (Furness, 1996).

However, it needs to be stressed that accumulation of Cd in internal organs was slow. This may in part be

because overall food consumption rates are low (compared to endothermic animals), and this will reduce Cd intake. However, a low level of consumption is compounded by a low overall assimilation rate and an even lower rate of assimilation across the gut (Mann et al., 2006). The differences between lacertids and at least some mammalian taxa are highlighted by the Cd residue data for lizards (*Lacerta vivipara*) and shrews (*Sorex minutes*) reported by Gutleb and Gutleb (1991). The shrews, which are even more short-lived than the lizards (several months versus several years), had kidney Cd-burdens an order of magnitude higher than the lizards.

4.2. Biological responses

Long-term Cd exposure did not have any effect on body weight or organ mass of lizards irrespective of the Cd treatment, presumably because Cd doses were not sufficient to cause significant changes in weight or organ mass. High dietary Cd-exposure (50–450 mg kg^{-1}) in ducks has previously been correlated with a reduction in the liver weight and increase of kidney weight after 42 days (Di Giulio and Scanlon, 1984). In addition, birds exposed to the highest dose showed a loss of body weight after the exposure period.

We measured AChE in brain and BChE in plasma to investigate whether long-term dietary Cd exposure leads to an inhibition of ChEs. In this study, no such inhibition was detected in either brain AChE or plasma BChE activities after 21-week dietary-Cd exposure. This is consistent with the absence of detectable Cd in brain and plasma samples of lizards. The levels of brain AChE activity and total protein in our lizards were lower than those reported for another lacertid lizard, *Gallotia galloti* (mean ± SD, 801 ± 115 nmol min^{-1} mg^{-1} total protein, Sánchez et al., 1997), and indicates a large degree of variability, even between closely related taxa. Normal plasma BChE activity of *P. carbonelli* were in the same range as measured in the lacertid lizard *G. galloti* (6.68 ± 1.02 μmol min^{-1} ml^{-1} - plasma; Sánchez-Hernández and Sanchez, 2002), and higher than BChE activity determined in the Australian dragon *Pogona vitticeps* (0.42 μmol min^{-1} ml^{-1} plasma, calculated by difference between mean AChE and total ChE activities determined in lizard plasma; Bain et al., 2004).

No significant increases in MT were demonstrated in either the gut or liver of Cd exposed lizards. Induction of MT within the gut following dietary administration of Cd has been demonstrated in mammals (for review, see Zalups and Ahmad, 2003) and more recently in rainbow trout (Chowdhury et al., 2005). Accumulation of Cd in liver tissue has also been correlated with increases of MT in the liver of mammals (Zalups and Ahmad, 2003) and amphibians (Vogiatzis and Loumbourdis, 1998). However, most of these studies have demonstrated MT induction after relatively short exposures to high concentrations of Cd. In the present study, the relatively low concentrations used may be adequate to explain the absence of significant elevations of MT.

In high dose studies, exposure to Cd will result in the induction of gut-MT and it has been suggested that this is a mechanism for the immobilisation and storage of Cd within the gut mucosal cells (Kimura et al., 1998; Zalups and Ahmad, 2003). However, Reeves et al. (2005) have recently demonstrated that MT induction does not necessarily coincide with exposure to dietary Cd if the dosage is low, and not necessary for retention of Cd within the rat gut mucosa. The doses of Cd in this study are relatively low (environmentally relevant), and MT induction might not necessarily be expected. The absence of MT within the liver is somewhat more intriguing, because Cd is known to be a potent inducer of MT within hapatocytes (Zalups and Ahmad, 2003). Also, MT-like proteins have been isolated from the liver of the closely related *P. muralis* (Flos et al., 1986), and also induced in embryos of *P. sicula* exposed to Cd (Riggio et al., 2003). In the present study, the actual quantities of Cd that reached the liver during the exposure were extremely low (Fig. 2). The highest concentrations of Cd were detected in the SCC group (1.29 μg g^{-1} ≡ 11.5 nmol g^{-1}). The mean concentrations of MT in the livers of the CON, BCC and SCC groups were ~290 μg g^{-1} (≅45 nmol g^{-1}). If we assume a binding coefficient of 1:8 (Berntssen et al., 2001), then there was already ample capacity for Cd binding within the livers of the lizards.

Metabolic trade-offs expressed as increased SMR have been found in various taxa exposed to metal contamination (Rowe, 1998; Rowe et al., 1998; Hopkins et al., 1999). Therefore, SMR presents itself as a valuable biomarker of toxic effect. The data presented in this study suggest that there were no metabolically demanding effects of an environmentally relevant accumulation of Cd.

5. Conclusions

Our results support a slow rate of Cd accumulation in the lizard *P. carbonelli* exposed to an environmentally relevant concentration of dietary Cd. In light of results on tissue distribution of Cd, the gut presents an effective barrier against Cd accumulation and the highest Cd concentrations were found in the gut tissue. In vertebrates, Cd will eventually accumulate preferentially within the kidney, where toxic effects are manifest. However, small lacertids are relatively short lived animals (3–4 years; Galán, 2004), and Cd accumulation may be so slow as to be inconsequential either for the lizards or their predators.

Biomarkers are an important element in ERA (exposure and effect assessment) because they can offer reliable information about bioavailability and toxicity of pollutants. In this study, although a significant accumulation of Cd took place, no responses in the analysed biomarkers were demonstrated. In accordance with other authors who indicate that MT induction in wild fauna is a phenomenon less common than under laboratory conditions, our results indicate that this biomarker of metal exposure may not provide a measure of Cd exposure in lizards (or reptiles

in general) when the exposure is low and prolonged. Also, our data do not support the use of cholinesterases as a biomarker for chronic low-level Cd exposure in lacertids. Lastly, despite previous studies indicating that SMR is a sensitive biomarker of metal-induced metabolic tradeoffs, we have been unable to demonstrate its value in this study.

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