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VITAMIN E, VITAMIN A, AND CAROTENOIDS IN MALE COMMON LIZARD TISSUES

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ABSTRACT: Vitamin E, vitamin A, and carotenoids are essential micronutrients for animals because of their antioxidant and immunostimulant functions and their implications for growth, development, and reproduction. In contrast to mammals and birds, information about their occurrence and distribution is generally lacking in reptiles, constraining our understanding of the use of these micronutrients. Using high-performance liquid chromatography, we determined the concentrations of vitamin E, vitamin A, and carotenoids in plasma, storage sites (liver and abdominal fat bodies), and in the colored ventral skin of male Common Lizards, *Lacerta vivipara*. All tissues shared a similar micronutrient profile, except the liver, which also showed traces of vitamin A_1. The main vitamin E compound present was α-tocopherol followed by lower concentrations of γ-(β-)tocopherol. Vitamin A_2 was the main vitamin A compound and it showed the highest concentration in the liver, where vitamin A_2 esters and traces of vitamin A_1 were found. Lutein was the main carotenoid, and it formed esters in the liver and the ventral skin. Zeaxanthin and low concentrations of β-carotene were also present. The liver was the main storage site for carotenoid and vitamin A, whereas hepatic vitamin E concentrations resembled those present in abdominal fat bodies. Compared with abdominal fat bodies, the ventral skin contained lower concentrations of vitamin A and vitamin E, but similar concentrations of carotenoids. These results suggest that important differences exist in micronutrient presence, concentration, and distribution among tissues of lizards and other taxa such as birds and mammals.

Key words: Coloration; Dehydroretinol; Nutrition; Oxidative stress; Retinol; *Squamata*; Vitamin A metabolism; Xanthophylls

VITAMIN E, vitamin A, and carotenoids are essential micronutrients required by animals for metabolic, immunological, reproductive, and developmental processes (Ames, 2006). Animals are not able to synthesize them de novo and thus depend on dietary intake to meet their basic nutritional requirements (Surai, 2002). Vitamin E (i.e., isomeric forms of tocopherol and tocotrienol) mainly functions as a potent antioxidant (Burton and Traber, 1990; Rock et al., 1996). Vitamin A compounds (retinoids that exhibit retinol activity; Blomhoff et al., 1990) enhance cellular proliferation and differentiation (McCaffery et al., 2003; Mora et al., 2008), promote embryonic growth and development and postnatal bone remodeling (Edem, 2009), and constitute the chromophore of visual pigments in the retina (Pepe, 1999). In animals, carotenoids serve as ornamental pigments (Olson and Owens, 1998), antioxidants, immune enhancers, and vitamin A precursors (Simpson, 1983; Olson, 1993; Surai, 2002; Chew and Park, 2004).

Current knowledge about the occurrence and implications of vitamin A, vitamin E, and carotenoids mostly comes from studies on humans, other mammals, and birds, whereas limited information is available in reptiles, particularly in squamates. This is especially surprising given that deficiency of these micronutrients impairs growth, reproduction, and survival and provokes skin, respiratory, and ocular diseases, both in captive (Boyer 1996; Ferguson et al., 1996; Goodman, 2007) and free-ranging reptiles (Holladay et al., 2001; Brown et al., 2004). These micronutrients may play an important role in reptile behavior, for example through femoral gland secretions and ornamental coloration (Steffen and McGraw, 2007; Fitze et al., 2009; Martín and López, 2010). Despite their importance, research mainly focused on egg and less importantly on blood (Thompson et al., 1999a,b,c; Dierenfeld et al., 2002) and studies investigating micronutrient content in other tissues are lacking.

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In this study, we determined the profile and concentrations of vitamin A, vitamin E, and carotenoids present in male Common Lizards, *Lacerta vivipara*. *Lacerta vivipara* is a small ground-dwelling lacertid lizard that inhabits peat bogs and moist heath lands. Its distribution ranges from northern Spain and Greece at the southern boundary up to northern Europe and Asia. It inhabits mainly cold climates with overwintering sites exposed to subzero temperatures (Sindaco and Jeremcenko, 2008). It feeds on small invertebrates, mostly spiders and *Homoptera* (Avery, 1966; Heulin, 1986) and also on larger prey like earthworms (L. M. San-Jose, personal observation). In the Pyrenean populations, where the study was conducted, adult *L. vivipara* males develop a conspicuous ventral coloration that ranges from white to yellow and orange (Sinervo et al., 2007). Coloration spreads from the base of the tail to the collar scales and rarely reaches throat scales. Yellow-orange ornamental pigmentation stems from carotenoids, which have also been detected in other tissues of *L. vivipara* (Czeczuga, 1980; Fitze et al., 2009). Nothing is known about the presence and content of vitamin A and vitamin E in this species, with the exception of α-tocopherol, which is present in femoral gland secretions (Gabirot et al., 2008). Using high-performance liquid chromatography (HPLC) analyses, we investigated the content of vitamin A, vitamin E, and carotenoids present in plasma, main reserve organs (liver and abdominal fat bodies), and ventral skin of male Common Lizards. In particular, we tested in each tissue whether different vitamin E compounds showed different concentrations and, similarly, whether different carotenoid species showed different concentrations. For each vitamin E and vitamin A compound, and for each carotenoid species, we also tested whether the concentration differed among liver, ventral skin, and abdominal fat bodies.

Materials and Methods

Lizard Handling

In June 2008, we captured eight adult male Common Lizards (snout-to-vent length [SVL] range: 50–56 mm) at Somport (Central Pyrenees, Huesca, Spain, 42°47′N, 0°31′W; datum = European Datum 1950). They were brought to the laboratory at the Instituto Pirenaico de Ecología (Jaca, Huesca, Spain), where we measured SVL (to the nearest 1 mm) and body mass (to the nearest 0.001 *g*). Lizards were individually housed in plastic terraria (25 × 15 × 15 cm) equipped with two shelters, a water pond, and peat bog as substrate. A 40-W bulb placed in a corner of each terrarium provided light and heat following a 10-h light:14-h dark photoperiod and an ultraviolet (UV) light source provided UVB and UVA for 2 h per day (Fitze et al., 2008, 2010). Lizards were fed every 2 d with one wax worm larva, *Galleria mellonella*, and water was provided ad libitum. Lizards were captured, housed, and decapitated according to the permits provided by the Gobierno de Aragón, Spain (see Acknowledgments for permit numbers).

Tissue Sampling

Twenty days after capture, a blood sample was collected from the retro-orbital sinus of each lizard using a heparinized microcapillary tube. An average (±SE) of 9.81 μL (±2.04) of plasma was obtained after blood centrifugation (5 min at 8900 *g*). After blood sampling, lizards were anesthetized by subcutaneously injecting 0.02 mL/g of a metomidine and ketamine solution (1:50) and thereafter decapitated. Abdominal fat bodies, liver, and the ventral skin between collar scales and anal plate were harvested. All tissues were rinsed twice with phosphate-buffered saline to avoid contamination with the remaining blood and weighed to the nearest 0.01 mg. On average (±SE) 37.76 mg (±6.55) of abdominal fat bodies, 98.05 mg (±6.46) of liver, and 87.03 mg (±4.78) of ventral skin were obtained and used for subsequent analyses. All samples were stored for 2 mo at −20°C and then moved to −80°C until HPLC analyses (ca. 1 mo).

HPLC Analyses

Vitamin E, vitamin A, and carotenoids of abdominal fat bodies, liver, ventral skin, and blood plasma were analyzed by HPLC using an adapted protocol of Olmedilla et al. (1997). Plasma samples were mixed with 0.1 mL

of distilled water and 0.2 mL of ethanol, vortexed, and extracted twice with 0.5 mL of methylene chloride–hexane (1:5). Organic phases were pooled, evaporated to dryness, reconstituted in tetrahydrofuran (THF)–ethanol (EtOH), and injected onto the HPLC column. Abdominal fat bodies, liver, and ventral skin samples were placed in EtOH for 25 min. Extraction was performed by ultrasound and intermittent vortex for 5 min. Water (1 mL) and 2 mL of methylene chloride–hexane (1:5) were added, vortexed, centrifuged, pooled, evaporated to dryness, reconstituted (THF–EtOH), and injected onto the HPLC column.

A subset of five liver and four ventral skin samples were analyzed twice (once saponified and once not saponified) and their HPLC chromatograms were compared to evaluate the presence of vitamin A and carotenoid esters. Ester forms show the same absorption spectra but longer retention times than free forms (Ladislav et al., 2005) and thus chromatograms where esters are present have more peaks than chromatograms where only free forms are present. Saponification results in ester hydrolysis, leading to the presence of free forms only. Consequently, chromatograms of saponified samples show fewer peaks and bigger absorption peaks of free forms compared with chromatograms of not saponified samples. Tissue samples were saponified with methanolic potassium hydroxide following the protocol of Granado-Lorencio et al. (2001). First, reconstituted extracts were evaporated and reconstituted with pyrogallic acid (0.3 M) dissolved in ethanol. Saturated methanolic potassium hydroxide was added and the mixture was vortexed for 3–5 min. Partition was carried out by adding distilled water and methylene chloride–hexane. Finally, the organic phases were pooled, evaporated to dryness, reconstituted (THF–EtOH), and injected onto the HPLC column.

The chromatographic system consisted of a Spheri-5-ODS column (Applied Biosystems, San Jose, CA) with gradient elution of acetonitrile–methanol (85:15) for 5 min to acetonitrile–methylene chloride–methanol (70:20:10) for 20 min. The injection volume was 10 μL and the flow rate was 1.8 mL/min. Ammonium acetate (0.025 M) was added to the methanol. Isomeric forms of tocopherol (vitamin E) were detected using a photodiode array detector (model 2996, Waters Associates, Milford, MA) set at 295 nm and fluorescence (excitation: 290 nm, emission: 330 nm). Under these assay conditions γ- and β-tocopherol (hereafter referred as γ-(β-) tocopherol) could not be distinguished and δ-tocopherol concentration could not be determined. By setting the photodiode array detector at 326 nm we could detect dehydroretinol (vitamin A_2 alcohol) and retinol (vitamin A_1 alcohol). Concentrations of vitamin A_2 were measured as relative response (absorbance units full scale per milligram of tissue). Carotenoid detection was carried out by setting the photodiode array at 450 nm. Using this method, *trans*-lutein, zeaxanthin, 13/15-*cis*-lutein, α-carotene, all-*trans*-β-carotene, 9-*cis*-β-carotene, and 13/15-*cis*-β-carotene, among other carotenoids, could be determined simultaneously. Compound identification was carried out by comparing retention times of the samples with those of authentic standards and with online UV–visible spectra. The short- and long-term precision and accuracy of the analytical method were verified periodically through participation in the Fat-Soluble Quality Assurance Program conducted by the National Institute of Standards and Technology (Gaithersburg, MD).

Statistics

For each tissue, we tested whether different vitamin E compounds showed different concentrations using Wilcoxon signed-rank tests for small sample sizes. We also tested for each tissue whether different carotenoid species showed different concentrations using Friedman tests and Wilcoxon signed-rank tests for post hoc comparisons.

To test whether compound concentrations differed among liver, ventral skin, and abdominal fat bodies we used Friedman tests. Wilcoxon signed-rank tests were used for post hoc comparisons between pairs of tissues. Quantitative comparisons between plasma and other tissues were not possible because plasma concentrations were volume referenced and tissue samples were mass referenced.

All the statistical analyses were conducted using R v 2.10.1 software (Free Software

Foundation, GNU Project, Boston, MA). For all statistical models the significance level was set at $\alpha = 0.05$ (two-tailed) and adjusted for post hoc comparisons using sequential Bonferroni procedures (Hochberg, 1988).

Results

Vitamin E

In all samples, vitamin E compounds α-, γ-(β-), and δ-tocopherol were detected. α-Tocopherol was the predominant compound showing significantly higher concentrations than γ-(β-) tocopherol in plasma ($W = 36$, $N = 8$, $P = 0.008$), liver ($W = 36$, $N = 8$, $P = 0.008$), fat bodies ($W = 36$, $N = 8$, $P = 0.008$), and ventral skin ($W = 36$, $N = 8$, $P = 0.008$; Table 1). There existed significant differences between liver, fat bodies, and ventral skin in the concentrations of α-tocopherol (Friedman's $\chi^2 = 9.75$, df = 2, $P = 0.008$) and γ-(β-) tocopherol (Friedman's $\chi^2 = 13$, df = 2, $P = 0.002$). Post hoc analyses revealed that both α-tocopherol and γ-(β-) tocopherol were less concentrated in the skin than in the liver and fat bodies (Table 1).

Vitamin A

In all samples, vitamin A was mainly present as dehydroretinol (vitamin A_2) and traces (<0.001 μg/mg) of retinol (vitamin A_1) were only found in five liver samples (Table 1). Our analyses therefore concentrate on vitamin A_2. There existed significant differences in vitamin A_2 concentrations among tissues (Friedman's $\chi^2 = 16$, df = 2, $P < 0.001$). The highest and lowest concentrations were found in the liver and in the ventral skin respectively, whereas fat bodies showed an intermediate concentration (Table 1). Esters of vitamin A_2 were detected in the liver but not in the other tissues (Fig. 1).

Carotenoids

Lutein and zeaxanthin were the most abundant carotenoids in all tissue samples (mean ± SE, 78.9 ± 0.1% and 20.2 ± 0.1% of the total carotenoid concentration, respectively; Table 1). β-Carotene (mean ± SE, 1.2 ± 0.1%) was found in the liver, skin, and fat bodies of all individuals, but only in one individual did we detect β-carotene in the plasma (0.04 μg/mL). Lutein esters were identified in ventral skin and liver samples. After hydrolysis, lutein ester peaks disappeared from the chromatograms, resulting in increased lutein concentrations (Fig. 2). Zeaxanthin concentration also increased after hydrolysis, suggesting that either zeaxanthin esters or lutein isomers (e.g., 9-*cis*-lutein coeluting with zeaxanthin) might exist at low concentrations.

In plasma, lutein was significantly more concentrated than zeaxanthin ($W = 36$, $N = 8$, $P = 0.008$). There also existed differences among lutein, zeaxanthin, and β-carotene concentrations of liver (Friedman's $\chi^2 = 16$, df = 2, $P < 0.001$), fat bodies (Friedman's $\chi^2 = 16$, df = 2, $P < 0.001$), and ventral skin (Friedman's $\chi^2 = 16$, df = 2, $P < 0.001$). In these tissues, lutein concentrations were significantly higher than zeaxanthin and β-carotene concentrations and zeaxanthin concentrations were significantly higher than β-carotene concentrations (for all Wilcoxon post hoc tests $W = 36$, $N = 8$, $P = 0.008$). The concentration of lutein, zeaxanthin, and β-carotene significantly differed among liver, fat bodies, and ventral skin (lutein: Friedman's $\chi^2 = 13$, df = 2, $P = 0.002$; zeaxanthin: Friedman's $\chi^2 = 12.25$, df = 2, $P = 0.002$; β-carotene: Friedman's $\chi^2 = 12.25$, df = 2, $P = 0.002$). Fat and ventral skin concentrations of lutein, zeaxanthin, and β-carotene were lower than liver concentrations (Table 1). Lutein concentration tended to be higher in the ventral skin than in fat bodies and there existed no significant differences between fat and ventral skin concentrations of zeaxanthin and β-carotene.

Discussion

Vitamin E

α-Tocopherol was the predominant vitamin E compound in all tissues of *L. vivipara* males. Its constant predominance together with the presence of γ-(β-) tocopherol in all analyzed tissues may indicate that *L. vivipara* males do not selectively absorb or store different vitamin E compounds, as previously observed in birds and mammals (Traber and Sies, 1996). The concentrations of vitamin E found in the different tissues of *L. vivipara* were within the range observed in birds (Crissey et al., 1998; Surai, 2002; Karadas

TABLE 1.—Concentration (mean ± SE) of vitamin E, vitamin A, and carotenoids measured in plasma, liver, abdominal fat bodies, and ventral skin of eight male Common Lizards (*Lacerta vivipara*) and results from post hoc (Wilcoxon signed-rank) tests among liver, fat bodies, and ventral skin.

	Lizard samples				Comparisons		
Micronutrients	Plasma	Liver	Abdominal fat bodies	Ventral skin	Liver vs. abdominal fat bodies	Liver vs. ventral skin	Abdominal fat bodies vs. ventral skin
Vitamin E							
α-Tocopherol	22.299 ± 2.548 (μg/mL)	2.833 ± 0.255 (μg/mg)	2.956 ± 0.714 (μg/mg)	0.807 ± 0.108 (μg/mg)	$W = 15$, $P = 0.742$	$W = 36$, $P = 0.023$	$W = 35$, $P = 0.031$
γ-(β-) Tocopherol	8.286 ± 1.134 (μg/mL)	0.832 ± 0.073 (μg/mg)	1.112 ± 0.150 (μg/mg)	0.205 ± 0.015 (μg/mg)	$W = 28$, $P = 0.195$	$W = 36$, $P = 0.016$	$W = 36$, $P = 0.016$
Vitamin A							
Vitamin A_1	—	<0.001 μg/mg	—	—			
Vitamin A_2	232.9 ± 34.7 (absorbance units full scale [AUFS]/μL)	985.0 ± 25.6 (AUFS/mg)	163.9 ± 26.94 (AUFS/mg)	90.70 ± 9.31 (AUFS/mg)	$W = 36$, $P = 0.008$	$W = 36$, $P = 0.008$	$W = 36$, $P = 0.008$
Carotenoids							
Lutein	4.928 ± 0.566 (μg/mL)	8.535 ± 2.225 (μg/mg)	0.153 ± 0.055 (μg/mg)	0.218 ± 0.019 (μg/mg)	$W = 36$, $P = 0.016$	$W = 36$, $P = 0.016$	$W = 26$, $P = 0.313$
Zeaxanthin	1.357 ± 0.128 (μg/mL)	1.436 ± 0.283 (μg/mg)	0.046 ± 0.016 (μg/mg)	0.054 ± 0.004 (μg/mg)	$W = 36$, $P = 0.016$	$W = 36$, $P = 0.016$	$W = 24$, $P = 0.461$
β-Carotene	—	0.039 ± 0.010 (μg/mg)	0.005 ± 0.003 (μg/mg)	0.003 ± 0.001 (μg/mg)	$W = 36$, $P = 0.016$	$W = 36$, $P = 0.016$	$W = 16$, $P = 0.844$

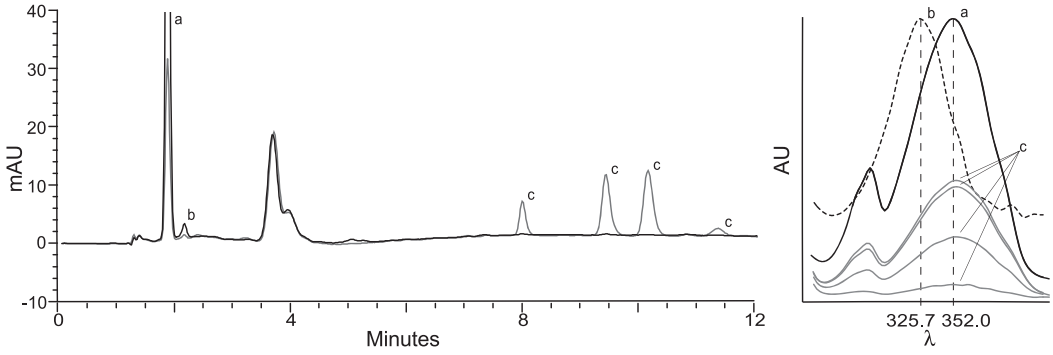

FIG. 1.—High-performance liquid chromatography chromatogram (at 326 nm and in miliabsorbance units, mAU) and absorption spectra (in absorbance units, AU) of vitamin A compounds (a, vitamin A_2 alcohol; b, vitamin A_1 alcohol; c, vitamin A_2 esters) present in the liver of male Common Lizards (*Lacerta vivipara*) before (gray line) and after (dark line) hydrolysis.

et al., 2005) and mammals (Rosa et al., 2007; Il'ina et al., 2008). Our data suggest that *L. vivipara* could differ from birds and mammals in how vitamin E is allocated among different reserve tissues. Birds preferentially store vitamin E in the liver and mammals in fat stores (Rock et al., 1996; Surai, 2002). In contrast, we found no significant differences in vitamin E concentrations between these two organs. Although concentrations in tail fat reserves are unknown, our results suggest that in *L. vivipara* males both liver and fat bodies function as the main vitamin E storage organs. However, more studies are needed to generalize this finding to other lizard species.

Vitamin E concentrations in *L. vivipara* did not resemble those reported in other insectivorous or related species. Namely, vitamin E concentrations in *L. vivipara* males were higher than in other insectivorous and herbivorous lizards (Cosgrove et al., 2002; Raila et al., 2002) and carnivorous, herbivorous, and omnivorous turtles (Ghebremeskel et al., 1991; Deem et al., 2006; Chaffin et al., 2008). They were, however, in the range reported for free-ranging alligators (Lance et al., 2001). Therefore, available data do not seem to support a phylogenetic or dietary origin of vitamin E concentrations in *L. vivipara*, although only studies specifically testing these hypotheses will allow understanding of the causes. High vitamin E concentration could be also a consequence of certain environmental conditions. For example, to maintain cellular membrane fluidity in cold-water environments, some fish present high levels of unsaturated fatty acids, which require exceptionally high levels of vitamin E to avoid

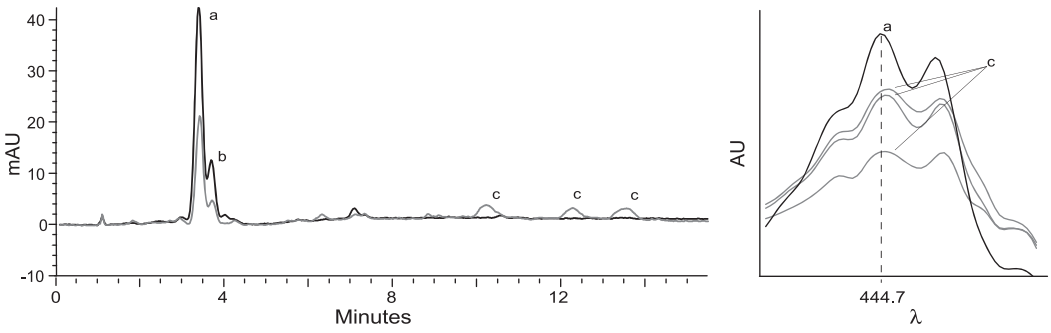

FIG. 2.—High-performance liquid chromatography chromatogram (at 450 nm and in miliabsorbance units, mAU) and absorption spectra (in absorbance units, AU) of different carotenoids (a, lutein; b, zeaxanthin; c, lutein esters) present in the ornamented ventral skin of male Common Lizards (*Lacerta vivipara*) before (gray line) and after (dark line) hydrolysis.

oxidation (Fujisawa et al., 2010). To endure low habitat temperatures and long overwintering periods, *L. vivipara* also develops highly efficient antioxidant defenses (Voituron et al., 2006). Thus, the particularly high concentrations of vitamin E found in the tissues of *L. vivipara* could be an adaptive response allowing lizards to occupy cold habitats across Eurasia. Alternatively, high vitamin E concentrations could be the consequence of feeding lizards with wax worms, which are rich in vitamin E (Barker et al., 1998; Surai et al. 1998). However, this alternative seems unlikely because in a different study where we fed *L. vivipara* with wax worms during 40 d, plasma α-tocopherol concentration decreased over time (estimated change ± SE; −9.86 ± 2.1 μg/mL, $F_{1,41} = 21.95$, $P < 0.001$), whereas plasma γ-(β-) tocopherol concentration slightly increased (estimated change ± SE; 0.41 ± 0.1 μg/mL, $F_{1,39} = 9.43$, $P = 0.004$; L.M. San-Jose, personal observation). These results show that wax worms cannot explain high α-tocopherol concentrations, suggesting that wax worms may be below the nutritional α-tocopherol requirements of *L. vivipara*.

Vitamin A

Vitamin A_2 was the main vitamin A compound in the tissues of *L. vivipara* and low concentrations of vitamin A_1 were detected in the liver. Vitamin A_2 predominace has been reported in some freshwater teleosts, amphibians, and crustaceans (Wald, 1939; Tsin et al., 1984; Suzuki and Eguchi, 1985). In reptiles, vitamin A_1 usually predominates over vitamin A_2 (Plack and Kon, 1961; Provencio et al., 1992), which is absent in most species (Thompson et al., 1999a,b,c; Raila et al., 2002). Only two studies show vitamin A_2 predominance and both studies exclusively focus on the retina (Atlas Day Gecko, *Quedenfeldtia trachyblepharus*, and Green Anole, *Anolis carolinensis*; Provencio et al., 1992; Röll, 2000). Thus, the predominance of vitamin A_2 in nonretinal tissues contrasts with findings reported in reptiles or in any terrestrial vertebrate.

Preferential accumulation of vitamin A_2 may occur in species feeding on vitamin A_2-rich species (Käkelä et al., 1997) or through its synthesis from provitamin A carotenoids (Gross and Budowski, 1966). Because insects and other small invertebrates commonly ingested by *L. vivipara* are poor vitamin A (both A_1 and A_2) sources (Barker et al., 1998; Finke, 2002), it is unlikely that vitamin A_2 predominance arises from direct vitamin A_2 intake and *L. vivipara* may therefore obtain vitamin A_2 through carotenoid metabolism (Dierenfeld et al., 2002). Among the carotenoids present in *L. vivipara* only β-carotene can be efficiently metabolized into A_2 (Morton and Creed, 1939; Simpson, 1983). Lutein and zeaxanthin are inefficient precursors of vitamin A, although lutein previously dehydrated to anhydrolutein can be metabolized into vitamin A_2 (Budowski and Gross, 1965; Goswami and Barua, 1981). However, the absence of anhydrolutein in the tissues of *L. vivipara* may discard this metabolic route. To synthesize vitamin A_2, β-carotene is first metabolized into vitamin A_1, which is finally dehydrogenated into vitamin A_2 (Gross and Budowski, 1966). This metabolic route would explain why we found some small traces of vitamin A_1, although it does not explain why vitamin A_2 predominated in our study. Therefore, our data do not support that vitamin A_2 prevails in the tissues of *L. vivipara* because of dietary constraints (i.e., direct intake or increased availability of direct vitamin A_2 precursors), indicating that *L. vivipara* may preferentially synthesize it, as observed in fish (Gross and Budowski, 1966). The implications of increased vitamin A_2 accumulation are not clear. In species that preferentially use vitamin A_1, vitamin A_2 has been suggested to inefficiently replace health-enhancing functions of vitamin A_1, since A_2 is 49 to 60% less active in inducing and promoting growth than vitamin A_1 (Lovern et al. 1939; Shantz and Brinkman, 1950). However, in an evolutionary context we would expect some beneficial effect of vitamin A_2 that counteracts its lower activity and explains why some species preferentially synthesize and accumulate it. More studies are clearly needed to completely understand the physiological and evolutionary implications of vitamin A_2 and, as shown here, *L. vivipara* can be an ideal model animal to investigate them.

Carotenoids

Similar to what has been reported in birds, amphibians, fish, and other reptiles (Czeczuga, 1980; Costantini et al., 2005; McGraw, 2006; Steffen et al., 2010), *L. vivipara* males seem to selectively retain more xanthophylls (lutein and zeaxanthin) than carotenes (β-carotene). This skewed carotenoid profile could result directly from the diet, given that insectivorous diets are usually richer in lutein and zeaxanthin than in β-carotene (Partali et al., 1987; Isaksson and Andersson, 2007; but see Eeva et al. 2010). Alternatively, the relatively low content of β-carotene could result from a preferential use for vitamin A synthesis, which could limit its presence (Chen and Huang, 2011). Xanthophyl predominance resulting from feeding lizards with wax worms (richer in lutein and zeaxanthin than in β-carotene; Barker et al., 1998; Surai et al. 1998) can be discarded since in a previous study, no differences in the carotenoid profile and in the relative carotenoid content before and after feeding lizards with wax worms for 2 mo were found (L.M. San-Jose, personal observation).

In addition to the carotenoid species detected in the current study, previous studies reported the occurrence of astaxanthin, canthaxanthin, β-cryptoxanthin, and diatoxanthin in other populations of *L. vivipara* (Czeczuga, 1980; Fitze et al., 2009). These different results may reflect interpopulation variation in the available carotenoid species, but differences in analytical methodology or in peak interpretation (e.g., interpretation of carotenoid ester forms as free forms; Ladislav et al. 2005) cannot be discarded. Interestingly, interpopulation differences in available carotenoid species have rarely been investigated, and to our knowledge only one study found significant differences among populations due to differences in prey diversity (Negro et al., 2000; but see Partali et al., 1987). In *L. vivipara*, prey diversity varies among populations (Avery, 1966; Pilorge, 1982; Heulin, 1986), indicating that differences among studies in detected carotenoid species may indeed reflect interpopulation variation. Given that different carotenoid species show different biological properties (mostly in provitamin A activity and antioxidant capacity; Simpson, 1983; Olson, 1993; Chew and Park, 2004), important implications for health are expected from interpopulation variation in carotenoid species and *L. vivipara* can be an ideal subject in which to investigate them.

The observed carotenoid concentrations in plasma were within the range observed in other free-ranging lizards (Costantini et al., 2005) and those in ornamental skin were similar to reported values in ornamental traits of other lizards (Steffen and McGraw, 2007) and birds (both colored plumage and bare parts; Stradi et al., 1995; Saks et al., 2003; Butler et al., 2011). However, relative carotenoid concentration of ornamental skin compared with reserve tissues importantly differed from birds. In *L. vivipara*, ornamental skin showed similar and lower carotenoid concentrations than fat bodies and liver, respectively, whereas birds commonly accumulate higher concentrations of carotenoids in ornamental traits compared with liver and fat stores (Isaksson and Andersson, 2008; McGraw and Toomey, 2010). Therefore, in contrast to birds, *L. vivipara* can invest a lot of carotenoids into coloration and simultaneously maintain relatively large reserves of carotenoids. This suggests that the allocation of carotenoids to ornamentation may be less limited than in birds and that it could explain why carotenoid supplementation does not affect coloration of *L. vivipara* (Fitze et al., 2009) and other lizards (Olsson et al., 2008; Steffen et al., 2010) while it has strong effects in birds (Tschirren et al., 2003; Fitze et al., 2003).

In conclusion, we have provided information about vitamin A, vitamin E, and carotenoid content in different tissues of the Common Lizard, *L. vivipara*. In reptiles, especially in free-ranging squamates, little information about these micronutrients has been available and rarely have several tissues been analyzed in the same species. Our study showed that lizards deposit high concentrations of vitamin E (α- and γ-[β-] tocopherol), vitamin A (mainly A_2), and carotenoids (lutein, zeaxanthin, and β-carotene) in the liver and, less importantly, in fat bodies. In general, *L. vivipara* differs from other well-studied animals in the concentration and distribution among tissues of micronutrients, suggesting

that important differences in nutritional access and requirements may exist and that reptiles deserve more attention and research. Our study showed vitamin A_2 predominance in all *L. vivipara* tissues, which has been observed only in some freshwater species and in the retinas of species of two lizards. Given the carotenoids found in the tissues of *L. vivipara*, our results suggested that vitamin A_2 may be synthesized from β-carotene. This metabolic route could explain why β-carotene occurred at lower concentrations than lutein and zeaxanthin and why only small amounts of vitamin A_1 were found. Together with other studies, our results indicate that important differences in carotenoid availability may exist between *L. vivipara* populations. Despite the high interest in the evolution of carotenoid-based ornaments, most studies focus on birds (Olson and Owens, 1998; Fitze and Tschirren, 2006; Jacot et al., 2010). Reptiles, mainly lizards, have received attention only in the recent years (Cote et al., 2008; Olsson et al., 2008; Fitze et al., 2009; Steffen et al., 2010). Our results showed that *L. vivipara* differs from birds in the allocation of carotenoids between ornamental and reserve tissues, indicating that reptiles may provide new insights into the evolution of carotenoid-based ornaments.

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