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Placental transfer of nutrients during gestation in the viviparous lizard, *Pseudemoia spenceri*

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Abstract Energy, ionic, protein and lipid contents and fatty acid profiles for the major lipid classes of freshly ovulated eggs and neonates of the viviparous lizard, *Pseudemoia spenceri*, were measured. Litter size is 1.7 ± 0.1, with larger females producing larger neonates. Placentotrophy results in approximately 23% more dry matter in the neonates than in the fresh egg. The increase in the quantity of protein and lipid during development is not significant and is reflected in the similarity of energy densities of eggs and neonates. As a percentage of dry matter, neonates have slightly lower proportions of lipid and protein than eggs because of significant uptake of ash, calcium, potassium and sodium, but not of magnesium, across the placenta. The amounts of triacylglycerol and phospholipid are not significantly different between the egg and the neonate, but neonates contain significantly more cholesterol and cholesteryl ester. The amounts of the major fatty acids, palmitic and oleic acids, recovered from the total lipids of the neonate do not differ significantly from the amounts present in the egg lipids, but the neonates contain significantly less linoleic and α-linolenic acids and more palmitoleic, stearic and arachidonic acids than the eggs. The amount of docosahexaenoic acid recovered from the lipids of the neonate is 2.6-times greater than the amount initially present in the egg. *P. spenceri* has a relatively larger egg and a smaller reliance on placentotrophy than other species in the same genus, all of which have a similar placental morphology. Nevertheless, the pattern of embryonic nutrition includes both obligative and facultative placentotrophy. All the major components of yolk of oviparous species are present in eggs of *P. spenceri*, but most are augmented during development by placental transfer.

Key words Placenta · Viviparity · Lipid · Embryo · Yolk

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Introduction

Even though the common reproductive mode in squamate reptiles (lizards and snakes) is oviparity, many taxa produce young through viviparity which has evolved independently within the Squamata on about 100 separate occasions (Blackburn 1992). Most viviparous taxa have very simple chorioallantoic placentae in which there is no morphological elaboration of maternal or embryonic tissue to enhance transfer of nutrients (placentotrophy). However, a few taxa have much more complex chorioallantoic placentae, with some species in the genus *Mabuya* having placentae that resemble those of some eutherian mammals (Blackburn et al. 1984). The early pioneering work on reptilian viviparity defined three main placental types based on the chorioallantoic placenta, denoted in increasing order of complexity, types I, II, and III (Weekes 1935), with the complex placentae of *Mabuya* (type IV) being added later (Blackburn 1993).

We are interested in the evolution of complex chorioallantoic placentae and have conducted a series of detailed studies of species with placental types II and III. All species that have been investigated in the genus *Pseudemoia* have complex placental structures (type III)

(Harrison and Weekes 1925; Weekes 1929, 1930; Stewart and Thompson 1996, 1998). The three species in which placentation has been described, *Pseudemoia entrecasteauxii*, *Pseudemoia pagenstecheri* and *Pseudemoia spenceri*, are similar in adult body size, but have different litter sizes (Rawlinson 1974; Stewart and Thompson 1996, 1998). *P. spenceri* has a small clutch size (mean of 1.9, range of 1–3, Rawlinson 1974), compared to 4.5 (range 2–7) for *P. pagenstecheri* (Thompson and Stewart 1994) and 3.6 (range 2–6) for *P. entrecasteauxii* (Stewart and Thompson 1993). Comparison of nutrient contents of freshly ovulated eggs and net uptake across the placenta in *P. entrecasteauxii* and *P. pagenstecheri* (Stewart and Thompson 1993; Thompson and Stewart 1994; Thompson et al. 1999a) shows that ovulation of smaller eggs in *P. pagenstecheri* is correlated with increased placental nutrient uptake. On the basis of these observations, we considered it likely that *P. spenceri* has a relatively larger egg and a relatively smaller reliance on placentotrophy than both *P. entrecasteauxii* and *P. pagenstecheri* (Thompson and Stewart 1994; Thompson et al. 1999a).

We tested these predictions using a series of female *P. spenceri* in which we measured litter size and chemical and energetic composition of freshly ovulated eggs and neonates for comparison with existing data for other species. At the same time, we were able to compare our data with published data for species with both simpler and more complex chorioallantoic placentae in an attempt to better understand the processes involved in the evolution of more complex placentotrophy from oviparous ancestors. By comparing the differences in key components of the yolk and neonates of species with different reproductive modes and different chorioallantoic placental types, we expect to identify components of the yolk that are difficult to transport across the placenta, and thus must be present in the egg at the time of oviposition, and those that readily cross the placenta and may be reduced in the yolks of species with complex placentae.

Materials and methods

Gravid female (n = 20) *P. spenceri* were collected in Kanangra-Boyd National Park at about the time of expected ovulation (austral spring – October) and another 17 females were collected in December 1995 for analysis of ions, energy and nitrogen in eggs and neonates. Further females were collected in September (n = 7) and December (n = 6) of 1997 for analysis of lipids.

Females collected in September and October were returned to the University of Sydney and killed by cervical dislocation prior to removal of eggs from the oviducts. Eggs were weighed and frozen prior to analysis of lipids or lyophilisation before analysis of energy content, ash, ions and nitrogen. Females collected in December of both years were kept in individual aquaria until parturition. Water was provided ad libitum in small plastic Petri dishes and mealworms (*Tenebrio molitor*) and crickets (*Acheta domestica*) dusted with calcium gluconate were provided 3 times per week. The aquaria were housed in a room at 19 °C, with a 25 W incandescent light bulb at one end which produced a thermal gradient within the aquarium for 9 h per day. The light regime followed that of the local environment and all aquaria were cooled to 19 °C each night.

All young were killed within 12 h of birth by rapid freezing. Dissection showed that none retained any residual yolk. Females and neonates were measured [snout-vent length (SVL) and tail length] to 1 mm using a ruler and weighed to 10 mg on a top-pan balance (females) or 0.1 mg using an analytical balance (neonates).

Lipids from eggs and neonates were analysed using the same methods employed in a similar study of *P. pagenstecheri* (Thompson et al. 1999a). Samples were homogenised in a suitable excess of chloroform-methanol (2:1, v/v) and the organic phase was washed with 0.88% (w/v) KCl (Christie 1984). The chloroform was evaporated from a portion of the sample and the total amount of lipid was determined gravimetrically. A separate portion of the total lipid extract was subjected to transmethylation (Noble et al. 1993) and analysed by gas-liquid chromatography (see below) for determination of the amount (mg per egg or per neonate) of each fatty acid derived from the total lipids of the eggs and neonates. The remainder of the extract was fractionated into the major lipid classes (triacylglycerol, phospholipid, cholesterol, cholesteryl ester, free fatty acid) by thin-layer chromatography on silica gel G using a solvent system of hexane-diethyl ether-formic acid (80:20:1, by vol.); visualisation of the bands and elution of the lipid classes from the silica were performed as described previously (Noble et al. 1993). The phospholipid fraction from eggs only was further fractionated by thin-layer chromatography on silica gel D using a solvent system of chloroform-methanol-acetic acid-water (25:15:4:2, by vol.). Classes of major phospholipid (phosphatidylcholine, phosphatidyl-ethanolamine, phosphatidyl-serine, sphingomyelin) were visualised and eluted from the silica (Noble et al. 1993). The isolated acyl-containing lipid and phospholipid classes were transmethylated to form fatty acid methyl esters that were analysed by gas-liquid chromatography using a capillary column (Carbowax, 30 m × 0.25 mm, film thickness 0.25 μm; Alltech, Carnforth, UK) in a CP9001 Instrument (Chrompack, Middleburg, The Netherlands) connected to an EZ Chrom Data System (Scientific Software, San Ramon, Calif., USA) as previously described (Noble et al. 1993). The identification of the peaks was confirmed by comparison with the retention times of standard mixtures of fatty acid methyl esters (Sigma Chemicals, Poole, UK). An enzymatic-colourimetric assay kit (Boehringer, Lewes, UK) was used to measure free cholesterol.

Inorganic ions in eggs and neonates were analysed as described by Thompson et al. (1999a). Sub-samples of homogenised dried eggs (n = 15) and neonates (n = 13) were ashed in a muffle furnace at 500 °C. Ash was digested in 3 ml concentrated sulfuric acid at 140 °C overnight. Excess sulphuric acid was evaporated and 5 ml 1:1 HCl:water added. Volume was made up to 100 ml in a volumetric flask with RO water to give 0.25 M HCl. All plastic and glassware had been washed in 1% nitric acid. Sodium and potassium were analysed in 1-ml samples to which 1.5 ml $CsCl_2$ was added using an atomic absorption spectrophotometer (GBC 906AA). Known concentrations of Ringers solution containing NaCl and KCl were added to 5.9 mmol/l $CsCl_2$ to make up 2.5 ml to generate a standard curve for comparison with the unknown samples. Concentrations of calcium and magnesium were measured using an induction coupled plasma quantometric analyser (ICP: Applied Research Laboratories). A 2-ml sample in 0.25 M HCl was introduced to the ICP and the concentrations of calcium and magnesium compared to commercial standards.

Dried samples of another six eggs and five neonates were pressed into pellets of approximately 10 mg and burned in a Phillipson microbomb calorimeter. The calorimeter was calibrated periodically using benzoic acid throughout these analyses.

Total nitrogen content was measured in dried sub-samples of eggs (n = 5) and neonates (n = 6) using an automated Kjeldahl procedure (Tecator system digestion unit 1009 and Kjeltec system 1026 distilling unit). A sample was added to a glass tube containing a Kjeldahl catalyst tablet (high selenium, 1.0 g sodium sulphate anhydrous and 0.05 g selenium, Labchem A, Cat. no. 2206-1000) and 5 ml concentrated (98%) sulfuric acid and heated to 400 °C for 90 min. The sample was left to cool slowly and then was introduced

to the distillation procedure. NaOH in water (40% w/v) was added automatically prior to distillation. The distillate, collected in a flask containing 25 ml 4%(w/v) boric acid, was automatically titrated with 1 M HCl and the nitrogen content of the original sample calculated using the method of Clare and Stevenson (1964). Protein content was calculated from the nitrogen values using a conversion factor of 6.25 (Thompson 1981).

Differences between the amounts of ions, lipid classes and individual fatty acids derived from eggs and neonates were tested using one-way analysis of variance and significance assumed if $P < 0.05$. Equality of variances was compared using Bartlett's test. If variances were not equal, data were log transformed prior to analysis. Linear regressions were fitted with least squares regression using STATISTIX. Differences in egg and neonatal inorganic compositions among species of *Pseudemoia* were tested by ANCOVA using BIOMSTAT. Significance was assumed if $P < 0.05$. Data are presented as means ±1 SE.

Results

Mass, composition and energy densities

The mass of post-partum female *P. spenceri* is 3.34 ± 0.13 g ($n = 17$ females that gave birth) and mean SVL of females is 56.8 ± 0.5 mm ($n = 20$ females from which eggs were dissected). Wet mass of neonates is 312 ± 11 mg and SVL of neonates is 24.8 ± 0.3 mm ($n = 17$ mean of litters). Mean clutch size is 1.7 ± 0.1 ($n = 37$, range = 1–3); there is no significant relationship between the number of young (eggs and neonates) and female mass ($F_{(1,33)} = 0.69$, $P = 0.41$) or between female mass and dry mass ($F_{(1,15)} = 1.78$, $P = 0.20$) and wet mass ($F_{(1,15)} = 0.56$, $P = 0.46$) of the entire litter of neonates. In contrast, there are significant positive relationships between female mass (g) (X) and mean dry mass (mg) (Y) ($Y = 12.680 \cdot X + 26.286$, $r^2 = 0.304$, $P = 0.022$) and mean wet mass (mg) (Y) ($Y = 39.446 \cdot X + 180.531$, $r^2 = 0.248$, $P = 0.042$) of neonates. There is no significant relationship between female mass and either mean egg wet mass or dry mass.

The dry mass of neonates is 23% larger than the dry mass of eggs (Table 1). Eggs are composed almost entirely of protein, lipid and ash (Table 1). Although there is apparently more protein and lipid in neonates than in eggs, the differences are not significant (Table 1). In contrast, there is significantly more ash in neonates than in eggs (Table 1).

Neonates do not contain significantly more energy than eggs and their energy densities are not different (Table 1). There is significantly more calcium, potassium, and sodium, but not magnesium, in neonates than in eggs (Table 2).

Lipids

As a percentage of total lipid mass, triacylglycerol was the major class of lipid present in both the egg (67.4%) and the neonate (63.3%). Phospholipid was the second most predominant lipid class in eggs and neonates, forming about 22% of total lipid in both cases. A significant proportion (4.7%) of cholesteryl ester was present in the egg lipids and a greater proportion (7.1%) was found in the lipids of the neonate. Only low levels of free cholesterol (2.3%) were detected in the egg, with a higher proportion (4.8%) present in the neonate. Some free fatty acid was also detected in both eggs and neonates (Table 3).

The amounts of the most abundant lipid fractions, triacylglycerol and phospholipids, were not significantly different in neonates and eggs (Table 3). By contrast, the neonates contained significantly greater amounts of cholesteryl ester, free cholesterol and free fatty acid than the eggs.

Oleic acid (18:1n-9) was the major fatty acyl component of the triacylglycerol fraction of both the eggs (52.6 ± 1.2%) and neonates (59.1 ± 0.6%) (Table 4). The proportion of palmitoleic acid (16:1n-7) was considerably higher in the neonate (7.7 ± 0.9%) than in the egg (2.7 ± 0.1%). The proportions of the polyunsaturated fatty acids, linoleic (18:2n-6) and α-linolenic

Table 2 Mean (±1 SE) of ion contents (mg) of fresh eggs ($n = 15$) and neonates ($n = 13$) of *P. spenceri*

Ion	Eggs		Neonates
Calcium	0.711 ± 0.081	*	1.721 ± 0.111
Potassium	0.077 ± 0.005	*	0.551 ± 0.033
Magnesium	0.071 ± 0.011	NS	0.071 ± 0.014
Sodium	0.124 ± 0.007	*	0.378 ± 0.020

Asterisks indicate that value for eggs is significantly different from neonate (*$P < 0.001$, *NS* not significantly different)

Table 1 Mean (±1 SE) dry mass, protein, lipid, ash and energy content of eggs and neonates of *Pseudemoia spenceri*. Percentages are based on dry mass. *P* refers to probability derived from one-way ANOVA comparison of eggs and neonates

	Eggs	*n*	Neonates	*n*	*P*
Wet mass (mg)	116.0 ± 3.3	20	312.1 ± 10.5	17	<0.001
Dry mass (mg)	55.8 ± 1.7	20	68.6 ± 3.1	17	0.001
Protein (%)	67.4 ± 0.5	5	61.4 ± 1.9	6	–
Protein (mg)	36.7 ± 1.6	5	38.9 ± 1.6	6	0.362
Lipid (%)	26.7 ± 1.8	4	23.9 ± 2.6	6	–
Lipid (mg)	14.3 ± 1.1	4	17.5 ± 2.6	6	<0.001
Ash (%)	5.7 ± 0.8	6	9.0 ± 0.6	5	–
Total ash (mg)	3.0 ± 0.4	6	5.3 ± 0.3	5	0.002
Energy density (kJ/g ash-free)	25.7 ± 0.3	6	25.1 ± 0.5	5	0.325
Total energy (J)	1295 ± 53	6	1409 ± 195	5	0.557

Table 3 Proportions (as a percentage) and absolute amounts (mg) of major components that make up total lipids in eggs ($n = 4$) and neonates ($n = 5$) of *P. spenceri*. Asterisks indicate that the mount of lipid class per egg is significantly different from amount per neonate (*$P < 0.05$, *NS* no significant difference)

Lipid	%		mg		
	Eggs	Neonates	Eggs		Neonates
Triacylglycerides	67.4 ± 3.4	63.3 ± 2.7	9.9 ± 0.6	NS	11.1 ± 0.5
Phospholipids	23.2 ± 2.1	20.1 ± 1.9	3.4 ± 0.3	NS	3.5 ± 0.3
Cholesteryl esters	4.7 ± 0.9	7.1 ± 0.8	0.7 ± 0.1	*	1.2 ± 0.1
Free cholesterol	2.3 ± 0.3	4.8 ± 0.6	0.3 ± 0.1	*	0.8 ± 0.1
Free fatty acid	2.5 ± 0.8	4.7 ± 0.8	0.4 ± 0.1	*	0.8 ± 0.2

(18:3n-3) acids, were lower in the neonates compared with the eggs. Minor proportions of the $C_{20–22}$ polyunsaturates were detected in the egg triacylglycerol with even lower proportions present in the triacylglycerol of the neonates.

Palmitic acid (16:0) and 18:1n-9 were major components of the phospholipid fraction (Table 4) and their proportions were similar in the eggs and neonates. By contrast, although 18:2n-6 was a major component in the eggs (22.7 ± 1.0%), its proportion was much lower in the neonate (8.5 ± 0.5%). The egg phospholipids were relatively rich in arachidonic acid (20:4n-6) but contained only low proportions of docosahexaenoic acid (22:6n-3). The proportions of both 20:4n-6 and 22:6n-3 were markedly higher in the phospholipids of neonates than in those of the egg. Low proportions of eicosapentaenoic (20:5n-3) and docosapentaenoic (22:5n-3) acids were detected in the phospholipids of both eggs and neonates.

Phosphatidyl-choline (69.4%) was the major phospholipid class present in the egg with a significant proportion of phosphatidyl-ethanolamine (24.4%) and low levels of phosphatidyl-serine (3.2%) also present (Table 5). In addition, sphingomyelin formed approximately 3% of the total phospholipid. The main saturated fatty acid of phosphatidyl-choline was 16:0 with high proportions of 18:1n-9 and 18:2n-6 also present. The proportions of the $C_{20–22}$ polyunsaturates in phosphatidyl-choline were relatively low compared with the other phospholipid classes. Stearic acid (18:0) was the major saturated fatty acid of phosphatidyl-ethanolamine and particularly of phosphatidyl-serine. These latter two phospholipid classes contained lower proportions of 18:2n-6 but much higher levels of 20:4n-6 than were found in phosphatidyl-choline. Also, phosphatidyl-ethanolamine contained the highest proportions of 20:5n-3, 22:5n-3 and 22:6n-3 among the phospholipid classes.

The main fatty acid of the cholesteryl ester from both eggs and neonates was 18:1n-9 (Table 4). Although 18:2n-6 was also a major component in the eggs (14.9 ± 0.8%), its proportion was much reduced in the

Table 5 Fractions of phospholipids [Fatty acid (%w/w)] in eggs ($n = 4$) of *P. spenceri*. (*PC* phosphatidyl choline, *PE* phosphatidyl ethanolamine, *PL* phospholipid, *PS* phosphatidyl serine). Sphingomyelin makes up another 3.0 ± 1.0%

Fatty acid	PC	PE	PS
16:0	22.6 ± 0.6	4.5 ± 0.5	8.4 ± 0.9
16:1n-7	1.5 ± 0.2	0.4 ± 0.2	–
18:0	3.8 ± 0.2	10.1 ± 0.9	26.4 ± 1.5
18:1n-9	30.2 ± 1.9	30.8 ± 1.6	16.8 ± 1.1
18:2n-6	24.7 ± 1.9	14.8 ± 1.2	10.3 ± 0.9
18:3n-3	1.0 ± 0.6	0.6 ± 0.0	0.6 ± 0.6
20:1n-9	2.5 ± 0.3	10.2 ± 0.8	2.9 ± 1.0
20:4n-6	6.6 ± 0.5	17.8 ± 1.4	22.9 ± 1.2
20:5n-3	1.3 ± 0.4	2.0 ± 0.4	–
22:5n-3	0.5 ± 0.1	1.4 ± 0.1	–
22:6n-3	0.8 ± 0.1	2.0 ± 0.5	0.9 ± 0.9
% of PL	69.4 ± 5.7	24.4 ± 5.2	3.2 ± 0.4

Table 4 Mean percentage (%w/w ±1 SE) of fatty acids that make up the major lipid fractions of eggs ($n = 4$) and neonates ($n = 5$) of *P. spenceri*

Fatty acid	Triacylglycerol		Phospholipid		Cholesteryl ester		Free fatty acid	
	Eggs	Neonates	Eggs	Neonates	Eggs	Neonates	Eggs	Neonates
16:0	12.1 ± 0.6	14.5 ± 0.7	21.5 ± 0.9	19.1 ± 0.4	7.7 ± 0.8	3.9 ± 0.3	17.6 ± 2.3	11.9 ± 0.9
16:1n-7	2.7 ± 0.1	7.7 ± 0.9	1.4 ± 0.1	1.6 ± 0.1	2.5 ± 0.4	1.0 ± 0.3	0.3 ± 0.3	2.7 ± 0.5
18:0	4.2 ± 0.0	6.1 ± 0.1	5.1 ± 0.3	11.8 ± 0.3	3.4 ± 0.4	4.5 ± 0.3	13.2 ± 0.7	11.6 ± 0.4
18:1n-9	52.6 ± 1.2	59.1 ± 0.6	30.7 ± 1.3	27.8 ± 0.6	52.2 ± 1.1	40.8 ± 1.0	30.2 ± 1.6	40.3 ± 0.4
18:2n-6	11.8 ± 1.5	4.6 ± 1.1	22.7 ± 1.0	8.5 ± 0.5	14.9 ± 0.8	7.3 ± 0.5	10.8 ± 0.8	8.0 ± 0.4
18:3n-3	1.0 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	1.1 ± 0.1	0.8 ± 0.6	1.3 ± 1.2	0.1 ± 0.1
20:1n-9	9.3 ± 1.0	5.8 ± 0.2	3.5 ± 0.3	2.4 ± 0.1	2.6 ± 0.3	1.6 ± 0.1	5.1 ± 0.8	3.7 ± 0.2
20:4n-6	1.4 ± 0.1	0.3 ± 0.0	7.8 ± 0.3	14.9 ± 0.8	4.0 ± 0.2	3.2 ± 0.3	5.2 ± 0.4	11.3 ± 0.7
20:5n-3	0.4 ± 0.1	0.1 ± 0.0	1.0 ± 0.3	1.5 ± 0.2	0.9 ± 0.3	0.4 ± 0.1	1.2 ± 0.5	1.1 ± 0.2
22:5n-3	0.4 ± 0.0	0	0.5 ± 0.0	0.9 ± 0.0	0.2 ± 0.2	0.7 ± 0.0	0.2 ± 0.2	0.7 ± 0.1
22:6n-3	0.4 ± 0.0	0.1 ± 0.0	1.1 ± 0.1	5.4 ± 0.3	6.1 ± 1.0	4.3 ± 0.1	6.3 ± 2.3	2.1 ± 0.1

Table 6 Mean quantity (mg) of fatty acids that make up the total lipid of eggs ($n = 4$) and neonates ($n = 5$) of *P. spenceri*. *NS* no significant difference, $*P < 0.05$

Fatty acid	Eggs	Neonates	
16:0	1.66 ± 0.05	1.75 ± 0.05	NS
16:1n-7	0.29 ± 0.01	0.70 ± 0.02	*
18:0	0.59 ± 0.02	1.03 ± 0.03	*
18:1n-9	6.38 ± 0.22	6.90 ± 0.19	NS
18:2n-6	1.78 ± 0.06	0.87 ± 0.01	*
18:3n-3	0.12 ± 0.01	0.04 ± 0.00	*
20:1n-9	0.96 ± 0.01	0.70 ± 0.01	*
20:4n-6	0.35 ± 0.01	0.51 ± 0.01	*
20:5n-3	0.08 ± 0.01	0.01 ± 0.00	*
22:5n-3	0.06 ± 0.00	0.01 ± 0.00	*
22:6n-3	0.07 ± 0.01	0.18 ± 0.00	*

neonates (7.3 ± 0.5%). Relatively high proportions of 20:4n-6 (3.2–4.0%) and 22:6n-3 (4.3–6.1%) were present in the cholesteryl ester of both eggs and neonates. The free fatty acid fraction of both eggs and neonates consisted largely of 16:0, 18:0, 18:1n-9 and 18:2n-6 with significant proportions of 20:4n-6 and 22:6n-3 also present (Table 4).

The absolute amounts (mg) of 16:0 and 18:1n-9 from the total lipid did not differ significantly between the egg and the neonate (Table 6). However, the neonates contained greater amounts of 16:1n-7 and 18:0 and lower amounts of 18:2n-6, 18:3n-3 and 20:1n-9 than were present in the egg. Among the C_{20-22} polyunsaturates, neonates contained more 20:4n-6 and 22:6n-3 but less 20:5n-3 and 22:5n-3 than did eggs.

Discussion

Mass, composition and energy densities

We chose *P. spenceri* for this study because, like other members of the genus *Pseudemoia*, it has a complex chorioallantoic placenta (Stewart and Thompson 1998) and preliminary data indicated that this species has a larger egg size relative to the size of the neonate than other species of *Pseudemoia*. That prediction is supported because the egg size of *P. spenceri* is 55.8 mg dry mass, compared to 32.5 mg for *P. entrecasteauxii* (Stewart and Thompson 1993) and 18.2 mg for *P. pagenstecheri* (Thompson and Stewart 1994). Even though smaller neonates are produced by species with smaller egg sizes (Table 7), the ratio of dry mass in neonates to eggs for *P. spenceri* (1.2) is still lower than for either *P. entrecasteauxii* (1.7) (Stewart and Thompson 1993) or *P. pagenstecheri* (2.4) (Thompson et al. 1999a).

The three species of *Pseudemoia* for which there are data are placentotrophic, but a smaller proportion of the dry mass of the neonate is transported across the placenta in *P. spenceri* than in either of the other two species, despite the similarity of their placentae (Stewart and Thompson 1996, 1998). Because we do not know how the energetics of development are influenced by the sources of nutrients for embryos, we cannot determine if these ratios accurately predict differences in quantity of placental uptake. For example, the metabolic cost of yolk utilisation may differ from the cost of metabolism of placental nutrients. If we compare total quantities per embryo, differences between neonate dry mass and egg dry mass for the three species (*P. spenceri* 12.8 mg, *P. entrecasteauxii* 22.7 mg, and *P. pagenstecheri* 21.8 mg) indicate that the level of matrotrophy based on quantity of nutrients is similar for *P. pagenstecheri* and *P. entrecasteauxii*. This estimate of net placental uptake per embryo also indicates that the level of matrotrophy is less for *P. spenceri* than in the other two species.

Species within three genera (*Chalcides, Mabuya,* and *Pseudemoia*), all within the lizard family Scincidae, rely significantly on matrotrophy for embryonic nutrition (Blackburn et al. 1984; Ghiara et al. 1987; Stewart and Thompson 1993; Thompson et al. 1999a), whereas species with less complex chorioallantoic placentae have no net uptake of dry matter (summarised by Stewart and Thompson 1993). Species of *Pseudemoia* are of interest in that there are no obvious morphological differences in placental structure (Stewart and Thompson 1996, 1998), yet there are differences in egg size, neonatal size, placental uptake of nutrients and litter size among species (Table 7) (Rawlinson 1974; Greer 1982, 1989; Stewart and Thompson 1993, 1998; Thompson and Stewart 1994). Because of coevolution of life history characteristics, there may be important differences in demographic features among these three species that directly influence the level of placentotrophy. The role of embryonic nutrition in the evolution of life histories is unknown for matrotrophic reptiles.

Although there are great differences in size of eggs among the three species of *Pseudemoia* (Table 7), differences in the inorganic composition relative to egg size of recently ovulated eggs are small (Table 8). Appar-

Table 7 Reproductive characteristics of three species of *Pseudemoia*. (*SVL* snout vent length)

	P. spenceri	*P. entrecasteauxii*[a]	*P. pagenstecheri*[b,c]
Female SVL (mm)	56.8 ± 0.5	58.8 ± 0.8	56.5 ± 0.7
Litter size	1.7 ± 0.1	3.6 ± 0.2	4.5 ± 0.2
Egg dry mass (mg)	55.8 ± 1.7	32.5 ± 0.8	18.2 ± 0.4
Neonate dry mass (mg)	68.6 ± 3.1	54.6 ± 1.0	43.3 ± 5.2
Neonate/egg	1.23	1.68	2.38

[a] Stewart and Thompson 1993;
[b] Thompson and Stewart 1994;
[c] Thompson et al. 1999a

Table 8 Adjusted means ± SE from analysis of covariance (covariate = dry mass) for ion content (mg) of eggs and neonates of species of *Pseudemoia*. Data for *P. entrecasteauxii* from Stewart and Thompson (1993) and for *P. pagenstecheri* from [1]Thompson and Stewart (1994), [2]Thompson et al. (1999a)

	n	Calcium	Magnesium	Sodium	Potassium
Eggs					
P. entrecasteauxii	19	0.335 ± 0.036	0.036 ± 0.006	0.091 ± 0.005	0.076 ± 0.003*
P. pagenstecheri[1]	15	0.386 ± 0.093	0.045 ± 0.015	0.077 ± 0.012	0.052 ± 0.007
P. spenceri	14	0.386 ± 0.110	0.037 ± 0.018	0.091 ± 0.014	0.048 ± 0.009
Neonates					
P. entrecasteauxii	16	1.096 ± 0.075	0.039 ± 0.002*	0.437 ± 0.013	0.405 ± 0.015[a]
P. pagenstecheri[2]	8[3]	1.237 ± 0.117	0.049 ± 0.003	0.403 ± 0.020	0.458 ± 0.023[a,b]
P. spenceri	13[4]	1.642 ± 0.095*	0.052 ± 0.002	0.334 ± 0.016*	0.491 ± 0.018[b]

* significantly different ($P < 0.05$) values within the same column
[a] means do not differ significantly ($P < 0.05$)
[b] means do not differ significantly ($P < 0.05$)
[3] sample size for magnesium = 7
[4] sample size for magnesium = 12

ently, the inorganic composition of yolk does not vary with egg size. However, differences were found in ion contents among neonates (Table 8), indicating variation in the placental transfer of ions. We found no apparent pattern of variation in inorganic composition of neonates among species relative to egg size or level of matrotrophy.

Embryos of all Reptilia and Aves acquire calcium from a source in addition to yolk (Packard and Packard 1984; Packard 1994; Packard and Seymour 1997). Among squamate reptiles, this source is either the eggshell or placental uptake depending on the mode of reproduction of the species. Calcium derived from yolk contributes 77–81% of offspring calcium for four species of snake (two oviparous, two viviparous) (Packard et al. 1984; Packard and Packard 1988; Stewart 1989; Stewart et al. 1990) and there are no apparent differences associated with reproductive mode. Embryos of lizards may rely more heavily on the provision of calcium from non-yolk sources than snake embryos. Among the seven species of lizards that have been studied, yolk provides between 10% and 61% of offspring calcium (Thompson 1977; Packard et al. 1985; Stewart and Thompson 1993; Shadrix et al. 1994; Thompson et al. 1999a). Sample size is too small to clearly define trends that might be associated with reproductive mode and placental specialisation. For example, while the two highest percentages are oviparous species and the two lowest are species with Type III chorioallantoic placentation, the percentages for the remaining three species are virtually identical and these species differ in reproductive mode and placental specialisation (Table 9).

The mechanism for regulation of placental transfer of calcium among squamate reptiles is unknown and either maternal or embryonic tissues may mediate calcium delivery. The pattern of calcium provision to embryos among three species of *Pseudemoia* suggest that both uterine and embryonic systems may be involved in the evolution of placental provision of calcium. The total mass of yolk varies among these species (Table 7), but the relationship between dry mass of yolk and mass of calcium in yolk is constant (Table 8). The common regression (yolk calcium = −0.23 + 0.017 egg dry mass, $F_{(1,47)} = 134$, $P \leq 0.001$) indicates that if the evolution of placentotrophy occurs by yolk reduction without a concomitant decrease in calcium content of neonates, then a net placental transfer of 0.17 mg calcium must occur for each 10 mg decrease in yolk dry mass. Differences between *P. entrecasteauxii* and *P. pagenstecheri* fit this prediction. Based on mean egg dry mass for each

Table 9 Summary of calcium provision to embryos of squamate reptiles. Parity: *O* oviparous, *VI* viviparous with type I chorio-allantoic placenta, *VIII* viviparous with type III chorio-allantoic placenta

	Parity	Egg (mg)	Neonate (mg)	Neonate/Egg	Non-yolk Ca^{++}
Coluber constrictor[1]	O	29.6	38.3	1.3	23%
Pituophis melanoleucus[2]	O	88	116	1.3	24%
Virginia striatula[3]	VI	2.6	3.2	1.2	19%
Thamnophis ordinoides[4]	VI	10.7	13.9	1.3	23%
Pogona barbatus[5]	O	9.4	15.8	1.7	40%
Eumeces fasciatus[6]	O	0.72	1.19	1.6	39%
Bassiana duperreyi[7]	O	0.44	1.01	2.3	56%
Sphenomorphus quoyii[8]	VI	2.1	4.8	2.3	56%
P. spenceri	VIII	0.71	1.72	2.4	59%
P. entrecasteauxii[7]	VIII	0.30	1.09	3.6	72%
P. pagenstecheri[9]	VIII	0.11	1.14	10.4	90%

[1] Packard et al. 1984; [2] Packard and Packard 1988; [3] Stewart 1989; [4] Stewart et al. 1990; [5] Packard et al. 1984; [6] Shadrix et al. 1994; [7] Stewart and Thompson 1993; [8] Thompson, 1977; [9] Thompson et al. 1999a

species (Table 7), the predicted differences in placental uptake of calcium between *P. entrecasteauxii* and *P. pagenstecheri* is 0.24 mg for neonatal calcium content to be the same between species. This is identical to the measured value of 0.24 mg (*P. pagenstecheri* 1.03 mg; *P. entrecasteauxii* 0.79 mg) (Table 9). Total calcium content of neonates is similar (Table 9) and there is no significant difference in relative calcium content of neonates between these species (Table 8). These data suggest that the relative composition of calcium in yolk does not vary and the likely pattern for the evolution of embryonic nutrition is for variation in yolk quantity to be compensated by changes in placental transfer of calcium. This pattern would be expected if calcium uptake was driven by embryonic metabolism.

In contrast, the quantity of calcium in neonates relative to neonatal dry mass is higher for *P. spenceri* than in the other two species (Table 8). Because the relative amounts of calcium in the yolk do not differ among species (Table 8), placental uptake of calcium for *P. spenceri* must be greater relative to increase in mass of neonates than in the other species. These data suggest that either there is a positive allometry for calcium content associated with larger size in neonatal *P. spenceri* or placental uptake of calcium is not coupled solely with embryonic metabolism. Calcium uptake may be regulated by placental delivery mechanisms in addition to embryonic metabolism.

The lack of a significant uptake of magnesium across the placenta in *P. spenceri* (Table 2) is similar to *P. pagenstecheri* (Thompson et al. 1999a). Even though there is significantly more magnesium in neonates than in eggs of *P. entrecasteauxii*, the absolute difference is very small (Stewart and Thompson 1993). We conclude that yolk provides a supply of magnesium sufficient for embryological development in these three species. In contrast, placental uptake of calcium, potassium and sodium is quantitatively large and probably biologically important. The net uptake of sodium across the placenta for species with type III chorioallantoic placentation is approximately twice that of most species with type I placentae and the net uptake of potassium is approximately five times that of other species. Thus, it seems that all the requirements for magnesium are met by provision in the yolk of matrotrophic species during vitellogenesis, whereas calcium, sodium and especially potassium are provided across the placenta.

A pattern of supply of inorganic ions to embryos of squamate species is beginning to emerge. In oviparous species, there is a net uptake of calcium and sodium, but not magnesium and potassium from the eggshell (Stewart and Thompson 1993; Table 9). Viviparous species with type I chorioallantoic placentae typically have net placental uptake of calcium, sodium and potassium, but rely on the yolk as a source of magnesium (Thompson 1977, 1981; Stewart 1989, 1992; Stewart et al. 1990). Previous studies for species with type III chorioallantoic placentae show a similar trend (Stewart and Thompson 1993; Thompson et al. 1999a) and our data for *P. spenceri* support this pattern.

Interestingly, the energy density of eggs and neonates of *P. spenceri* is similar, reflecting their similar compositions. Although there is more energy in neonates than in eggs as expected by their larger size, the difference is not significant. The energy content of neonates is, however, influenced by the inclusion of a relatively small neonate in the sample. Nevertheless, the difference in energy content of eggs and neonates is smaller than the difference in their dry masses. Part of this difference results from the higher proportion of ash in neonates than in eggs (Table 1). Energy densities of eggs are generally higher than those of neonates in viviparous (Thompson et al. 1999a) and oviparous species (Thompson and Stewart 1997; Thompson and Russell 1998), reflecting a higher proportion of lipid in the eggs than in the neonates. *P. spenceri* is unusual in having a higher proportion of protein in the egg than in the neonate (Table 1). Nevertheless, the energy density of both eggs and neonates of *P. spenceri* is within the range for other lizards (Ballinger and Clark 1973; Booth and Thompson 1991; Ji 1992; Thompson and Stewart 1997; Thompson and Russell 1998). Furthermore, the lipid content of yolk is similar to that in other viviparous species, which tend to be lower than some, but not all, oviparous species (Thompson 1981; Ji 1992).

Although there is no statistically significant net uptake of protein or lipid across the placenta, there must be a biologically relevant gross uptake. By assuming that the organic mass of a neonate is 83% of the organic mass required for its development (Stewart and Thompson 1993), we have calculated that 23.5 mg of organic matter, or 45% more than was in the egg at ovulation, must be transported across the placenta during development in *P. spenceri* to provide energy for embryonic development.

P. spenceri may exhibit both categories of placental nutritional provision that have been proposed to describe the diversity of patterns of embryonic nutrition in squamates (Stewart 1989). Since its neonates are larger by a factor of 1.2 than the eggs, and considerable organic matter is taken up across the placenta, *P. spenceri* probably exhibits obligate placentotrophy i.e. placental nutrition is required for the production of viable offspring. Although our experiment was not designed specifically to determine whether *P. spenceri* also exhibits facultative placentotrophy [i.e. where placental provision of nutrients supplements (enhances) embryonic nutrition but this supplementation is not required for successful development] we can draw some inferences. Like *P. pagenstecheri* (Thompson et al. 1999a), larger female *P. spenceri* produce larger offspring, but do not ovulate larger eggs. Thus, larger females enhance the size of neonates by providing a greater quantity of placental nutrition than smaller females. As these larger neonates are larger than the minimum size for the population, i.e. those produced by smaller females, the females producing these large neonates exhibit facultative

placentotrophy. Since facultative placentotrophy also occurs in other obligate placentotrophic species (*P. entrecasteauxii*, Stewart and Thompson 1993; *P. pagenstecheri*, Thompson et al. 1999a) as well as species with simple chorioallantoic placentae (Stewart 1989), this result is not surprising, yet highlights an important characteristic of placentotrophic reptiles.

Lipids

The amount of lipid recovered in the neonates probably represents merely a portion of the lipid transferred to the embryo during development. In oviparous species such as the domestic chicken (Noble and Cocchi 1990), the turtle, *Emydura macquarii* (Thompson et al. 1999b) and the lizards *Eumeces fasciatus* and *Menetia greyii* (Thompson and Stewart 1997; Thompson and Russell 1998), approximately 50% of the total lipid originally present in the egg is used for energy during embryonic development. Thus, it is likely that considerably more lipid than the difference between that in the neonate and the egg of *P. spenceri* must have passed across the placenta during gestation to provide an energy substrate for embryonic development. In addition, it is likely that protein was also taken across the placenta and, as in eggs of oviparous lizards, used as an energy substrate (Thompson and Stewart 1997; Thompson and Russell 1998). However, since we do not know the relative proportions of oxidation of lipid and protein for provision of energy during development in *P. spenceri*, we cannot calculate how much of the estimated 23.5 mg of organic matter taken up across the placenta is lipid compared to protein.

Specific evidence for placental transfer of lipids is provided by the observation that the absolute amounts (mg per egg or per neonate) of several lipid components are greater in the neonate than in the egg. In particular, the amounts of free and esterified cholesterol are respectively 2.0-times and 2.7-times greater in the neonate than in the egg. Since cholesterol can not be oxidised for energy, it may be more appropriate than the other lipid classes as a marker for placental transfer. The form and mechanism by which cholesterol is transported across the placenta (e.g. via the receptor-mediated uptake of low- or high-density lipoproteins from the maternal circulation) is a subject for future studies.

The absolute amounts of the major fatty acids, 16:0 and 18:1n-9, of the total lipid in the egg and neonate are not significantly different. However, all the other fatty acids listed are present in significantly greater or lesser amounts in the lipids of the neonates than in the eggs. An unequivocal interpretation of these changes is not possible because of the multiple factors which could determine the fatty acid profile of lipids in neonates. Thus the differences between the egg and neonate could reflect a combination of the preferential oxidation of certain fatty acids for energy by the embryo, the conversion by the embryo of one fatty acid to another by desaturation/elongation and the selective transfer of certain fatty acids across the placenta. Increases of 16:1n-7 and 18:0 during development, however, could indicate the net transfer of these fatty acids across the placenta during gestation as their de novo synthesis is unlikely. On the other hand, the depleted proportions of 18:2n-6 and 18:3n-3 in the neonate (Table 6) may be due to the preferential use of these fatty acids for energy during embryonic development. Studies in mammals and birds (Neuringer et al. 1988) have suggested that 20:4n-6 and 22:6n-3 have important roles during embryonic life with particular relevance to the development of the central nervous system. Thus, it may be of note that the proportions of both these fatty acids are greater in the phospholipids of the neonate than in those of the egg. Moreover, there is a significantly greater amount of these long-chain polyunsaturates in the lipids of the neonate than in those of the egg (Table 6). This biomagnification is particularly marked for 22:6n-3 since the amount of this fatty acid in the neonate is 2.6-times greater than in the egg. It should be pointed out that the increase in the 22:6n-3 content of the neonate compared with the egg, amounting to some 0.11 mg, could be almost exactly accounted for by the combined decrease in the amounts of 20:5n-3 and 22:5n-3. Thus, it is feasible that the additional 22:6n-3 may be synthesised by the desaturation and/or elongation of 20:5n-3 and 22:5n-3 by the embryo. The proportion of 22:6n-3 in the egg lipids of *P. spenceri* is far lower than in the eggs of chickens and alligators (Noble 1991), which possibly reflects the largely insectivorous diet of the lizard (Brown 1986).

The proportions of triacylglycerol and phospholipid in the eggs of *P. spenceri*, at about 67% and 23% of total egg lipid, respectively, are similar to those of eggs of chickens and alligators (Noble 1991). However, a unique feature of these lizard eggs is the relatively high proportion of cholesteryl ester (4.7% compared with only about 1% in birds) and the low proportion of free cholesterol (2.3% compared with about 5% in birds) (Noble 1991). A comparison with the egg lipid compositions of related species of oviparous lizards may provide insights into possible adaptations related to the evolution of viviparity.

In conclusion, although *P. spenceri* has a complex placenta, it has a larger relative egg size and is both relatively and quantitatively less placentotrophic than other species that have been studied with similar placentae. Nevertheless, *P. spenceri* almost certainly is an obligate placentotroph and also exhibits facultative placentotrophy. There is a large net uptake of calcium, sodium and particularly potassium across the placenta. Additional lipid and probably protein must have been transported to the embryo across the placenta but oxidised to provide energy for developmental processes. Our data provide evidence for a net transfer of cholesterol and at least some fatty acids across the placenta in addition to the inter-conversion (desaturation/elongation) of fatty acids by the embryo. However, some

nutrients appear to be provided in the egg with no net uptake across the placenta (e.g. magnesium).

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