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PLACENTAL NUTRITION IN THE VIVIPAROUS LIZARD *NIVEOSCINCUS METALLICUS*: THE INFLUENCE OF PLACENTAL TYPE

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Summary

The ion, energy, lipid, nitrogen and fat-soluble vitamin contents of freshly ovulated eggs and neonates of the viviparous lizard *Niveoscincus metallicus* were measured to quantify uptake of nutrients across the placenta. This species is particularly interesting because it has a chorio–allantoic placenta that is intermediate in complexity compared to viviparous species that have been the focus of other studies. Newly ovulated eggs have a wet mass of 79.6±4.6 mg and a dry mass of 41.8±2.8 mg, compared to the neonates that have a wet mass of 224.2±8.2 mg and dry mass of 37.9±1.2 mg. Thus, there is no significant net uptake of dry matter across the placenta. Neonates have significantly less lipid (6.2±0.4 mg) than eggs (12.7±0.5 mg), but no significant difference in nitrogen (4.1±0.3 mg) compared to eggs (4.5±0.2 mg). Energy densities reflect the protein and lipid composition and the relative dry masses of the eggs and neonates. There is significantly more energy (1029.1±80.0 J) in the egg than in the neonate (858.2±38.6 J). The increase in the ash content of the neonates (2.9±0.2 mg) compared to fresh eggs (2.1±0.3 mg) was not significant, even though there was an approximately threefold increase in the amount of sodium (0.11±0.01 mg in neonates, 0.34±0.01 mg in eggs) and potassium (0.12±0.017 in neonates, 0.40±0.01 mg in eggs) in neonates compared to eggs. There was no significant uptake of calcium and magnesium during development. The egg lipids consisted of triacylglycerol (66.7±2.3 %), phospholipid (18.9±0.7 %), cholesteryl ester (4.9±1.6 %) and free cholesterol (5.6±1.5 %). The egg phospholipid contained comparatively high proportions of arachidonic and eicosapentanoic acids but low levels of docosahexaenoic acid (DHA), whereas the phospholipid of the neonate was greatly enriched in DHA. In the egg, the predominant vitamin E was α-tocopherol (62.6±3.4 mg g^{-1}), although there was some γ-tocotrienol (3.5±0.3 mg g^{-1}), and vitamin A was present (1.5±0.2 mg g^{-1}). The ratio of neonate dry mass to egg dry mass of *N. metallicus* (0.91) lies between that of species with type I (0.78) and type III (1.70) chorio–allantoic placentae, confirming our conclusion that the placenta of *N. metallicus* is functionally intermediate, as well as intermediate in complexity, between these other two types.

Key words: placenta, viviparity, fetal nutrition, lipid, calcium, sodium, egg, lizard, *Niveoscincus metallicus.*

Introduction

Although the ancestral, and most common, mode of reproduction in squamate reptiles is oviparity (Blackburn, 1992), viviparity has evolved often in squamates (Blackburn, 1982, 1985; Shine, 1985). There is, however, little chorio–allantoic placental complexity (Stewart, 1992) and probably little exchange of organic nutrients across the placenta of most viviparous species (Blackburn et al., 1984; Stewart and Thompson, 1993). Nevertheless, some species are highly placentotrophic, the most extreme being the complex, eutherian-like chorio–allantoic placentae of lizards of the genus *Mabuya* (Blackburn et al., 1984). Study of functionally intermediate forms is often instructive as it has the potential to expose information about possible steps involved in the evolution of a functional complex. Four types of chorio–allantoic placentation have been described among viviparous Squamata, termed, in increasing order of complexity, types I–III (Weekes, 1935; Blackburn, 1993; Stewart and Thompson, 1994) and type IV (Blackburn, 1992, 1993). Type I placentae, the most common form in viviparous squamates, involve little modification to basic oviparity, except that the eggshell is reduced and the eggs are retained *in utero* throughout embryonic development (Weekes, 1935).

Placental morphology of the next two most complex placental types, designated types II and III, has been described (Weekes, 1929, 1930, 1935; Stewart and Thompson, 1994, 1996, 1998) and the pattern of embryonic nutrition is known

for species with type III placentae (Stewart and Thompson, 1993; Thompson and Stewart, 1994; Thompson et al., 1999). The type II chorio–allantoic placenta has superficial placement of uterine blood vessels, which bulge into the uterine lumen, forming folds against which the chorionic cells of the embryo are apposed (Weekes, 1930). In contrast, type III placentae have considerable elaboration of both embryonic and maternal tissue (Weekes, 1935; Stewart and Thompson, 1996, 1998), with significant uptake of nutrients across the placenta (Stewart and Thompson, 1993; Thompson and Stewart, 1994; Thompson et al., 1999). In this study, we characterise the fatty acid, protein, energy and ionic content of recently ovulated yolks and of neonates for the Tasmanian skink *Niveoscincus metallicus*, which has a complex chorio–allantoic placenta with some features of Weekes' (1930) type II (Stewart and Thompson, 1994). The reproductive biology of *N. metallicus* is well studied (Swain and Jones, 1994; Jones and Swain, 1996) and placental transfer of the amino acid leucine and embryonic utilisation of some lipid fractions have been documented (Swain and Jones, 1997; Jones et al., 1998). By using comparative analyses of composition, we are able to identify uptake of nutrients across the placenta, which we can then compare to species with more (type III) or less (type I) elaborate placentae (Hadley and Christie, 1974; Thompson, 1981; Stewart and Castillo, 1984; Stewart and Thompson, 1993; Stewart, 1989, 1992; Stewart et al., 1990; Thompson and Stewart, 1994; Thompson et al., 1999).

This study is the first analysis of the pattern of embryonic nutrition in *N. metallicus*, although previous work has revealed that transfer of organic molecules does occur (Swain and Jones, 1997; Jones et al., 1998). Evolution of the eutherian-like placentation of squamates with type IV placentae must have involved reduction of yolk size with a concomitant increase in nutrient provision across the placenta. The reduction in ovum size may have occurred by a simple reduction of the quantity of yolk. Alternatively, some components of the yolk could have been maintained in the yolk while others were proportionally reduced compared to the concentration in eggs of their oviparous ancestors. The study of yolk composition and net placental uptake in a species with intermediate complexity of the chorio–allantoic placenta will provide the comparative data needed for analyses of patterns of variation in yolk quantity among viviparous species.

Materials and methods

Gravid female *Niveoscincus metallicus* (O'Shaughnessy, 1874) (N=37) were collected at The Thumbs, about 6 km south of Orford on the east coast of Tasmania (42°36′S, 147°54′E) at an elevation of 200–300 m, at the time of expected ovulation in the austral spring (9–10 October), 1996. The females were returned to the University of Sydney and accommodated in groups of four or five in aquaria containing 10–20 mm depth of peat moss, pine bark and flat rocks to provide shelter. Water was provided *ad libitum* in 40 mm diameter plastic Petri dishes and meal worms (*Tenebrio molitor*) and crickets (*Acheta domestica*) dusted with calcium gluconate were provided three times per week. The aquaria were housed in a room at 19 °C. A 25 W incandescent light bulb provided heat at one end of each aquarium for 9 h per day to create a thermal gradient within the aquarium and the light regime followed that of the local environment.

Females were assigned to one of three treatments: experimental (N=16), sham-operated controls (to control for possible effects of the surgery) (N=10) and unoperated controls (N=11). In this way, eggs and neonates could be obtained from each female. Females were distributed among treatments so that snout–vent lengths (L_{SV}) were not significantly different (ANOVA, F=2.42, 36 d.f., P>0.05). After anaesthetic (5 % halothane in oxygen), one oviduct containing freshly ovulated eggs was removed from the experimental females through a small incision on the right flank. Embryos were at an early stage of development and were not visible by eye. The incision was closed using monofilament polypropylene 5/0 sutures and the female allowed to recover. The sham-operated controls were treated in the same way, except that the oviduct was not removed. Females were separated just prior to parturition so that the offspring of individual females could be identified. All young were killed by rapid freezing within 24 h of birth. Dissection showed that none retained any residual yolk. Females and neonates were measured (snout–vent length and tail length) to an accuracy of 1 mm using a ruler and weighed to an accuracy of 10 mg on a top-pan balance (females) or 0.1 mg using an analytical balance (neonates).

Lipid was extracted from newly ovulated eggs (N=10) and neonates (N=8 from each treatment) by homogenisation in a suitable excess of chloroform–methanol (2:1, v/v) and the composition of the lipid fractions was analysed exactly as described by Thompson et al. (1999). Vitamin E fractions and vitamin A were extracted using petroleum spirit and concentrations were measured in eggs (N=3) using high-pressure liquid chromatography (HPLC) as described by Thompson et al. (1999). Eggs (N=10) and neonates (N=3 experimental; N=5, sham-operated; N=5, control) from different litters were lyophilised and then homogenised using a mortar and pestle. Dried sub-samples of the homogenate were ashed in a muffle furnace at 500 °C; the amounts of Na and K in the ash were measured using an atomic absorption spectrophotometer (GBC 906AA) and Ca and Mg in the ash were measured using an Induction Coupled Plasma Quantometric Analyser (Applied Research Laboratories) as described by Thompson et al. (1999).

Sub-samples from another five dried eggs and eight dried neonates (three from each of the sham-operated and experimental females and two controls) were pressed into pellets of approximately 10 mg and completely combusted in a Phillipson Microbomb Calorimeter. The calorimeter was calibrated using benzoic acid periodically throughout these analyses.

Further homogenised dried sub-samples (N=5, eggs; N=7, neonates) were used to determine the total nitrogen content of the samples using an automated Kjeldahl procedure (Tecator

System Digestion Unit 1009 and Kjeltec System 1026 Distilling Unit). Samples were added to glass tubes together with a Kjeldahl catalyst tablet (High Selenium; 1.0 g anhydrous sodium sulphate and 0.05 g selenium, Labchem A, Cat. No. 2206–1000) and 5 ml of concentrated (98 %) sulfuric acid. The mixture was heated to 400 °C for 90 min and left to cool slowly. NaOH in water (40 % w/v) was added automatically and the sample was distilled. The distillate, collected in a flask containing 25 ml of 4 % (w/v) boric acid, was automatically titrated with 1 mol l^{-1} 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 estimates of size of females from different treatments and between contents and concentrations of ions in eggs and neonates were tested by one-way analysis of variance or *t*-test using STATISTIX. Differences between experimental, sham-operated and control groups for estimates of neonate size were tested by nested analysis of variance using BIOMstat (1996). Linear regressions were computed using Excel 5. Significance was 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. Percentage data for lipids were arcsin-transformed prior to analysis by MANOVA using SAS. Linear regressions were fitted by using least squares regression with multiple *y*-values for each independent *x*-value using BIOMstat. Data are presented as means ± S.E.M.

Results

Mass, composition and energy densities

There was no significant difference in the post-partum size of females in snout–vent length (L_{SV}) or mass (Table 1) and no significant difference in the number of young (young or eggs removed + neonates) for females from each treatment. There was no significant relationship between neonate wet mass and post-parturient female mass or between neonate L_{SV} and post-parturient female mass. Litter size was weakly positively correlated with female L_{SV} (litter size=0.075$f_{L_{SV}}$–1.426, $F(1,29)=4.65$, $P=0.039$, $r=0.372$), but not with post-parturient mass of females for the combined data set (all treatments).

Although there was no significant difference in L_{SV} of neonates from females in different treatments, neonates from control females were significantly heavier (wet mass) than neonates from the other two treatments ($F(2,34)=4.37$, $P=0.02$) (Table 1). Only a sub-sample of neonates was available for dry mass determination ($N=8$, experimental; $N=7$, sham-operated; $N=6$, control) and they were not significantly different in dry mass between treatments. Hence, neonates were pooled for bomb calorimetry. Dry mass of neonates was about 10 % less than the dry mass of eggs (Table 2). Eggs were composed predominantly of protein with a significant contribution of ash. The remainder of the egg was lipid. Neonates were composed predominantly of protein, but with substantial proportions of lipid and ash.

Neonates contained about 17 % less energy than eggs, commensurate with their smaller size and lower lipid content (Table 2). Neonates had a lower energy density than eggs. The contents of calcium, potassium, magnesium and sodium were not significantly different in neonates from each of the treatments, so data for all neonates were pooled to compare with ion contents of eggs. There was significantly more potassium ($T(21)=20.27$, $P<0.001$) and sodium ($T(21)=14.29$, $P<0.001$), but not ash, calcium or magnesium, in neonates than in eggs.

Lipids

The MANOVA analysis of arcsin-transformed data showed no significant treatment effect for any of the lipid analyses, except triacylglycerol (Wilks' Lambda=0.087, $F=2.167$, $P=0.043$). Hence data for neonates from each of the three treatment groups were pooled for comparison with eggs. As the treatment effect for triacylglycerol was weak and involved only three out of twelve fatty acids, these data were pooled across treatments, also.

Triacylglycerol was the predominant lipid fraction in both eggs (67 %) and neonates (47–59 %). Phospholipids were also major components, representing 18 % of the total lipid. Although present in eggs, both free and esterified cholesterol represented larger fractions of the lipids in neonates than in eggs. The proportion of free fatty acids was approximately fourfold higher (18 %) in neonates than in eggs (4 %) (Table 3).

The triacylglycerol fatty acid profiles of eggs and neonates were very similar (Table 4). The major fatty acid components

Table 1. *Combined post-partum size of females and neonates from females of* Niveoscincus metallicus *from all experimental groups*

	Neonate			
	Control	Sham-operated	Experimental	All females
L_{SV} (mm)	21.2±0.2	20.8±0.3	21.0±0.2	48.9±0.50
Wet mass (g)	0.243±0.004*	0.218±0.008	0.223±0.005	2.722±0.058
Dry mass (g)	0.0388±0.0009	0.0376±0.0025	0.0413±0.0019	–

L_{SV}, snout to vent length.
$N=11$, controls; $N=10$, sham-operated; $N=16$, experimental females.
*Wet masses of neonates from control females are significantly larger than those from other treatments ($P=0.02$).

Table 2. *Measurements of variables of eggs and neonates of* Niveoscincus metallicus

Variable	Eggs (*N*)	Neonates (*N*)	Significance
Wet mass (mg)	79.6±4.6 (5)	224.2±8.2 (8)	*
Dry mass (mg)	41.8±2.8 (5)	37.9±1.2 (8)	NS
Ash (mg)	2.1±0.3 (5)	2.9±0.1 (8)	NS
Ash (%)	5.1±0.6 (5)	8.0±0.5 (8)	–
Total energy (J)	1029.1±80.0 (5)	858.2±38.6 (8)	*
Energy density ($kJ\,g^{-1}$ ash-free)	25.1±0.2 (5)	24.1±0.6 (8)	NS
Nitrogen (mg)	4.5±0.2 (5)	4.1±0.3 (7)	NS
Lipid (mg)	12.7±0.5 (8)	6.2±0.4 (6)	*
Protein of dry mass (%)	67.8±0.8 (5)	66.9±2.7 (7)	–
Lipid of dry mass (%)	30.4±1.2 (8)	16.4±1.0 (6)	–
Calcium	0.66±0.08 (10)	0.72±0.02 (13)	NS
Potassium	0.12±0.01 (10)	0.40±0.01 (13)	*
Magnesium	0.12±0.02 (10)	0.16±0.06 (13)	NS
Sodium	0.11±0.019 (10)	0.35±0.01 (13)	*

Values are mean ± S.E.M.

Mass and ash data are means for specimens used in bomb calorimetric analyses.

NS, not significantly different; *$P<0.001$.

Table 3. *Proportions (%) of major components of total lipids in eggs and neonates from control, sham-operated control and experimental female* Niveoscincus metallicus

Lipid	Egg	Neonates
Triacylglycerides	66.7±2.3	51.3±2.6
Free fatty acids	3.8±0.9	15.6±1.1
Phospholipids	18.9±0.7	17.1±0.8
Cholesteryl esters	4.9±1.6	8.4±0.6
Free cholesterol	5.6±1.5	7.6±0.6

N=10, eggs; *N*=23, neonates (8 sham+8 controls+7 experimental animals).

of the triacylglycerol fraction of both eggs and neonates were palmitic (16:0), oleic (18:1*n*-9) and linoleic (18:2*n*-6) acids. Similar proportions of stearic (18:0) and α-linolenic (18:3*n*-3) acids were also found in the eggs and neonates. The egg triacylglycerol contained significant proportions of arachidonic (20:4*n*-6) and eicosapentaenoic (20:5*n*-3) acids; lower proportions of these two fatty acids were present in the triacylglycerol of neonates.

The major fatty acids of the egg phospholipid were 16:0, 18:1*n*-9 and 18:2*n*-6 (Table 4). Relatively high proportions of 20:4n-6 and 20:5*n*-3, but only low proportions of docosapentaenoic (22:5*n*-3) and docosahexaenoic (22:6*n*-3) acids, were present in the egg phospholipid. The phospholipid of the neonates differed in composition from that of the eggs, containing lower proportions of 18:2*n*-6, 18:3*n*-3 and 20:5*n*-3 but higher proportions of 18:0, 20:4*n*-6, 22:5*n*-3 and particularly 22:6*n*-3.

Phosphatidylcholine was the major class of phospholipid in both eggs and neonates with substantial proportions of phosphatidylethanolamine and a lower proportions of phosphatidylserine also present (Table 5). Phosphatidylcholine of both eggs and neonates was characterised by a high content of 16:0 and 18:2*n*-6 and lower amounts of 18:0 and of the $C_{20–22}$ polyunsaturates compared with the other phospholipid classes. Phosphatidylethanolamine and phosphatidylserine, by contrast, were rich in 18:0 and 20:4*n*-6 with lower proportions of 16:0 and 18:2*n*-6. Phosphatidylserine was particularly rich in 20:4*n*-6, whereas phosphatidylethanolamine had the highest proportions of the $C_{20–22}$*n*-3 polyunsaturates. A key difference between the eggs and neonates was the presence, in the latter, of far higher proportions of 22:6*n*-3 in the phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine fractions.

The cholesteryl ester fraction of the eggs contained 18:1*n*-9 as the major fatty acid, but also contained high proportions of

Table 4. *Mean percentage of fatty acids in the major lipid fractions of eggs and neonates of* Niveoscincus metallicus

Fatty acid (% w/w)	Triacylglycerol		Phospholipids		Cholesteryl ester		Free fatty acid	
	Eggs	Neonates	Eggs	Neonates	Eggs	Neonates	Eggs	Neonates
14:0	1.0±0.2	1.2±0.1	0	0.9±0	0	0.4±0.1	0.7±0.2	0.4±0
16:0	13.7±0.3	16.3±0.4	24.2±0.4	25.4±0.3	7.2±0.4	8.1±0.3	14.6±1.1	11.1±0.2
16:1*n*-7	4.1±0.3	3.8±0.3	1.3±0.2	1.3±0	2.3±0.4	0.9±0.1	2.5±0.4	1.7±0.2
18:0	5.0±0.2	6.2±0.3	5.3±0.2	13.3±0.2	4.6±0.7	3.7±0.1	8.4±0.9	8.2±0.1
18:1*n*-9	42.0±1.8	40.5±1.1	22.6±1.3	20.3±0.3	51.0±1.3	36.5±0.8	29.0±1.9	25.7±0.8
18:2*n*-6	19.5±1.0	19.0±0.8	28.9±0.7	10.6±0.3	15.6±0.6	21.6±0.7	14.5±0.5	18.5±0.5
18:3*n*-3	5.1±0.9	5.5±0.6	1.8±0.5	0.6±0.1	1.8±0.8	0.9±0.1	6.4±2.2	2.5±0.2
20:1*n*-9	0.9±0.2	1.3±0.1	0.5±0.1	1.1±0	3.0±0.6	6.1±0.2	1.2±0.1	1.2±0
20:4*n*-6	2.6±0.2	1.5±0.1	8.9±0.7	12.8±0.4	11.3±1.1	7.2±0.4	7.1±1.3	21.0±0.7
20:5*n*-3	1.8±0.2	0.5±0.1	3.4±0.3	1.8±0.1	4.6±0.7	1.1±0.1	4.3±0.5	0.3±0
22:5*n*-3	0.8±0.1	0.7±0	0.6±0.2	1.6±0	0.2±0.2	0.4±0.1	1.6±0.3	2.5±0.1
22:6*n*-3	0.7±0	0.6±0	0.8±0.1	6.7±0.1	0	2.2±0.1	0.8±0.1	3.2±0.1

Values are given as % w/w ± S.E.M.

N=10, eggs; *N*=23, neonates (8 sham+8 controls+7 experimental animals).

Table 5. *Mean percentage of fractions of phospholipids in fresh eggs of* Niveoscincus metallicus

Fatty acid (% w/w)	Eggs			Neonates		
	PC	PE	PS	PC	PE	PS
% of PL	46.8±0.9	34.6±2.6	6.3±2.0	54.7±1.4	26.1±1.1	11.0±1.0
14:0	0.2±0	0	0	0.9±0.1	0.4±0.1	0
16:0	25.7±0.6	6.1±0.7	7.6±0.9	33.3±0.5	9.1±0.4	7.5±0.4
16:1*n*-7	1.6±0.1	1.0±0.2	0	1.4±0.1	0.8±0.1	1.1±0.3
18:0	4.1±0.2	11.9±0.6	28.8±1.1	8.8±0.1	20.7±0.3	27.4±1.6
18:1*n*-9	21.1±1.8	29.5±1.6	22.4±2.7	24.5±0.4	18.3±0.7	17.7±1.6
18:2*n*-6	29.9±0.7	19.8±1.5	13.3±0.4	12.6±0.4	7.2±0.7	7.4±0.3
20:1*n*-9	0.4±0	1.6±0.2	0	1.0±0	1.6±0.1	1.1±0.1
20:4*n*-6	8.2±0.9	18.1±1.8	27.2±2.9	8.6±0.4	20.8±0.6	23.6±0.9
20:5*n*-3	3.2±0.6	5.6±0.8	0.8±0.8	1.1±0.2	1.6±0.2	2.0±0.2
22:5*n*-3	0.8±0.1	2.0±0.6	0	1.0±0.1	2.0±0.2	2.3±0.2
22:6*n*-3	1.0±0.1	2.8±0.7	0	3.6±0.1	11.2±0.3	7.9±0.7

Values are given as % w/w ± S.E.M.
PL, phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.
N=4, eggs; *N*=24, neonates.

18:2*n*-6 and 20:4*n*-6 and a significant proportion of 20:5*n*-3 (Table 4). The cholesteryl ester of the neonates differed from that of the egg in several respects, most notably in the reduced proportions of 18:1*n*-9, 20:4*n*-6 and 20:5*n*-3 and increased proportions of 18:2*n*-6 and 22:6*n*-3.

The free fatty acid fraction of the eggs consisted of 16:0, 18:1*n*-9 and 18:2*n*-6 as the main components, with significant proportions of 20:4*n*-6 and 20:5*n*-6 also present (Table 4). In the neonates, this fraction contained a much greater proportion of 20:4*n*-6 and was also relatively enriched in 22:5*n*-3 and 22:6*n*-3 but relatively depleted in 18:3*n*-3 and 20:5*n*-3.

Among the lipid-soluble vitamins, α-tocopherol was the main form of vitamin E present in the eggs at a concentration of 62.6±3.4 mg g^{-1} wet yolk. γ-tocotrienol was also present at low concentrations (3.5 mg g^{-1}), as was vitamin A (1.5±0.2 mg g^{-1}). No other forms of vitamin E were detected in the eggs.

Discussion

Mass, composition and energy densities

A positive relationship between female L_{SV} and litter size has been observed previously in *N. metallicus* (Greer, 1982; Stewart and Thompson, 1994; Jones and Swain, 1996). Such a relationship is common among lizards, including species closely related to *N. metallicus* (Greer, 1982; Stewart and Thompson, 1993). The wet and dry masses of eggs and neonates of *N. metallicus* in this study are similar to those in an earlier report that was based on a very small sample size (Stewart and Thompson, 1994). The statistically significant difference in wet mass of neonates from different treatments is probably not biologically important because no other size parameter differed significantly and the ranges of percentage water content of neonates from the three treatments mostly overlap (control, 81.6–85.6 %; treatment, 80.1–84.6 %; sham-operated, 80.1–85.3 %).

Clearly, there is no net gain of dry mass by neonates of *N. metallicus* during embryonic development (Jones et al., 1998), as the neonates are approximately 10 % smaller in dry mass than the newly ovulated eggs (Table 2). The proportion of ash in both eggs and neonates of *N. metallicus* is similar to other species of lizards (Stewart and Thompson, 1993; Thompson and Russell, 1999; Thompson et al., 1999), but unlike other placental species (Thompson, 1982; Stewart and Thompson, 1993; Thompson et al., 1999) there is no significant uptake of inorganic ions across the placenta during development. Unusually, there is no significant uptake of calcium across the placenta during development, although there is a small trend in that direction. Calcium is often taken up from the eggshell of oviparous lizards (e.g. Packard et al., 1992) or the placenta of viviparous species (Stewart and Thompson, 1993; Thompson et al., 1999). The relative calcium content of eggs of *N. metallicus* is higher than that of oviparous and some viviparous species (Thompson et al., 1999). However, the relative calcium content of the neonates is almost identical to that in neonates of oviparous *Lampropholis guichenoti* and *Lampropholis delicata* (M. B. Thompson, B. K. Speake, K. J. Russell and R. J. McCartney, unpublished data) and viviparous *Pseudemoia entrecasteauxii* (Stewart and Thompson, 1993).

In contrast to calcium, there is clear uptake of sodium and potassium during development (Table 2), with about three times as much of each element in the neonates as in the fresh eggs. Nevertheless, uptake of these elements is quantitatively less in *N. metallicus* than in *P. entrecasteauxii* (Stewart and Thompson, 1993) or *P. pagenstecheri* (Thompson et al., 1999), which have a type III chorio–allantoic placenta.

Previously, a ratio of dry mass of neonate to dry mass of freshly ovulated egg has been used as an index of the degree of matrotrophy (e.g. Blackburn, 1992, 1994; Stewart and Thompson, 1993). We have summarised data for seven species of oviparous lizards, five species of squamates with a type I chorio–allantoic placenta and three species with a type III

Table 6. *Summary of dry masses of freshly ovulated eggs and neonates for squamate reptiles exhibiting different modes of parity*

Parity mode	*N*	Egg (mg)	Neonate (mg)	Neonate/egg
Oviparous[1,2,3,4,5]	7	75.2±22.9	61.5±15.9	0.75±0.03
Range		21.4–174.0	16.0–68.0	0.64–0.85
Type I chorio-allantois[6,7,8,9]	5	948.2±647.7	737.2±504.1	0.78±0.03
Range		139.6–3520.0	96.9–2740.0	0.69–0.84
Niveoscincus metallicus[10]	1	41.8	37.9	0.91
Type III chorio-allantois[1,3,11]	3	35.7±10.7	55.5±8.8	1.70±0.25
Range		19.0–55.5	41.1–71.5	1.29–2.19
Type IV chorio-allantois[12,13]	2	0.44±0.03	188.2±34.2	429.1±44.1
Range		0.40–0.47	154.0–222.4	385.0–473.2

N, number of species in each parity mode studied.

[1]M. B. Thompson, B. K. Speake, K. J. Russell and R. J. McCartney (unpublished data); [2]Florian (1990); [3]Stewart and Thompson (1993); [4]Thompson and Russell (1998); [5]Ji (1992); [6]Stewart et al. (1990); [7]Stewart (1989); [8]Thompson (1977); [9]Stewart and Castillo (1984); [10]This study; [11]Thompson et al. (1999); [12]Blackburn et al. (1984); [13]Vitt and Blackburn (1991).

chorio–allantoic placenta for comparison with *N. metallicus* (Table 6). There is no apparent difference in this ratio for oviparous (0.75±0.03) species and viviparous species with type I placentae (0.78±0.03) ($T(10)=0.53$, $P=0.61$). Species with type III placentae range from 1.29–2.16 with a mean of 1.70±0.44. *N. metallicus*, with a type II chorio–allantoic placenta lies between these extremes with a ratio of 0.91. The pattern of embryonic nutrition of *N. metallicus* is functionally intermediate between species that develop type I and type III chorio–allantoic placentae and the morphology of the chorio–allantoic placenta of this species is intermediate in complexity between type I and type III as well (Weekes, 1930; Stewart and Thompson, 1994). In addition to *N. metallicus*, placental morphology has been described for three species of *Niveoscincus* (Weekes, 1930; Stewart and Thompson, 1998). Among these species, the chorio–allantoic placenta of *N. coventryi* is less complex structurally than that of the other three species. From these results, we predict that the neonate/egg mass ratio in *N. coventryi* will be less than 0.91.

Whereas the energy density of neonates of *N. metallicus* is similar to that of oviparous and other viviparous species (Thompson et al., 1999), the energy density of fresh eggs of *N. metallicus* is at the lowest extreme of the range known for lizards (Booth and Thompson, 1991). The significance of the low energy density in eggs is not known, but a net uptake of energy across the placenta does not occur as there is less energy in the neonates than in fresh eggs.

Although there is slightly less protein in the neonate than in the fresh egg (Table 2), it is likely that there was uptake of protein across the placenta during development. Several species of oviparous lizards utilise protein as a major energy source during embryonic development, with protein contributing approximately half of the energy consumed (Thompson and Stewart, 1997; Thompson and Russell, 1998, 1999). By assuming that protein is used as an energy substrate by embryonic *N. metallicus* also, we conclude that protein is taken up by embryos across the placenta. The transfer could be in the form of amino acids (Swain and Jones, 1997), although placental uptake of labelled amino acids need not be an indication of net uptake of organic molecules. In the skink *Eulamprus quoyii*, there is no net uptake of organic nutrients across the simple type I placenta (Thompson, 1981), even though amino acids do move across the placenta (Thompson, 1977). Identification of the energy substrates and quantification of their energetic contributions to embryonic development in *N. metallicus* would provide an interesting comparison with oviparous species and may provide information useful to understanding of evolution of viviparity.

Lipids and lipid-soluble components

The amount of total lipid recovered in the neonates of *N. metallicus* is approximately half the amount originally present in the egg (this study; Jones et al., 1998). In addition to indicating that a significant proportion of yolk lipid is oxidised for energy by the embryo, this result does not provide any clear evidence for the uptake of lipid across the placenta. However, the placental transfer of at least a small proportion of the embryonic lipid requirements, or of certain specific lipid components, cannot be precluded at this stage.

The relative proportions of triacylglycerol, phospholipid and free cholesterol in the eggs of *N. metallicus* are very similar to those reported for the egg yolks of the chicken and the alligator (Noble, 1991). Interestingly, the lipid class profile that we found in both eggs and neonates of *N. metallicus* varies from that reported in another population of the same species (Jones et al., 1998). While both studies found approximately the same proportion of total lipid to be composed of phospholipids, Jones et al. (1998) found a much lower proportion of triacylglycerol (and hence higher proportion of phospholipid) in neonates than we did. These differences may reflect a greater abdominal fat storage of triacylglycerol in neonates in our study than in Jones et al. (1998), perhaps reflecting a maternal diet richer in triacylglycerol in our captive animals, with a greater placental uptake of triacylglycerol, than for mothers collected from the wild by Jones et al. (1998). However, the

lipid class profile of the *N. metallicus* eggs differs considerably from that reported for eggs of another lizard, *S. jarrovi* (Hadley and Christie, 1974). In particular, *Sceloporus jarrovi*, which has a type I placenta, produces eggs with a higher proportion of triacylglycerol (87 %) and a lower proportion of phospholipid (9 %). The possibility that differences in the degree of placentotrophy are accompanied by changes in the relative proportions of lipid classes in the egg awaits further studies on a greater range of lizard species.

The fatty acid composition of the egg lipids presumably reflects the fatty acid profile of the insectivorous diet of the mother during vitellogenesis. The major polyunsaturated fatty acid was 18:2*n*-6, although a relatively high proportion of 18:3*n*-3 was also present, particularly in the triacylglycerol. The egg lipids were also characterised by relatively high proportions of 20:4*n*-6 and 20:5*n*-3 but low proportions of 22:6*n*-3. This latter fatty acid is believed to have an important role in the development of the central nervous system and the phospholipids of the developing brain and retina of most vertebrates are characterised by high proportions of this *n*-3 polyunsaturate (Neuringer et al., 1988). Specific mechanisms for ensuring the selective delivery of 22:6*n*-3 to the developing neural tissues have apparently evolved in mammals and birds (Noble and Cocchi, 1989). Avian embryos take up 22:6*n*-3 preferentially from the yolk lipids and apparently express a series of mechanisms, unique to this fatty acid, to ensure its tissue-specific delivery to the brain (Speake et al., 1998). The alligator embryo preferentially takes up total phospholipid, rich in 22:6*n*-3, from the yolk (Noble et al., 1990, 1991). It is noteworthy that, in the present study, the proportion of 22:6*n*-3 in the phospholipid in neonates was 8.5-fold greater than in phospholipid of the egg. The extent of this biomagnification during development was far higher than for any other fatty acid. Possible mechanisms for this relative increase in 22:6*n*-3 include: (1) biosynthesis (desaturation/elongation) from 18:3*n*-3 and/or 20:5*n*-3, since the proportions of both these precursors decrease during development; (2) relative resistance of 22:6*n*-3 to β-oxidation by the embryo; (3) the selective transfer of 22:6*n*-3 across the placenta.

The concentration of vitamin E in eggs of *N. metallicus* is similar to that in eggs of turtles (Thompson et al., 1998) but about double that in eggs of *P. pagenstecheri* (Thompson et al., 1999). This may reflect an inability of *N. metallicus* to transport vitamin E across the placenta, in contrast to *P. pagenstecheri*, which has a more complex placenta. The concentration of vitamin A in eggs of *N. metallicus* is also similar to that in eggs of turtles (Thompson et al., 1998), and half that in eggs of *P. pagenstecheri* (Thompson et al., 1999). However, the concentration of vitamin A is much lower in eggs of *N. metallicus* than in egg yolks of chickens (Naber and Squires, 1993) and some other birds (Ionov et al., 1994).

Conclusion

This study clearly shows that the pattern of embryonic nutrition, based on neonate:egg dry mass ratio, of *N. metallicus* is functionally intermediate between that of *Pseudemoia* spp. with more complex chorio-allantoic placentae (Stewart and Thompson, 1993; Thompson and Stewart, 1994; Thompson et al., 1999) and that of species with simple chorio–allantoic placentae (Table 6), as implied by the placental morphology. It seems that certain nutrients such as potassium and sodium, most probably protein and possibly certain lipid components, are not provided in sufficient quantities to meet the demands of the developing embryo and must be supplemented across the placenta. Hence, the yolks of *N. metallicus* are not merely smaller versions of the yolks expected in the oviparous ancestors of *N. metallicus*, but are at least partly modified. The details of how individual nutrients are transported across the placenta of lizards are completely unknown, but an understanding of these mechanisms is required to explain modifications to yolk composition during the evolution of viviparity.

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