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Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch

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Early nutrition has recently been shown to have pervasive, downstream effects on adult life-history parameters including lifespan, but the underlying mechanisms remain poorly understood. Damage to biomolecules caused by oxidants, such as free radicals generated during metabolic processes, is widely recognized as a key contributor to somatic degeneration and the rate of ageing. Lipophilic antioxidants (carotenoids, vitamins A and E) are an important component of vertebrate defences against such damage. By using an avian model, we show here that independent of later nutrition, individuals experiencing a short period of low-quality nutrition during the nestling period had a twofold reduction in plasma levels of these antioxidants at adulthood. We found no effects on adult external morphology or sexual attractiveness: in mate-choice trials females did not discriminate between adult males that had received standard- or lower-quality diet as neonates. Our results suggest low-quality neonatal nutrition resulted in a long-term impairment in the capacity to assimilate dietary antioxidants, thereby setting up a need to trade off the requirement for antioxidant activity against the need to maintain morphological development and sexual attractiveness. Such state-dependent trade-offs could underpin the link between early nutrition and senescence.

Keywords: carotenoids; development; senescence; sexual selection; *Taeniopygia guttata*; vitamins

1. INTRODUCTION

The quality of early nutrition has recently been shown to profoundly affect fitness-related traits at adulthood. In marked contrast to the positive effects on lifespan of adult dietary restriction (Merry 1995; Sohal & Weindruch 1996; Beckman & Ames 1998; Jennings *et al.* 2000; Aihie Sayer & Cooper 2002), low-quality nutrition during foetal and/or neonatal development generally reduces adult lifespan (Aihie Sayer *et al.* 2001; Aihie Sayer & Cooper 2002). This occurs even if affected individuals actually attain normal adult size and appearance (Lucas *et al.* 1996).

The mechanisms underlying these downstream effects are poorly understood. One possible explanation is that low-quality neonatal nutrition permanently impairs an individual's capacity to prevent oxidative damage to biomolecules, an important factor in the ageing process. The rate at which organisms' age is thought to be determined by a balance among oxidant production, antioxidant defences and repair of oxidative damage. Oxidants, which comprise free radicals, non-radical oxidants and singlet oxygen species generated in metabolic processes, are major causes of damage to living cells (Beckman & Ames 1998). The system of antioxidant defences appears to be highly conserved across the vertebrates, with lipophilic antioxidant vitamins and carotenoids derived from the diet being a key component (Beckman & Ames 1998). Low-quality nutrition during development may invoke changes in the programming of cells and organs (Barker 1998; Lucas 1998) that adversely influence the capacity to assimilate dietary antioxidants.

Dietary antioxidants have several other roles within the body. In birds, key antioxidants such as the carotenoids are frequently involved in the production of sexual ornaments, leading to potential trade-offs between the need for defence against oxidants and the need to attract a mate. It has been suggested that carotenoid pigmentation, the development of song repertoire and sexual ornament size and shape are all influenced by oxidants, the negative effects of which are ameliorated by antioxidants (von Schantz *et al.* 1999). Sexual ornament development can be influenced by early nutritional quality (Nowicki *et al.* 1998; Ohlsson *et al.* 2002), but whether this is a consequence of nutritionally mediated changes in antioxidant defence systems has not hitherto been investigated.

In this study, we experimentally investigated the links among the quality of early nutrition, plasma antioxidant defences and sexual attractiveness at adulthood using the zebra finch, *Taeniopygia guttata*. Previous work on this species has shown that, as in other vertebrates (Aihie Sayer *et al.* 2001), a short period of low-quality neonatal nutrition, while having no apparent effect on sexual maturation or adult body size, has a substantial effect on adult lifespan: *ca.* 50% of sexually mature non-breeding male zebra finches that had been reared on low-quality diets as nestlings died before 500 days of age, compared with only 20% in a control group that had experienced standard-quality early nutrition (Birkhead *et al.* 1999). The aim of this study was therefore to investigate whether low-quality nutrition early in life induces a reduction in adult antioxidant levels.

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2. MATERIAL AND METHODS

(a) *Manipulation of early nutrition*

Adult zebra finches were taken from our stock population, paired randomly and allowed to breed under standardized conditions. Pairs were housed in individual breeding cages (60 cm × 45 cm × 40 cm) with an external nest-box, in a room maintained at 20.5 ± 2.0 °C under full spectrum, artificial light (Bird Lamp; Arcadia, Croydon, UK; 16 : 8, L : D cycle). The diet provided to these parents prior to their chicks hatching comprised mixed seeds (foreign finch mixture; J. E. Haith, Cleethorpes, UK), grit, cuttlefish and water *ad libitum*. They also received 1.5 g of conditioning food per breeding pair twice weekly (a mixture of Rearing and Conditioning Food (J. E. Haith), Daily Essentials vitamin supplement (The Birdcare Company, Nailsworth, UK) and water; 200 : 1 : 100 w/w/v), 1.5 g of green vegetables per pair twice weekly, and Calcivet calcium supplement in the water five times weekly (1 : 100 v/v; Vetafarm, Wagga Wagga, Australia).

We randomly allocated families to receive either a standard-quality or a lower-quality rearing diet for 15 days after the chicks hatched. The first eggs in a clutch normally hatch a day ahead of the last (Zann 1996), and the treatment diets were provided on the day that hatching started. The standard-quality rearing diet comprised mixed seeds *ad libitum*, and 1.5 g per family daily of a mixture of the conditioning food (see above) and mixed seed that had been soaked overnight to saturation in water (1 : 1 w/w), whereas the lower-quality diet included pearl white millet *ad libitum*, and 1.5 g per family of a mixture of conditioning food and water-soaked pearl white millet (1 : 1 w/w) only once per week. Both rearing diets included grit, cuttlefish and water *ad libitum*, and neither diet included green vegetables. The seeds fed *ad libitum* to the two treatments contained similar levels of protein and lipophilic antioxidants (mixed seeds: protein (percentage dry mass), 11.60%; total carotenoids, 1.26 μg g^{-1}; vitamin E (α- and γ-tocopherol), 11.41 μg g^{-1}; pearl white millet: protein, 12%; total carotenoids, 2.54 μg g^{-1}; vitamin E, 10.76 μg g^{-1}; neither seed type contained vitamin A). The main difference between the standard- and lower-quality rearing diets was in the amount of nutrients provided in the conditioning food (protein, 13.6%; total carotenoids, 0.77 μg g^{-1}; vitamin E, 27.50 μg g^{-1}; vitamin A, 2.92 μg g^{-1}). The values for protein levels were obtained from Birkhead *et al.* (1999) and the values for antioxidant levels were derived from our own laboratory analysis (see § 2b). The diet provided after the 15 day chick-rearing period was the same for all birds, and was the usual one for non-breeding birds (mixed seed, grit, cuttlefish and water *ad libitum*, and 0.75 g per bird of conditioning food once weekly).

All birds remained in their family groups throughout the experiment. Zebra finches are altricial birds, hatching in an embryo-like state typical of songbirds, and leave the nest at 16–18 days (Zann 1996). The manipulation of neonatal nutrition therefore corresponded to the period during which the chicks developed from being naked and blind at hatching to being fully feathered and able to fly; after this point all birds had an identical diet. Ten randomly selected and unrelated offspring per sex were used from each dietary treatment in the analyses of antioxidant levels and external adult morphology.

(b) *Measurement of antioxidants*

Zebra finches become sexually mature before 80 days of age and, if allowed, both sexes will breed for the first time at *ca.* 90 days (Zann 1996). We collected a small blood sample (up to 140 μl) under UK Home Office licence shortly after fledging (25 days) and again from the same individuals at adulthood (99.0 ± 0.2 days; mean ± s.e.m.; the exact age at which samples were taken did not differ between treatments; two-way ANOVA: rearing diet, $F_{1,36} = 0.67$, n.s.; sex, $F_{1,36} = 0.41$, n.s.; rearing diet by sex interaction, $F_{1,36} = 2.81$, n.s.). We followed the guideline that total blood volume in birds is *ca.* 10% of body mass (w/v), and up to 10% of this pool can be safely withdrawn (Campbell 1995).

Whole blood was collected from the brachial vein into heparinized capillary tubes, centrifuged and plasma stored at –20 °C until biochemical analysis. We measured blood plasma concentrations of lipophilic antioxidants (vitamins A, E and total carotenoids) using high-performance liquid chromatography (HPLC). Plasma (20 μl) was mixed with 5% sodium chloride (20 μl), then ethanol was added (40 μl) and the mixture was vortexed for 20 s. This step extracts both free and protein-bound antioxidant molecules. Hexane (700 μl) was added and the mixture was vortexed for a further 20 s. The hexane phase, containing vitamins A, E and carotenoids, was collected and dried under a stream of nitrogen gas then redissolved in 200 μl methanol ready for HPLC. Triplicate samples of mixed seeds, pearl white millet and conditioning food, respectively, were ground to a fine powder using a mortar and pestle then treated before HPLC as follows. Samples (0.5 g) were mixed with 5 ml 10% (w/v) pyrogallol in ethanol, and 1.25 ml 60% potassium hydroxide, then capped under nitrogen gas, vortexed and saponified at 70 °C for 30 min in a water bath. After heating, samples were cooled on ice, and 7 ml 5% sodium chloride and 5 ml hexane were added and vortexed. The mixture was left to stand on ice in the dark for 30 min to precipitate, after which the hexane phase containing the lipophilic antioxidants was collected. Extraction using hexane was performed twice, and the combined extracts were dried under a stream of nitrogen gas then redissolved in 500 μl of dichloromethane–methanol (1 : 1) ready for HPLC.

Samples (20 μl) were injected into an HPLC system fitted with a 5μ C_{18} reverse-phase column (25 cm × 4.6 mm) (Spherisorb S5NH2; Phase Separations, Clwyd, UK) with a mobile phase of methanol-distilled water (97 : 3) at a flow rate of 1.5 ml min^{-1}; carotenoids were identified as a single peak at 445 nm, and concentrations were calculated in relation to a lutein standard (Sigma-Aldrich, Poole, UK). Vitamins A (retinol) and E (α- and γ-tocopherol) were determined from the same extract, using a 3μ C_{18} reverse-phase column (15 cm × 4.6 mm) (Spherisorb S30DS2; Phase Separations, Clwyd, UK) and a mobile phase of methanol-distilled water (97 : 3) at a flow rate of 1.05 ml min^{-1}. The excitation and emission wavelengths were 325 nm and 480 nm (vitamin A), and 295 nm and 330 nm (vitamin E), respectively. Standard solutions of retinol and α-tocopherol in methanol were used for instrumentation calibration and tocol was used as an internal standard.

(c) *Measurements of adult morphology*

To see whether the period of lower-quality neonatal nutrition had influenced the external morphology of the birds at adulthood, we made morphological measurements when collecting the second blood sample. Body mass was measured by using an electronic balance (± 0.01 g), tarsus length using a sliding calliper and wing length (maximum chord) using a wing rule (± 0.2 mm). In addition to measurements of body size we also measured bill colour, a secondary sexual characteristic that is carotenoid based in this species (McGraw *et al.* 2002) and there-

fore likely to be influenced by an individual's ability to assimilate dietary carotenoids. Bill coloration in zebra finches is thought to represent a condition-dependent signal of quality (Birkhead *et al.* 1998, 1999), with some studies showing that females prefer males with the reddest bills (Burley & Coopersmith 1987; De Kogel & Prijs 1996). Bill redness has also been hypothesized to serve as a signal in male–male competition (for a review see Collins & Ten Cate 1996). Adult bill colour was scored on a rank scale ranging from 1 (light orange) to 9 (dark red) using standard colour chips (Dulux Trade Colour Palette; Dulux, UK) that characterize the full range of bill colours observed in our study population. Specifically, we used the following chips: rank 1 (68YR 34/780), 2 (55YR 28/778), 3 (44YR 26/756), 4 (33YR 20/708), 5 (31YR 18/648), 6 (15YR 16/594), 7 (19YR 13/558), 8 (09YR 11/476), 9 (14YR 10/434), where the first number and letters indicate the hue (colour family), the numerator is the brightness and the denominator is the chroma (degree of saturation with hue). The coefficients of variation of colour parameters in this scale were 63.3% (hue), 42.2% (brightness) and 20.4% (chroma), respectively. Thus, we relied mainly on hue (redness) to measure bill colour, as in earlier work (Burley *et al.* 1992; Birkhead *et al.* 1998). There was a high level of agreement between colour ranks scored independently by two of us (Spearman's correlation, $r_s = 0.91$, $n = 39$, $p < 0.0001$); mean values were used for subsequent analysis (for one bird only one score was available).

We carried out mate-choice experiments in which sexually experienced, stock females were offered a choice between adult males of the two neonatal diet treatments. We put a female and two unrelated males (one from each rearing diet) into a standard mate-choice arena with electronically monitored perch arrays as described previously (Jones *et al.* 2001). Males were matched for bill colour and body mass and did not differ between treatments (bill colour rank: Wilcoxon $Z = 0.11$, $n = 10$, $p = 0.914$; body mass: paired $t = 0.09$, d.f. = 9, $p = 0.934$; Zann 1996). We allowed 15 min for the finches to settle before beginning 90 min data collection. Then, to control for any confounding effect of female preference between perch arrays, we switched the position of the males in the apparatus, allowed them 15 min to settle, and then collected data for a further 90 min. We recorded the percentage of time a female spent perched in front of each male, and her activity in front of each male in terms of the rate of hopping between perches, two standard measures of mate preference in zebra finches (Jones *et al.* 2001). Trials were carried out under full spectrum, artificial light (Bird Lamp). All birds were used in only a single trial.

3. RESULTS

There was a small (11%) decline in brood size between hatching, fledging and sexual maturity, but this did not differ between treatments (repeated-measures ANOVA: change in brood size over time, $F_{2,37} = 5.84$, $p = 0.006$; change in brood size by rearing diet interaction, $F_{2,38} = 1.39$, $p = 0.26$), nor did the average brood size during the rearing period ($F_{1,38} = 0.17$, $p = 0.69$). Therefore, the size of family groups did not differ between treatments.

Plasma antioxidant levels when the birds were aged 25 days (i.e. *ca.* 10 days after having all being put on to the same standard diets) were low and did not differ between the treatments (figure 1; $p > 0.09$ in all cases). However, by adulthood (i.e. *ca.* 85 days after being put on the same diets), when plasma antioxidant levels had generally increased, birds that had experienced lower quality nutrition as neonates had, on average, half the plasma concentrations of antioxidants compared with

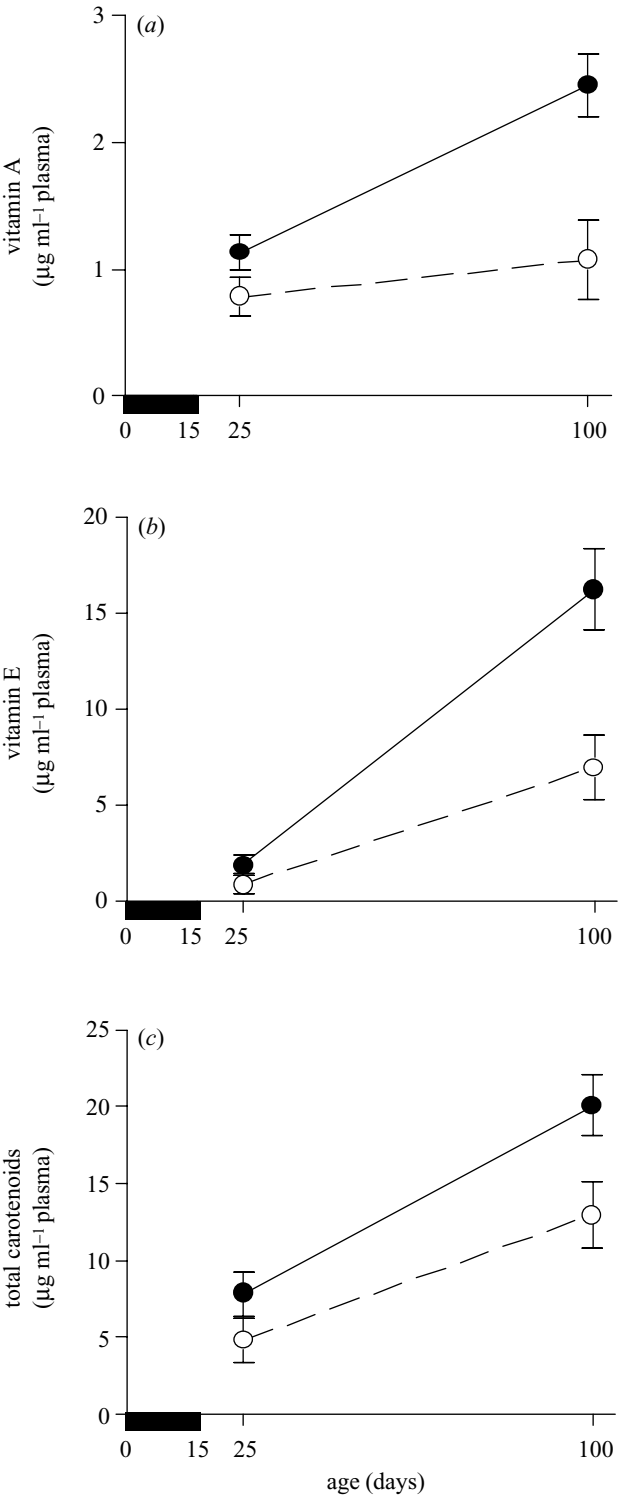

Figure 1. Effects of standard-quality (filled symbols and solid lines) and lower-quality (open symbols and broken lines) neonatal nutrition on plasma concentrations of antioxidants in zebra finches. (*a*) Vitamin A (retinol), (*b*) vitamin E (α- and γ-tocopherol), (*c*) total carotenoids. Values are means ± s.e.m.; see § 3 for statistical analyses. The thick black bars denote the period when the diets differed between treatments.

Table 1. A summary of the comparison of external morphology at adulthood in zebra finches exposed to standard- and lower-quality neonatal nutrition.
(All statistical tests controlled for sex and the interaction between neonatal nutrition and sex. Only effects of neonatal nutrition are reported in the table; sex had a significant effect in one analysis[d] (in all other analyses $p > 0.08$), but the neonatal nutrition × sex interaction term was always non-significant ($p > 0.21$).)

	neonatal nutrition			
adult morphological trait	standard quality	lower quality	statistic	p
body mass (g)[a]	19.74 ± 0.67	19.65 ± 0.72	0.01[c]	0.93
tarsus length (mm)[a]	14.95 ± 0.13	14.60 ± 0.15	2.88[c]	0.10
wing length (mm)[a]	60.50 ± 0.34	60.27 ± 0.48	0.17[c]	0.68
bill colour rank[b]	3.50 (2.75, 4.94)	4.00 (2.88, 5.44)	0.64[d]	0.42

[a] Mean ± s.e.m.
[b] Median on a scale of 1–9 (first and third quartiles).
[c] F statistic resulting from two-way ANOVA, d.f. = 1,36.
[d] H statistic resulting from two-way Kruskal–Wallis ANOVA, d.f. = 1; males had redder bills than females ($H = 23.18$, d.f. = 1, $p < 0.0001$).

Table 2. Summary of mate-choice trials using sexually mature male zebra finches from the standard- and lower-quality neonatal nutrition treatments as stimulus subjects, and sexually experienced stock females as test subjects.

	male neonatal nutrition			
female behaviour	standard quality	lower quality	statistic	p
per cent time with male[a]	48.42 ± 8.46	46.58 ± 6.54	0.13[c]	0.90
hop rate (hops min^{-1})[b]	0.92 (0.15, 1.08)	0.88 (0.41, 3.43)	0.76[d]	0.45

[a] Mean ± s.e.m.
[b] Median (first and third quartiles).
[c] t statistic resulting from one-sample t-test based on lower-quality minus standard-quality values; d.f. = 9.
[d] Z statistic resulting from Wilcoxon's signed-ranks test, $n = 10$ pairs of males.

those that had received the standard-quality diet during the same period (figure 1). This effect was found separately for vitamin A (change in plasma vitamin A over time by neonatal nutrition interaction, $F_{1,36} = 5.22$, $p = 0.028$, effect of neonatal nutrition, $F_{1,36} = 15.38$, $p < 0.0001$), vitamin E (change over time by neonatal nutrition interaction, $F_{1,36} = 8.36$, $p = 0.006$, effect of neonatal nutrition, $F_{1,36} = 14.41$, $p = 0.001$) and total carotenoids (change over time by neonatal nutrition interaction, $F_{1,36} = 6.74$, $p = 0.014$; effect of neonatal nutrition, $F_{1,36} = 17.06$, $p < 0.0001$; all analyses are repeated-measures ANOVAs that controlled for sex and are based on $n = 10$ males and 10 females of each treatment; in all cases sex and its interaction with neonatal nutrition was non-significant ($p > 0.22$)).

However, neonatal nutrition had no effect on adult body mass, tarsus or wing length (table 1). As expected, males had redder bills than females, but there was no difference in bill colour between treatments (table 1). Thus, birds that received either standard-quality or lower-quality neonatal nutrition were indistinguishable to us at adulthood in terms of external morphology. Furthermore, in mate-choice trials sexually experienced females taken from our stock population did not distinguish between adult males of the two rearing diets (table 2). Interestingly, although adult bill colour rank tended to be positively correlated with plasma carotenoid levels in birds that had received standard-quality neonatal nutrition (Spearman's correlations, $n = 10$ in all cases: males, $r_s = 0.624$, $p = 0.054$; females, $r_s = 0.710$, $p = 0.021$), there was no suggestion of such a relation in birds that had received lower-quality neonatal nutrition (males, $r_s = 0.049$, $p = 0.89$; females, $r_s = -0.305$, $p = 0.39$; figure 2).

4. DISCUSSION

Whereas the external appearance of the birds as adults was not apparently affected by early nutrition, there was a very marked effect on plasma antioxidant levels. Birds that had experienced the relatively low-quality diets during their early growth had half the levels of lipophilic antioxidants as adults than birds reared on the standard-quality diet. This difference developed despite the birds having been on the same diet continuously since 15 days of age, in fact for 85% of their lives.

These results suggest that the quality of the rearing diet permanently affected the capacity of birds to assimilate lipophilic antioxidants from the diet. Obviously, the rearing diets, although provided *ad libitum*, differed in several components (see § 2a). Further experimental work would be needed to tease apart the effects of different dietary constituents. Earlier studies have demonstrated long-term effects of foetal protein and lipid intake on hormone and nutrient metabolism in vertebrates (see Snoek *et al.* 1990; Desai *et al.* 1995). It has been suggested that this reflects the importance of the early nutritional environment in

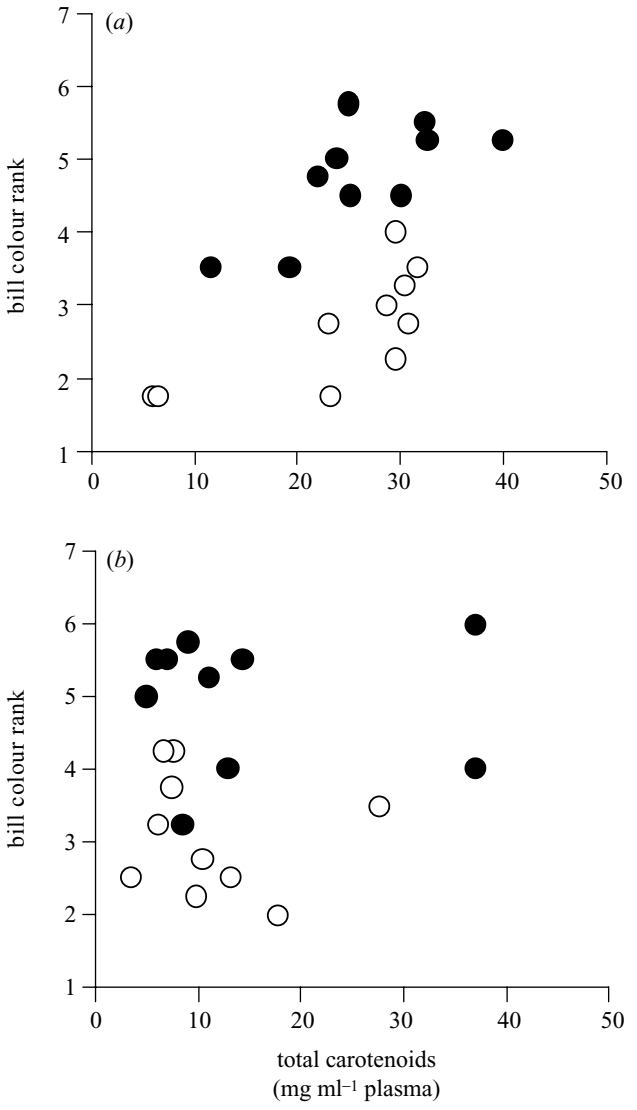

Figure 2. Relation between adult bill colour rank and plasma carotenoid concentration in birds that had received either (*a*) standard-quality neonatal nutrition or (*b*) lower-quality neonatal nutrition. Males are denoted by filled symbols and females by open symbols; see § 3 for statistical analyses.

stimulating gene expression, clonal selection or differential proliferation of cells, with permanent consequences for the quantities of cell populations in tissues (Barker 1998; Lucas 1998). The uptake and transportation of lipophilic antioxidants is dependent on lipoproteins, the formation of which in neonatal enterocytes has recently been found to be influenced by early nutritional quality (Nutting *et al.* 2002). A putative explanation for our results is that formation of such lipoproteins was reduced in neonates on lower-quality diets, thus impairing the capacity for uptake and transport of lipophilic antioxidants. Expression of such proteins does not reach adult levels until two weeks after weaning in rats (Kim *et al.* 1996), which may explain why we did not already see treatment differences in plasma antioxidant levels in fledgling (25 days) zebra finches. Although we did not measure other aspects of the antioxidant defence system, including hydrophilic and enzymatic antioxidants that birds can synthesize *de novo*, it seems unlikely that such defences could compensate for an impaired capacity to assimilate lipophilic antioxidants. Different types of antioxidant serve specific roles. For example, antioxidant enzymes are a first line of defence being specifically involved in preventing oxidant formation, whereas lipophilic antioxidants inactivate oxidants and prevent the chain reaction of oxidant formation that results in lipid peroxidation (Surai 2002). We therefore anticipate that lower-quality neonatal nutrition birds will show reduced lifespans compared with controls. However, it is currently too early to assess the ultimate outcome of our experiment.

Despite the large difference in plasma levels of carotenoids, we did not find any difference in bill coloration between the treatment groups. However, as it is possible that birds may have differed in more subtle ways that our measurements could not detect, we also examined whether adult female zebra finches could themselves distinguish between adult males from different rearing treatments in mate-choice trials. This was not the case. Thus, in terms of their external appearance, those birds reared on the lower-quality diets appeared to show no adverse effects. This is consistent with earlier work on a range of taxa (Lucas *et al.* 1996; Desai *et al.* 1996; Birkhead *et al.* 1999; Metcalfe & Monaghan 2001).

Interestingly, it has been hypothesized that susceptibility to damage caused by oxidants is revealed to prospective female partners through the expression of male secondary sexual traits such as carotenoid-based bill coloration (von Schantz *et al.* 1999). We found that higher bill colour ranks were indeed associated with higher plasma carotenoid levels in birds of both sexes that had received the standard-quality neonatal diet. By contrast, however, no such relation was found in lower-quality neonatal diet birds, suggesting that such birds could no longer maintain high carotenoid levels both in bill coloration and in plasma. These data, therefore, show that carotenoid-based signals can be uncoupled from plasma levels and hence from antioxidant defence levels. The maintenance of bill pigmentation, despite relatively low plasma levels of carotenoids, suggests active uptake of carotenoids from circulation, presumably facilitated by specific carotenoid-binding proteins (e.g. as in the human retina; Yemelyanov *et al.* 2001). This uncoupling has important potential implications for our understanding of how the expression of secondary sexual traits signals state. Individuals with different developmental backgrounds, and thereby differing capacities to assimilate antioxidants, may allocate compounds such as carotenoids differentially to competing requirements; where the availability of antioxidants is reduced, maximizing the ability to acquire a mate and reproduce in the short term may be more important to fitness than maximizing antioxidant defences and hence lifespan. Uncoupling of antioxidant distribution between ornaments and the bloodstream could be dynamic, changing over time in response to shifting demands and priorities. When oxidant production increases (e.g. immune activation), low-quality neonatal nutrition birds may need to allocate a greater share of their antioxidant pool to preventing oxidative damage rather than into sexual signals.

Compared with our finches, male ring-necked pheasants (*Phasianus colchicus*) that received a lower protein diet as chicks had reduced expression of carotenoid-based ornaments at adulthood compared with chicks that received higher protein nutrition (Ohlsson *et al.* 2002).

Our results from finches suggest that such an effect could be mediated by changes in antioxidant assimilation capacity. Why effects of the early nutritional environment on expression of secondary sexual traits apparently differ between species is interesting, and may relate to differences in the magnitude of the nutritional deficit experienced, or to ecological factors such as differences in extrinsic mortality risk which influence the fitness consequences.

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REFERENCES

Aihie Sayer, A. & Cooper, C. 2002 Early diet and growth: impact on ageing. *Proc. Nutr. Soc.* **61**, 79–85.

Aihie Sayer, A., Dunn, R., Langley-Evans, S. & Cooper, C. 2001 Prenatal exposure to a maternal low protein diet shortens life span in rats. *Gerontology* **47**, 9–14.

Barker, D. J. P. 1998 *Mothers, babies and health in later life*. Edinburgh: Churchill Livingstone.

Beckman, K. B. & Ames, B. N. 1998 The free radical theory of aging matures. *Physiol. Rev.* **78**, 547–581.

Birkhead, T. R., Fletcher, F. & Pellatt, E. J. 1998 Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. *Behav. Ecol. Sociobiol.* **44**, 179–191.

Birkhead, T. R., Fletcher, F. & Pellatt, E. J. 1999 Nestling diet, secondary sexual traits and fitness in the zebra finch. *Proc. R. Soc. Lond.* B **266**, 385–390. (DOI 10.1098/rspb.1999.0649.)

Burley, N. T. & Coopersmith, C. B. 1987 Bill color preferences of zebra finches. *Ethology* **76**, 133–151.

Burley, N. T., Price, D. K. & Zann, R. A. 1992 Bill color, reproduction and condition effects in wild and domesticated zebra finches. *Auk* **109**, 13–23.

Campbell, T. W. 1995 *Avian haematology and cytology*. Iowa State University Press.

Collins, S. A. & Ten Cate, C. 1996 Does beak colour affect female preference in zebra finches? *Anim. Behav.* **52**, 105–112.

De Kogel, C. H. & Prijs, H. J. 1996 Effects of brood size manipulations on sexual attractiveness of offspring in the zebra finch. *Anim. Behav.* **51**, 699–708.

Desai, M., Crowther, N. J., Ozanne, S. E., Lucas, A. & Hales, C. N. 1995 Adult glucose and lipid metabolism may be programmed during fetal life. *Biochem. Soc. Trans.* **23**, 331–335.

Desai, M., Crowther, N. J., Lucas, A. & Hales, C. N. 1996 Organ-selective growth in the offspring of protein-restricted mothers. *Br. J. Nutr.* **76**, 591–603.

Jennings, B. J., Ozanne, S. E. & Hales, C. N. 2000 Nutrition, oxidative damage, telomere shortening, and cellular senescence: individual or connected agents of aging? *Mol. Genet. Metab.* **71**, 32–42.

Jones, K. M., Monaghan, P. & Nager, R. G. 2001 Male mate choice and female fecundity in zebra finches. *Anim. Behav.* **62**, 1021–1026.

Kim, H. S., Arai, H., Arita, M., Sato, Y., Ogihara, T., Tamai, H., Inoue, K. & Mino, M. 1996 Age-related changes of alpha-tocopherol transfer protein expression in rat liver. *J. Nutr. Sci. Vitaminol.* **42**, 11–18.

Lucas, A. 1998 Programming by early nutrition: an experimental approach. *J. Nutr.* **128**, 401S–406S.

Lucas, A., Baker, B. A., Desai, M. & Hales, C. N. 1996 Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. *Br. J. Nutr.* **76**, 605–612.

McGraw, K. J., Adkins-Regan, E. & Parker, R. S. 2002 Anhydrolutein in the zebra finch: a new metabolically derived carotenoid in birds. *Comp. Biochem. Physiol.* B **132**, 811–818.

Merry, B. J. 1995 Effect of dietary restriction on aging—an update. *Rev. Clin. Gerontol.* **5**, 247–258.

Metcalfe, N. B. & Monaghan, P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260.

Nowicki, S., Peters, S. & Podos, J. 1998 Song learning, early nutrition and sexual selection in songbirds. *Am. Zool.* **38**, 179–190.

Nutting, D. F., Kumar, N. S., Siddiqi, S. A. & Mansbach, C. M. 2002 Nutrient absorption. *Curr. Opin. Gasteroenterol.* **18**, 168–175.

Ohlsson, T., Smith, H. G., Råberg, L. & Hasselquist, D. 2002 Pheasant sexual ornaments reflect nutritional conditions during early growth. *Proc. R. Soc. Lond.* B **269**, 21–27. (DOI 10.1098/rspb.2001.1848.)

Snoek, A., Remacle, C., Reusens, B. & Hoet, J. J. 1990 Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol. Neonate* **57**, 107–118.

Sohal, R. S. & Weindruch, R. 1996 Oxidative stress, caloric restriction, and aging. *Science* **273**, 59–63.

Surai, P. F. 2002 *Natural antioxidants in avian nutrition and reproduction*. Nottingham University Press.

von Schantz, T., Bensch, S., Grahn, M., Hasselquist, D. & Wittzell, H. 1999 Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond.* B **266**, 1–12. (DOI 10.1098/rspb.1999.0597.)

Yemelyanov, A. Y., Katz, N. B. & Bernstein, P. S. 2001 Ligand-binding characterisation of xanthophylls carotenoids to solubilized membrane proteins derived from human retina. *Exp. Eye Res.* **72**, 381–392.

Zann, R. A. 1996 *The zebra finch: a synthesis of field and laboratory studies*. Oxford University Press.