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Effects of experimentally manipulated yolk thyroid hormone levels on offspring development in a wild bird species

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ABSTRACT

Maternal effects are a crucial mechanism in a wide array of taxa to generate phenotypic variation, thereby affecting offspring development and fitness. Maternally derived thyroid hormones (THs) are known to be essential for offspring development in mammalian and fish models, but have been largely neglected in avian studies, especially in respect to natural variation and an ecological context. We studied, for the first time in a wild species and population, the effects of maternally derived THs on offspring development, behavior, physiology and fitness-related traits by experimental elevation of thyroxine and triiodothyronine in ovo within the physiological range in great tits (*Parus major*). We found that elevated yolk TH levels had a sex-specific effect on growth, increasing male and decreasing female growth, relative to controls, and this effect was similar throughout the nestling period. Hatching or fledging success, motor coordination behavior, stress reactivity and resting metabolic rate were not affected by the TH treatment. We conclude that natural variation in maternally derived THs may affect some offspring traits in a wild species. As this is the first study on yolk thyroid hormones in a wild species and population, more such studies are needed to investigate its effects on pre-hatching development, and juvenile and adult fitness before generalizations on the importance of maternally derived yolk thyroid hormones can be made. However, this opens a new, interesting avenue for further research in the field of hormone mediated maternal effects.

1. Introduction

Maternal effects are a crucial mechanism in a wide array of vertebrate taxa to generate phenotypic variation, potentially adapting offspring to their expected environment (Mousseau and Fox, 1998). Especially prenatal maternal effects, such as maternal effects mediated via prenatal exposure to hormones, are important as there can be organizing effects on the developing embryo (see McCormick, 1999; Uller and Olsson, 2006; von Engelhardt and Groothuis, 2011; Helle et al., 2013). Oviparous species, such as birds, are good model species for studying prenatal maternal effects, as eggs facilitate manipulation of maternal resources and signals. So far, the literature on maternal hormones in bird eggs, especially in non-domesticated bird species, has almost solely focused on steroid hormones: There is extensive data on their effects (within physiological range) on development, phenotype, behavior and even fitness, as well as variation among and within clutches due to environmental factors (reviewed by Groothuis et al., 2005; von Engelhardt and Groothuis, 2011). However, from poultry (reviewed by McNabb, 2007), and recently also from few wild species (great tit, Ruuskanen et al., 2015), it is known that bird eggs also contain the thyroid hormones (THs), L-thyroxine (T4) and 3,5,3′-triiodothyronine (T3, the biologically more active form). Interestingly, our recent results show that in wild populations of great tits (*Parus major*), there is variation in yolk TH levels among females, in relation key environmental conditions, temperature and timing of breeding (Ruuskanen et al. unpubl), and yolk TH levels increase over the egg laying sequence within clutches (Ruuskanen et al., 2015). If this variation in yolk THs within physiological range is associated with offspring fitness-related traits, it opens up the possibility for an adaptive maternal effect mediated via maternal deposition of thyroid hormones in wild populations. Nevertheless, knowledge of the importance and function of maternally derived THs in birds is limited, and data on the significance of natural variation of yolk TH levels in ecological or evolutionary contexts is lacking in wild bird species.

There are several reasons to expect maternal THs to play an important role in offspring development in avian species: Exposure to THs is indispensable for normal development and metabolism in vertebrates (Norris and Carr, 2013), including birds (McNabb and Wilson, 1997; Decuypere et al., 2005; McNabb, 2007; McNabb and Darras, 2014), as they are needed for differentiation and maturation of the central nervous system, muscle, skeletal, skin/feather, gut and lung tissues, and

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are important in the hatching process (McNabb and Wilson, 1997; McNabb, 2007). In adult birds THs play multiple important roles in the initiation of gonadal maturation, egg-laying, molt, and multiple aspects of thermogenesis and metabolism, including metabolic rates (McNabb, 2007; McNabb and Darras, 2014). Furthermore, (bio)medical research in humans and rodent models suggests that exposure to *maternal* THs in early foetal stages is crucial for normal growth and development, especially neural development, as hypothyroid conditions lead to severe pathological consequences on brain and behavior (e.g. Zoeller et al., 2007; Patel et al., 2011). Also in fish eggs, maternal THs play an important role in early development (hatching, growth, pigmentation) and even survival (Power et al., 2001; Campinho et al., 2014). However, data on the effects of THs within the natural variation is scarce. Nevertheless, extensive data from other egg hormones, such as steroids, suggests that variation within physiological range can affect multiple offspring traits (von Engelhardt and Groothuis, 2011). Importantly, there is good evidence that also the early avian embryo is sensitive to maternal THs: essential components for TH action (transporters, deiodinase enzymes and receptors) are present in the avian (chicken) embryo from a very early age, before the development of its own TH production, and expression of these proteins changes in response to TH administration already during the first days of embryonic development (Flamant and Samarut, 1998; Darras et al., 2009; Geysens et al., 2012; Van Herck et al., 2013, 2012). This opens the intriguing possibility that maternally derived yolk THs could substantially affect embryonic development (and have potential long-lasting effects) also in birds. To our knowledge, in the only experimental study where yolk THs were artificially elevated prior to incubation (via dosing females with T4 and T3), supraphysiological doses led to increased cartilage growth and differentiation in quails (Wilson and McNabb, 1997). However, to understand the ecological and evolutionary significance of maternal THs in the egg and their adaptive potential, the potential effects of natural variation within the physiological range in yolk THs on fitness-related traits in wild populations need to be addressed.

We studied the effects of yolk THs on offspring development, physiology, behavior and fitness-related traits by experimentally manipulating yolk TH (T4 and T3) levels within the physiological range in a field population of great tits, and cross-fostered chicks to create a within-clutch experimental design. To our knowledge, this is the first such study in a wild species in a field population – where the fitness-related effects can be investigated. We measured various traits, all of which have been previously found to be affected by THs (either maternal or produced by the embryo/nestling itself) in birds or other taxa. The measured traits are important in a wild population, as they are likely be related to nestling pre- or post-fledging survival. We measured: 1) Hatching success: embryonic thyroid hormones stimulate a variety of metabolic and developmental processes necessary for successful hatching process, and exogenous T4 close to hatching increases hatching success (Christensen, 1985; McNabb, 2007), thus likely maternal THs can also play a role; 2) Growth: embryo circulating THs are known to be positively associated with growth pre-and post-hatching (but severe hypo- and hyperthyroidism may decrease growth) (King and May, 1984; McNabb and Wilson, 1997; McNabb, 2007). We measured both skeletal size and body mass as THs are found to be important for both skeletal and muscle growth in many taxa. Furthermore, in precocial quails, also maternal THs were associated with increased embryonic skeletal growth (Wilson and McNabb, 1997). Importantly, offspring size is related to post-fledging survival, thus offspring growth is directly related to fitness; 3) Metabolic rates: In adult birds, higher circulating TH levels are associated with higher metabolic rates (Bishop et al., 1995; Welcker et al., 2013), thus we hypothesize that maternal THs could also affect offspring metabolic rates. Increased nestling metabolic rates may be related to increased energy consumption, and, especially in poor rearing conditions, this may cause a fitness cost in wild populations; 4) Stress reactivity: Maternal hyperthyroidism has been found to impair stress coping in e.g. rat offspring (Zhang et al., 2008). Individuals in wild populations are likely to encounter many types of stressors (abiotic, resource-based, inter/intraspecific), and coping with these challenges is likely related to their fitness; 5) Motor coordination: Establishment of brain architecture during central nervous system development in vertebrates (including birds) is critically affected by THs and several studies in mammals point to the effect of maternal THs on behavior (Rainwater et al., 2008; Patel et al., 2011). Maternal THs have been found to play a role in the early stages of nervous system differentiation in chicken embryos prior to the onset of their own thyroid function (see Flamant and Samarut, 1998). We hypothesized that adverse effects of altered maternal THs could also be manifested in traits such as motor coordination or cognition/learning in birds. In wild populations, chick motor coordination such as coordination of the begging behavior is important for food solicitation from the parents; 6) Fledging success: This is a crucial fitness –related trait in wild populations. We hypothesize that the effect of maternal THs on fledging success is likely to be indirect, mediated via increased growth or other effects of THs on development. In short, we predicted that nestlings from eggs with higher yolk TH levels would show 1) increased hatching success 2) increased growth 3) higher metabolic rates 4) higher stress reactivity 5) poorer motor coordination and 6) higher fledging success. We also determined nestling sex and studied the potential sex-dependent effects, as growth is sex-dependent in great tits (Råberg et al., 2005; Nicolaus et al., 2009), and there is some indication for variation in plasma THs across the sexes in birds (Newcombe et al., 1992).

2. Methods

The experiment was conducted in a nest box population (155 nestboxes) in Bennekom in the Netherlands (51° 59′ 53 N, 5° 40′ 35 E). The study species, the great tit, is an abundant, non-migratory, insectivorous hole-breeding passerine, and a common model species in ecological research. Both parents take part in nestling feeding, and nestlings usually fledge at age of 18–21 days.

2.1. Yolk thyroid hormone treatment

The nest boxes were monitored with three day intervals for initiation of nest building, and as soon as nest-building was halfway, monitored every day. From 44 nests, we collected the 4th egg and from 14 nests the 1st and the 7th egg (in total 34 eggs from CO nests and 38 eggs from TH-treatment nests, see below) for the purposes of another study. The rest of the eggs of these clutches were used for the manipulation experiment, see Table 1 for sample sizes. Yolk T3 and T4 levels were elevated by injection into the egg yolk before incubation had started (i.e. a combined injection of the two hormones). All eggs of the clutch received the same treatment (hereafter control or yolk TH treatment). We conducted a between-clutch design instead of a within-clutch design as the latter was practically not feasible: it would have required tracking which chick hatches from which egg. In practice, monitoring the hatching of each egg in great tit nests in a wild population is not possible, as this would require many visits within a day, which is too much disturbance for the female and will lead to desertion of the nest.

The injection dose was calculated based on a sample of 113 eggs (58 clutches) from the same population from the previous year (in 2013, see Ruuskanen et al., 2015). Average concentrations were: T4 2.97 pg/mg (SD 0.84, range 1.4–6.6) and T3 0.33 pg/mg, (SD 0.13, range 0.12–0.6).

Table 1
Sample sizes; numbers of nests, injected eggs and chicks in each treatment. CO = control, TH = thyroid hormone elevation.

Sample sizes	CO	TH	Total
Nests	30	32	62
Eggs injected	198	222	420
Chicks hatched	101	111	212