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RESEARCH ARTICLE

Testing different forms of regulation of yolk thyroid hormone transfer in pied flycatchers

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ABSTRACT

Hormones transferred from mothers to their offspring are considered a maternal tool to prepare progeny for expected environmental conditions, increasing maternal and offspring fitness. To flexibly influence offspring, mothers should be able to transmit the hormonal signals independent of their own hormonal status. However, the ability to regulate hormone transfer to the next generation is under debate. We studied the transfer of thyroid hormones (THs) to eggs in a bird model. We elevated thyroxine (T_4, the prohormone for the biologically active triiodothyronine, T_3) during egg laying using T_4 implants in females of a wild population of pied flycatchers (*Ficedula hypoleuca*), and measured the resulting plasma and yolk T_4 and T_3 levels. We found an increase in plasma and yolk T_4 and no change in plasma or yolk T_3 concentration, leading to a decrease in yolk T_3/T_4 ratio in response to the T_4 treatment. The yolk T_3/T_4 ratio was similar to the plasma ratio in females during the yolking phase. This suggests that mothers are not able to regulate TH transfer to yolk but may regulate the T_4 to T_3 conversion to avoid potential costs of elevated exposure to the active hormone to herself and to her progeny. The absence of regulation in hormone transfer to eggs is in contrast to our predictions. Future studies on deiodinase activity that converts T_4 to T_3 in maternal and embryonic tissues may help our understanding of how mothers regulate circulating THs during breeding, as well as the embryos' role in converting maternal T_4 to its biologically active T_3 form during development.

KEY WORDS: Maternal hormones, Maternal effects, Thyroxine, Triiodothyronine, Trade-offs, Birds

INTRODUCTION

Maternal effects are the non-genetic influences of a mother on her progeny and are thought to be adaptive (Moore et al., 2019; Mousseau and Fox, 1998; Yin et al., 2019). Maternal hormones transferred to the next generation are a potential prenatal pathway for mothers to shape their offspring phenotype (Groothuis et al., 2005; Groothuis et al., 2019; Ruuskanen and Hsu, 2018). Transfer of maternal hormones may be adaptive when the environment of the offspring can be predicted by the mothers ('anticipatory maternal effects'; Marshall and Uller, 2007). Mothers transfer thyroid hormones (THs), which have so far received little attention compared with glucocorticoids and androgens (Ruuskanen and Hsu, 2018). THs are produced by the thyroid gland and are present in two main forms: thyroxine (T_4) and triiodothyronine (T_3). T_4, a precursor of T_3, is converted to T_3 in tissues. T_3 exerts most of the TH action, as a result of its much greater affinity to TH receptors than T_4 (ca. 50-fold greater; Zoeller et al., 2007), although T_4 can also exert non-genomic actions (Davis et al., 2016). Thyroid hormones have pleiotropic effects that serve several biologically important functions across vertebrates, including growth, reproduction and metabolism (Behringer et al., 2018; Ruuskanen and Hsu, 2018).

Regulatory mechanisms of maternal hormone transfer are essential to minimise physiological trade-offs between optimal hormone exposure in the mother versus that in the offspring (Groothuis and Schwabl, 2008). For example, circulating THs increase with low temperature to stimulate metabolism and hence heat production (McNabb and Darras, 2015). If elevated maternal circulating THs increase yolk THs, this could stimulate embryo metabolism, which could be detrimental if the resources in the egg are insufficient to cope with the increased metabolism. The evidence for a regulatory mechanism for several hormones, including corticosterone and THs, is mixed (Groothuis and Schwabl, 2008), but such regulation could take place at the circulating level in the mothers and/or at the follicle level. Regulation at the follicle level may happen by controlling the transfer or conversion of THs or by producing THs independently from the thyroid gland. These mechanisms have been suggested to exist in human ovaries (Monteleone et al., 2017; Rae et al., 2007). Such regulatory mechanisms may allow mothers to regulate the deposition of THs in their eggs independently from their own circulating TH levels. This would free mothers from the possible constraint to optimise their own circulating levels of THs and the levels in their eggs independently of each other. A few studies in birds have shown some preliminary evidence that mothers may indeed be able to regulate yolk TH transfer. In Japanese quails, a low-dose oral administration of T_4 resulted in an increase in yolk T_3 but not in circulating T_3, whereas T_4 increased in both tissues (Wilson and McNabb, 1997). Administration of T_3 in turn increased plasma T_3 but not yolk T_3 (Wilson and McNabb, 1997). Furthermore, artificial blocking of TH production in hens led to a decrease in yolk T_3 but not in plasma T_3, while T_4 decreased in both tissues (Van Herck et al., 2013). These studies on domesticated precocial birds respectively induced hyperthyroidism for 3–6 weeks and hypothyroidism for 16 weeks. Such a long exposure to hormone concentrations outside of the natural range may have triggered responses one would not observe under natural variations. Therefore, there is a need for complementary studies under shorter time scales and within the physiological range of wild altricial species.

In this experiment, we tested whether mothers are able to regulate their transfer of yolk THs, at the circulating and/or at the follicle level. We experimentally manipulated TH levels with T_4 implants using a within-subject design in female pied flycatchers (*Ficedula hypoleuca*) during egg laying and collected plasma samples and unincubated pre- and post-implantation eggs for the analysis of T_3 and T_4. Implanting the prohormone T_4 enabled us to test the possible differential conversion of this hormone to the biological

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active T_3 in the mother as a regulatory mechanism to protect herself or the egg from increased exposure to these hormones. It also allowed us to test whether mothers can regulate the transfer of hormones to the egg. First, if the implant successfully increased circulating T_4, we would expect a higher availability of the substrate (i.e. the prohormone T_4) in tissues and subsequent conversion to T_3. Some of the increased T_3 in tissues may be released back into the circulation, leading to increased plasma T_3 (e.g. Escobar-Morreale et al., 1995). Alternatively, females may buffer the increase in plasma T_4 by downregulating the conversion of T_4 to T_3, thus yielding no increase in tissue or plasma T_3. Second, we predicted that if mothers were able to regulate yolk TH transfer independently from their circulating levels, only one of these two compartments (i.e. plasma or yolk) would be affected by exogenous T_4, or one would be more affected than the other (Groothuis and Schwabl, 2008). In this case, the T_3/T_4 ratio may be different between the two tissues. Conversely, if mothers were unable to regulate yolk TH transfer, one would expect both plasma and yolk THs to vary in the same direction and with a similar magnitude in response to exogenous T_4 (Groothuis and Schwabl, 2008). Thus, the T_3/T_4 ratio would not differ between the tissues. If implants increased plasma THs (i.e. T_4 and/or T_3), they may also have increased female metabolism, thus affecting their body mass and percentage of red blood cells (haematocrit). One would expect a decrease in body mass if resources are not sufficient, and an increase in haematocrit as a result of increased energy expenditure, although the latter relationship may not be so straightforward (Fair et al., 2007).

MATERIALS AND METHODS

The experiment was conducted in 2016 and 2017 in Turku, Finland (60°26′N, 22°10′E). The study species, the pied flycatcher, *Ficedula hypoleuca* (Pallas 1764), generally lays a single clutch of 6–7 eggs. Into egg-laying females we inserted either a T_4 implant or a control implant (see below for more details on the dose and implantation).

Preparation of T_4 implants and implantation

Two types of sterile implants (ca. 3 mm of diameter) were used for this experiment: ready-made T_4 pellets (10 µg, hereafter T_4 implant) and respective controls, which were identical, but without T_4 (both implants from Innovative Research America, Sarasota, FL, USA). The amount of T_4 in the implants was based on the natural production rate of T_4 measured in chickens, quail and pigeons (1–3 µg T_4 100 g^{-1} body mass per day; McNabb and Darras, 2015) and adjusted to the average body mass of pied flycatchers. The T_4 was embedded in a matrix that is designed to steadily release the hormone for 21 days.

Before implantation between the scapula, the skin was disinfected with a cotton pad dipped in 70% ethanol. An incision was made with an 18 gauge needle (BD Microlance™) and the implant was inserted and pushed away from the incision to avoid losing the implant. The wound was sealed with veterinary tissue adhesive (3M Vetbond™), which is commonly used in experiments with pit tags and shown to have no effect on birds.

Experimental design – captive females

First, to validate that implants increased circulating THs in a short time window after implantation, we conducted an experiment with female pied flycatchers in captivity in 2016. As the yolk formation takes approximately 3.5–4 days in passerines (Williams, 2012), implants inserted during egg laying need to increase hormone levels within days to enable quantification of their effect on newly formed eggs. We captured egg-laying female flycatchers from the wild population and housed them on a natural photoperiod and *ad libitum* food for the validation experiment as repeated disturbance during egg laying in the wild could cause nest desertion. Because of the similar treatment and breeding stage, we expect the data on wild-caught captive birds to be similar to plasma levels of wild birds in the main experiment (see below). On the 4th day after capture, each female received either a subcutaneous control or T_4 implant (n=4 per group). Blood samples were taken before the implant was inserted, and at 24 and 72 h after the implant (between 09.30 h and 11.30 h). After the last blood sample, females were released to the site where they were captured. Circulating TH levels in response to the implants are presented in Table 1.

Experimental design – wild females

In the wild population, the experiment was conducted in 2016 and 2017. The first egg of a clutch was collected freshly on the day it was laid and replaced by a dummy egg as a within-clutch control (hereafter 'pre-implant'). On the morning that the second egg was laid (07:00–09:00 h), females were captured and weighed (±0.1 g), and received a T_4 (n=11) or a control implant (n=10) as above. The last egg of the clutch (mean±s.d. egg rank 6±0.25, hereafter 'post-implant') was collected on the day it was laid, as it is mostly formed under the influence of the hormone implant (see above). In total, we collected 26 eggs from 13 clutches (13 pre- and 13 post-implant eggs) in the T_4 implant group, and 22 eggs from 11 clutches (11 pre- and 11 post-implant eggs) in the control group. Early in the incubation, on average 1.3±1.0 days (mean±s.d.) after the last egg was laid, females were blood sampled (08:00–12.30 h) for the analysis of circulating T_4 and T_3 (n=11 for T_4 implant and n=10 for control, respectively, blood sample analysis failed for 3 females) as well as haematocrit (proportion of red blood cells obtained by centrifugation of the capillaries). Body mass was also recorded (±0.1 g) to analyse potential body mass loss following the insertion of the implant. During blood sampling, the implants were still visible, which indicates that the implants were still releasing T_4 at that time.

Hormone analysis

Blood samples (ca. 40 µl) were taken from the brachial vein. Plasma was collected via centrifugation and frozen at −20°C until analysis.

Table 1. Circulating thyroxine (T_4), triiodothyronine (T_3) and T_3/T_4 ratio in captive female pied flycatchers in response to T_4 or control implants

	Control implant			T_4 implant		
Time (h)	0	24	72	0	24	72
n	3	4	4	3	3	3
T_4 (pg µl^{-1})	4.9±1.7	3.5±1.3	4.9±1.9	5.3±2.6	28.9±18.0	8.4±3.3
T_3 (pg µl^{-1})	0.7±0.5	0.5±0.4	0.9±0.5	0.7±0.1	0.7±0.2	1.1±0.2
T_3/T_4 ratio	0.16±0.10	0.20±0.19	0.17±0.09	0.18±0.14	0.04±0.04	0.15±0.09

Females were sampled prior to insertion of the implant, and 24 and 72 h later. Thyroid hormone (TH) analysis of one sample (time 0 in control group) failed as a result of too low a plasma volume. TH data are means±s.d.

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Eggs were thawed, the yolks separated and homogenised in MilliQ water (1:1), and a small sample (ca. 50 mg) was used for TH analysis. Yolk and plasma THs were analysed using nano-LC-MS/MS, following Ruuskanen et al. (2018, 2019). TH concentration, corrected for extraction efficiency, is expressed as pg mg^{-1} yolk or pg ml^{-1} plasma.

Statistical analysis

Data were analysed with the software R version 3.6.2 (http://www.R-project.org/). Linear mixed models were fitted using the R package *lme4* (Bates et al., 2015). *P*-values were obtained by model comparison using Kenward–Roger approximation from the package *pbkrtest* (Halekoh and Højsgaard, 2014). Estimated marginal means and standard errors (EMMs±s.e.) were derived from models using the package *emmeans* (https://CRAN.R-project.org/package=emmeans). Effect size estimates (Cohen's *d*) obtained from marginal means were computed with the package *emmeans*. Effect size estimates obtained from the raw data were calculated with the package *effsize* (https://CRAN.R-project.org/package=effsize). Model residuals were checked for normality and homogeneity with the package DHARMa (https://CRAN.R-project.org/package=DHARMa).

Yolk THs were ln-transformed to achieve normal distribution of the residuals. Yolk TH concentrations and T_3/T_4 ratio were analysed by fitting linear mixed models that included the treatment (i.e. T_4 or control implant) as the predictor, hormone levels in the pre-implant egg and year as covariates, and the hormone assay as a random intercept. Pre-implant egg was added to account for the variation in yolk THs among females.

Plasma TH levels of the incubating females were analysed using linear regressions with treatment as a fixed factor and body mass, ambient temperature and time of the day as covariates, as these covariates are known to influence circulating levels (McNabb and Darras, 2015). Covariates were centred and scaled. Year was not included in the model as it covaried with ambient temperature (variance inflation factor, VIF>2), and the latter is known to affect circulating THs (McNabb and Darras, 2015). Plasma T_3/T_4 ratio was ln-transformed and analysed with an identical linear regression to that for plasma THs.

Female haematocrit and loss of body mass were analysed by fitting linear models with treatment as the fixed factor, and year and clutch size (as a proxy for reproductive investment) as covariates.

Effect sizes (Cohen's *d*) of the treatment on yolk and wild female plasma THs were estimated from marginal means. Effect size estimates of the treatment on the T_3/T_4 ratio in the yolk and in captive female plasma were computed from the raw data. To avoid nest abandonment, we did not blood sample wild females during egg laying. Therefore, we used the data from captive birds in the following way: plasma samples from captive females averaged over day 1 and 3 after the implantation (reflecting the yolking phase of the last egg in wild birds) were compared with the post-implant last eggs collected from wild females.

Ethical note

The experiments were conducted under licence from the Animal Experiment Board of the Administrative Agency of South Finland (ESAVI1018/04.10.07/2016) and South-Western Finland Centre for Economic Development, Transport and Environment (VARELY/412/2016).

RESULTS

Yolk THs of pre-implant eggs (first eggs of a clutch) did not differ between females with control or T_4 implants (mean±s.e. yolk T_4, control 8.29±0.61 pg mg^{-1} yolk versus T_4 implant 7.63±0.43 pg mg^{-1} yolk; yolk T_3, control 2.85±0.20 pg mg^{-1} yolk versus T_4 implant 2.94±0.38 pg mg^{-1} yolk; all $t \leq 0.89$ and all $P \geq 0.39$). After receiving a T_4 implant, females produced eggs with ca. 2 times higher yolk T_4 concentration than control implanted females (EMM±s.e. post-T_4 implant egg 17.14±2.07 pg mg^{-1} yolk versus post-control implant egg 8.54±1.08 pg mg^{-1} yolk; Table 2, Figs 1A and 2A). Pre-implant yolk T_4 did not predict post-implant yolk T_4 (Table 2). In contrast, post-implant yolk T_3 did not differ between the groups (mean±s.e., control 2.34±0.27 pg mg^{-1} yolk versus T_4 implant 2.72±0.29 pg mg^{-1} yolk; Table 2, Figs 1B and 2A), but pre-implant yolk T_3 predicted post-implant yolk T_3 (Table 2).

Regarding circulating THs, captive females implanted with a T_4 implant had higher circulating T_4 than captive control females during the first 3 days after the implant (Table 1). There was a similar, but non-significant, trend in wild female plasma T_4 early in the incubation (when egg laying was finished, ca. 10 days after implantation) (Table 2). Plasma T_3 and T_3/T_4 ratio were not affected by the implants in wild females (Table 2).

The treatment decreased the T_3/T_4 ratio in the post-implant egg (Table 2) and the trend was similar for plasma levels in captive females sampled during egg formation (Table 1, Fig. 2B). The effect sizes for T_3/T_4 ratios in plasma and in yolk largely overlapped, suggesting no clear difference between the two tissues (Fig. 2B).

Table 2. Linear models of yolk and plasma THs in wild female pied flycatchers in response to T_4 implants

	Estimate±s.e.	F_{ddf} or T	*P*-value
(A) Yolk THs			
Yolk T_4			
Implant (T_4)	0.75±0.19	$14.96_{17.6}$	**0.001**
Pre-implant egg	0.07±0.06	$1.09_{20.0}$	0.31
Year (2017)	0.09±0.20	$0.18_{18.2}$	0.67
Yolk T_3			
Implant (T_4)	0.12±0.11	$1.09_{17.1}$	0.31
Pre-implant egg	0.20±0.05	$13.33_{18.1}$	**0.002**
Year (2017)	−0.01±0.12	$0.01_{17.8}$	0.92
T_3/T_4 ratio			
Implant (T_4)	−0.12±0.03	$12.28_{17.6}$	**0.003**
Pre-implant egg	−0.01±0.11	$0.01_{19.3}$	0.91
Year (2017)	−0.01±0.03	$0.10_{18.2}$	0.76
(B) Plasma THs			
Plasma T_4			
Implant (T_4)	1.16±0.75	1.54	0.14
Body mass	−0.90±0.38	−2.33	**0.03**
Temperature	0.11±0.39	0.29	0.78
Time	−0.46±0.39	−1.80	0.26
Plasma T_3			
Implant (T_4)	0.14±0.15	0.95	0.36
Body mass	−0.02±0.08	−0.26	0.80
Temperature	−0.05±0.08	−0.26	0.58
Time	−0.09±0.08	−1.16	0.26
Plasma T_3/T_4 ratio			
Implant (T_4)	−0.13±0.29	−0.45	0.66
Body mass	0.23±0.15	1.54	0.14
Temperature	−0.10±0.15	−0.64	0.53
Time	0.02±0.15	0.15	0.89

(A) Full linear mixed models of yolk THs in response to T_4 implants in wild pied flycatchers (T_4 implant n=13 pre-implant and n=13 post-implant and n=13 eggs; control n=11 pre-implant and n=11 post-implant eggs). Hormone assay was included as a random intercept. *F*-value denominator degrees of freedom (ddf) are shown; numerator degrees of freedom (ndf)=1 in each case. (B) Full linear models of plasma THs in response to T_4 implants, in wild female pied flycatchers (T_4 implant n=11 females; control n=10 females). Covariates in B were centred and scaled. *P*-values in B were obtained by separate *t*-test for each predictor. Significant *P*-values are shown in bold.

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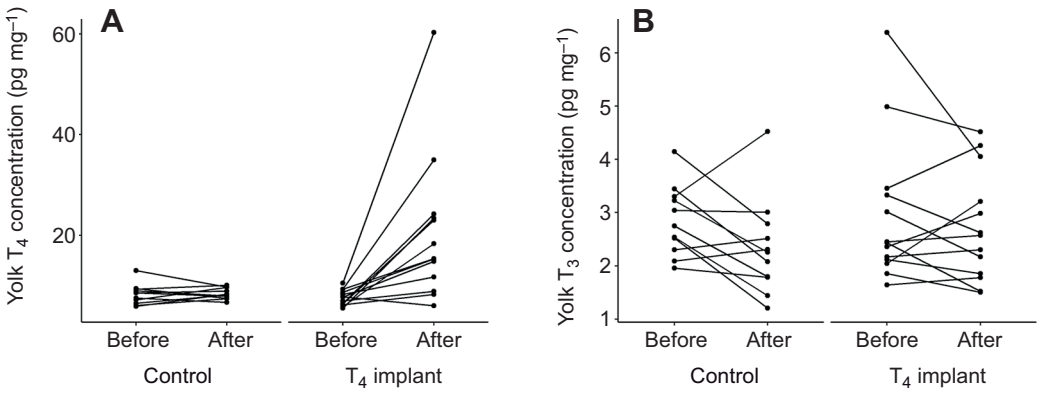

Fig. 1. Experimental manipulation of thyroid hormone (TH) levels in female pied flycatchers. Concentrations of thyroxine (T4; A) and triiodothyronine (T3; B) in eggs of female pied flycatchers implanted with a control implant (n=11 pre-implant and n=11 post-implant eggs) or 10 µg T4 implant (n=13 pre-implant and n=13 post-implant eggs). 'Before' and 'after' respectively refer to eggs collected before or after the females had received an implant.

The T_4 implant did not affect female body mass loss or haematocrit (Table 3). Female body mass loss and haematocrit did not differ between the sampling years (Table 3). Clutch size was not associated with female body mass loss, but was positively related to haematocrit (Table 3).

DISCUSSION

To our knowledge, this study is the first to manipulate circulating THs of a wild bird species during egg laying, to study potential regulation of maternal TH transfer at the level of the mother's circulation and at the follicle level. Contrary to previous studies on other maternal hormones (e.g. steroid hormones), we looked not only at the response in the implanted hormone T_4 but also at its more active metabolite T_3. To our knowledge, studies that manipulated a prohormone and measured the change in its active metabolites have rarely been conducted. We detected no effects of exogenous T_4 on female haematocrit or body mass. We found an increase in plasma and yolk T_4 in response to exogenous T_4. This result would indicate an absence of regulation in the transfer of T_4 from mothers to their eggs, supporting the epiphenomenon hypothesis of Groothuis and Schwabl (2008), namely that yolk hormones merely reflect or mirror the maternal circulating levels. Yet, there was a brief peak in T_4 levels, with relatively high plasma T_4 levels occurring during the yolking phase (Table 1), and we cannot fully exclude the potential explanation that any regulatory mechanism would have failed under high T_4. We predicted that elevated plasma T_4 would increase plasma or yolk T_3, the more potent hormone, because of the increased amount of its precursor, T_4. However, we observed no changes in circulating or yolk T_3. Our study therefore probably failed to induce a trade-off in mothers between plasma and yolk T_3, and we thus cannot conclude on the presence or absence of a regulatory mechanism for maternal transfer of yolk T_3. The unchanged plasma T_3 concentrations together with the rapid decrease in plasma T_4 after implantation observed in captive females (Table 1) suggest a change in the peripheral TH metabolism to quickly remove excess T_4. In rats, hyperthyroidism increases the conversion of T_4 and T_3 into inactive metabolites (Bianco et al., 2002). Likewise, increased circulating T_4 rapidly decreases the conversion of T_4 into T_3 (Bianco et al., 2002). Both mechanisms prevent the production of T_3, which may explain why we observed no increase in plasma T_3. Because plasma T_3 is known to positively correlate with basal metabolic rate in wild birds (Chastel et al., 2003; Elliott et al., 2013; Welcker et al., 2013), these mechanisms may allow individuals to cope with elevated THs and could be important tools for mothers to protect themselves and their progeny from the potentially detrimental consequences of elevated T_3. As T_3 binds with 50 times greater affinity to TH receptors than does T_4 (Zoeller et al., 2007), it is

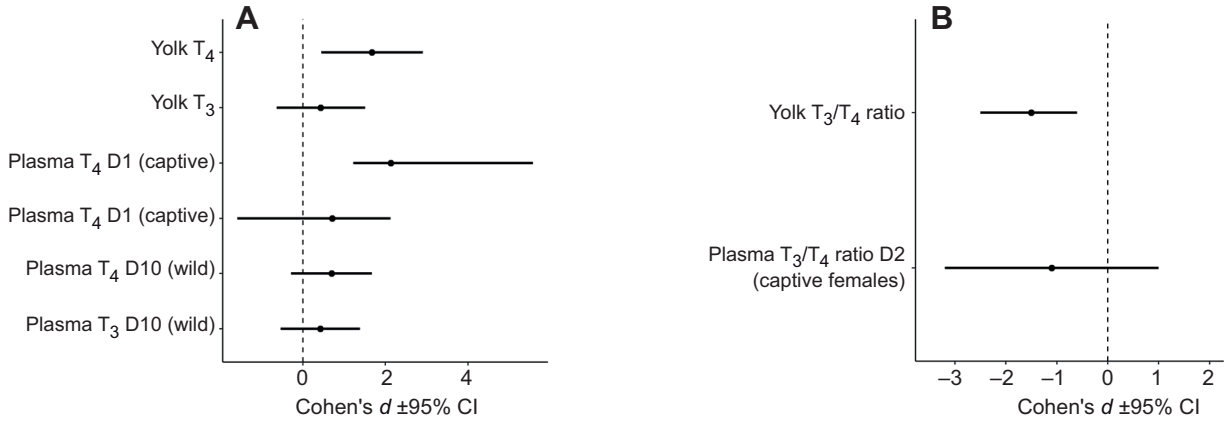

Fig. 2. Yolk and plasma levels of T_3 and T_4 in captive and wild females. (A) Cohen's d and 95% confidence intervals (CIs) for yolk (post-implant egg) and plasma (wild females) T_3 and T_4 were calculated from the marginal means of the respective models. Cohen's d and 95% CIs for plasma of captive females were calculated from the raw data. D1, 1 day after implantation; D10, 10 days after implantation. (B) Cohen's d and 95% CIs for the T_3/T_4 ratio in the yolk (post-implant egg) and plasma (captive females) calculated from the raw data. D2, 2 days after implantation, obtained by averaging the values for day 1 and day 3 after implantation. This period overlaps with the timing of yolk formation of the post-implant eggs, and thus reflects the circulating TH levels during yolk formation.

Table 3. Linear models of body mass loss and haematocrit in wild female pied flycatchers in response to T_4 implants

	Estimate±s.e.	*T*	*P*-value
Body mass loss			
Implant (T_4)	−0.16±0.56	−0.28	0.79
Year (2017)	0.04±0.65	0.06	0.95
Clutch size	−0.24±0.35	−0.68	0.51
Haematocrit			
Implant (T_4)	0.03±0.02	1.53	0.14
Year (2017)	0.04±0.02	1.52	0.15
Clutch size	0.04±0.02	2.66	**0.02**

T_4 implant n=11 females; control n=10 females. Significant *P*-value is shown in bold.

reasonable to expect a stricter regulation of the more potent hormone. This is indeed in line with previous studies reporting that yolk T_3 showed low within-individual variation, contrary to yolk T_4 (Hsu et al., 2019b), and that yolk T_3 was heritable whereas yolk T_4 was not (Ruuskanen et al., 2016a). To ascertain the hypothesis of a regulation of the T_4 to T_3 conversion, one should analyse the expression and activity of different enzymes involved in TH metabolism in response to exogenous THs, both in mothers and in embryos.

In addition to regulating their own plasma levels of THs, we hypothesised that mothers may be able to regulate the exposure of the developing follicles to THs. Elevated yolk THs (within the natural range) can affect hatching success, offspring growth and metabolism as found by studies in altricial species (Hsu et al., 2017; Hsu et al., 2019a; Ruuskanen et al., 2016b). We found no evidence for such a regulatory mechanism, as the T_3/T_4 ratio appeared not to differ between female plasma at the time of yolking (data from captive birds) and yolk. This result is contrary to that of Wilson and McNabb (1997), where yolk T_3 but not circulating T_3 was increased in response to long-term T_4 administration. This contradiction may be caused by different time scales between the two studies, the method of administration and/or the timing of sampling. In our study, the peak in T_4 rapidly decreased after implantation. Conversely, Wilson and McNabb (1997) administrated exogenous T_4 for longer periods of time, which might have forced females to deposit T_3 in their eggs to maintain normal plasma T_3.

We found no evidence for regulation of plasma T_4 concentration or T_4 transfer to the yolk, suggesting that yolk T_4 levels reflect circulating levels in the mother. Nevertheless, we found evidence that females regulated plasma T_3 concentration, while the results on yolk T_3 regulation remain inconclusive because of the lack of change in plasma T_3. Whether the potential regulation of plasma T_3 is due to changes in the T_4 to T_3 conversion and whether it has been selected to benefit the mother or the offspring is as yet unclear. This could be tested by elevating plasma and yolk T_3 and measuring whether potential detrimental effects are larger in the mother or the offspring. Further studies could also aim at investigating the changes in TH metabolism (enzyme production and activity) in response to increased hormone levels.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: T.S., S.R.; Methodology: T.S., S.R.; Formal analysis: T.S.; Investigation: T.S., S.R.; Writing - original draft: T.S.; Writing - review & editing: T.S., B.H., T.G.G., S.R.; Supervision: T.G.G., S.R.; Project administration: S.R.; Funding acquisition: T.G.G., S.R.

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Data availability
Data are available from the Zenodo digital repository: https://doi.org/10.5281/zenodo.3747401

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