Research

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Physiology

Born to be young? Prenatal thyroid hormones increase early-life telomere length in wild collared flycatchers

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The underlying mechanisms of the lifelong consequences of prenatal environmental condition on health and ageing remain little understood. Thyroid hormones (THs) are important regulators of embryogenesis, transferred from the mother to the embryo. Since prenatal THs can accelerate early-life development, we hypothesized that this might occur at the expense of resource allocation in somatic maintenance processes, leading to premature ageing. Therefore, we investigated the consequences of prenatal TH supplementation on potential hallmarks of ageing in a free-living avian model in which we previously demonstrated that experimentally elevated prenatal TH exposure accelerates early-life growth. Using cross-sectional sampling, we first report that mitochondrial DNA (mtDNA) copy number and telomere length significantly decrease from early-life to late adulthood, thus suggesting that these two molecular markers could be hallmarks of ageing in our wild bird model. Elevated prenatal THs had no effect on mtDNA copy number but counterintuitively increased telomere length both soon after birth and at the end of the growth period (equivalent to offsetting *ca* 4 years of post-growth telomere shortening). These findings suggest that prenatal THs might have a role in setting the 'biological' age at birth, but raise questions about the nature of the evolutionary costs of prenatal exposure to high TH levels.

1. Introduction

Prenatal environmental conditions can have lifelong consequences on health and ageing, but much remains to be done to uncover the mechanisms linking the pre- and postnatal stages [1]. Thyroid hormones (THs) are master regulators of development, health and ageing [2–4]. They are transferred from the mother to the embryo [4] and thyroid disorders during pregnancy can induce developmental pathologies in humans [5]. There is natural variation in the amount of TH being transferred from the mother to the embryo, and some of this variation is linked to the environmental conditions experienced by the mother (e.g. food availability or temperature [4]). This suggests that maternal THs could be a potential signal transferred from the mother to the embryo to adjust offspring phenotype to current environmental cues in order to maximize fitness [4]. While the potential for prenatal THs to mediate adaptive maternal effects remains mostly untested, some of our recent data in wild collared flycatcher

Figure 1. Age-related variation in potential hallmarks of ageing in wild collared flycatchers: (a) decrease in relative mtDNA copy number mtDNAcn, (b) decrease in relative telomere length measured with qPCR, and (c) decrease in absolute telomere length measured with in-gel TRF. Data are cross-sectional, adult birds were of known-age and chicks (i.e. age 0) were 12 days old (from control group only, 1 chick per nest). rTL and mtDNAcn have been z-transformed, Pearson's coefficients of correlation r and associated p -values are presented along with the age-related regression equations. Regression lines are plotted $\pm$ 95% CI, $N = 58$ (44 adults, 14 nestlings). See also the main text for statistical analyses restricted to adult individuals.

(*Ficedula albicollis*) suggest that increasing prenatal TH exposure increases both hatching success and early-life growth [6]. From an evolutionary perspective, such apparent beneficial effects are likely to come with associated costs if there is no increase in resource acquisition. This could occur, for instance, through a reduction in resource allocation towards somatic maintenance, which could impair subsequent health and accelerate ageing [7]. Yet, we currently lack experimental data on the effects of prenatal THs on postnatal health and ageing.

Avian models offer a great potential to study this question since prenatal conditions can be directly manipulated through hormonal injection in the egg [4]. Yet, one important challenge remains to be able to assess long-term consequences on ageing under an ecologically-realistic scenario. When direct effects on survival and lifespan cannot be estimated, the use of biomarkers that are mirroring age-related health impairments could be useful. Two promising biomarkers are telomere length and mitochondrial DNA (mtDNA) copy number since both markers are frequently reported to decrease with age and to be associated with increased mortality risks [8–10]. There is evidence that telomere length could reflect phenotypic quality since early-life telomere length has been shown for instance to predict lifespan [11] and fitness [12] in avian species. Interestingly, a large part of the inter-individual variation in telomere length could already be set at birth, and thus may be partially caused by different exposure to maternal hormones [13,14].

In this study, we aim to evaluate whether mtDNA copy number and telomere length decline with age in a free-living bird population of collared flycatchers, and to test the effects of prenatal THs on those potential hallmarks of ageing. To this aim, we first investigated age-related changes in telomere length and mtDNA copy number using cross-sectional data covering most of the lifespan spectrum for this population. While it is established that telomeres shorten with age in most bird species, there is no information to date regarding age-related variation of mtDNA copy number in avian models [15]. Then, using egg-injection of THs, we investigated the effect of prenatal THs on postnatal mtDNA copy number and telomere length. Based on the known stimulation of mitochondrial biogenesis by THs (among other means, through the upregulation of PGC1 α/β [16]), we predicted that increasing prenatal THs might increase early-life mtDNA copy number, which could be a cellular pathway supporting the transient

growth-enhancing effect we previously demonstrated in this species [6]. Conversely, since THs have been reported in some studies to increase oxidative stress and in our model species to enhance growth (two pathways accelerating telomere shortening [7,17]), we predicted that increasing prenatal THs levels should shorten telomere length at birth, and/or increase early postnatal telomere shortening.

2. Material and methods

This study was conducted in the long-term monitored population of collared flycatchers on Gotland, Sweden. In 2016, 44 randomly selected adult birds (30 females and 14 males) of known age (1–7 years old, i.e. cross-sectional data; maximum lifespan = 9.8 years) were sampled across different woodlands. Data and samples from 30 nests of a previous study [6] were used to investigate the effects of a prenatal TH manipulation on telomeres and mtDNA copy number. We used 14 Control (vehicle-injected) and 16 TH nests in which all eggs were injected with a *ca* a 2 s.d. increase of TH egg content based on natural range, following the procedure described in detail in [6] and electronic supplementary material, S1. The original study [6] demonstrated that TH-injected eggs had a higher hatching success, that early-life survival was not significantly affected by prenatal TH manipulation, and that chicks from TH-eggs were heavier at day 2 and larger at day 8 but did not significantly differ from controls in mass or size at fledging [6]. Two chicks per nest were randomly selected among those surviving to day 12. These chicks were blood sampled twice: soon after hatching (day 2; less than 10 μ l of blood) and at the end of growth (i.e. day 12; less than 50 μ l of blood). Relative mtDNA copy number of blood cells was measured as described in [18] and electronic supplementary material, S2. Both relative telomere length (rTL measured using qPCR) and absolute telomere length (TL, measured using in-gel telomere restriction fragment analysis (TRF) from day 12 samples only) were measured as described in [19] and electronic supplementary material, S2. Two day 2 samples failed to meet DNA quality control criteria, three samples failed to amplify for the mtDNA copy number analysis, and one day 12 DNA sample was excluded from TRF analysis owing to insufficient DNA quantity, explaining the observed differences in sample sizes among analyses. Age-related variation in mtDNA copy number and telomere length were tested using Pearson correlation tests, and age-related regression equations were calculated to infer the amount of telomere length or mtDNA copy number lost per year post-growth (figure 1). The effects of prenatal TH elevation and age on mtDNA copy number and telomere length (rTL and TL) were

Figure 2. Effects of experimental prenatal thyroid hormone elevation on: (a) early-life dynamics of mtDNA copy number, (b) early-life dynamics of relative telomere length and (c) absolute telomere length at the end of growth period (day 12). rTL and mtDNA copy number have been z-transformed. Means are plotted as symbols $\pm$ s.e., individual responses are plotted as solid lines, and p -values are indicated within each panel. See electronic supplementary material, table S1 for details on statistics.

tested using linear mixed models, with nest identity and bird identity as random effects (to control for multiple birds per nest and multiple samples per bird), as well as age (2 versus 12 days), treatment (TH versus Control) and their interaction as fixed effects. mtDNA copy number and rTL were z-transformed prior to statistical analysis [20]. Standardized effect sizes (ESs) for mixed models were calculated using the emmeans package in R [21]. Non-significant interactions were removed from final models. The effect of sex was tested but excluded from final analyses since it was never significant.

3. Results and discussion

We found strong evidence that both mtDNA copy number ($r = -0.57$, $p < 0.001$, figure 1a) and telomere length measured using either a relative qPCR method (rTL, $r = -0.30$, $p = 0.022$, figure 1b) or an absolute in-gel quantification (TL, $r = -0.48$, $p < 0.001$, figure 1c) significantly decreased from growth completion (i.e. day 12) to late-adulthood using a cross-sectional approach. Restricting our analyses to adult individuals only (i.e. age ≥ 1) reveals that mtDNA copy number ($r = -0.42$, $p = 0.004$) and absolute telomere length ($r = -0.43$, $p = 0.003$) still significantly decrease with age, while this relationship is not significant any more for relative telomere length ($r = -0.23$, $p = 0.14$). These findings in our avian model are in accordance with reports from the human literature [9,10]. Since individuals exhibiting short telomeres (in both human and various avian species [8,10]) or low mtDNA copy number (only tested in humans to the best of our knowledge [9]) have been shown to disappear earlier from the population, the age-related slopes from our cross-sectional sampling presented in figure 1 could underestimate the real age-related decline in both telomere length and mitochondrial density.

We found no significant impact of prenatal THs on mtDNA copy number (TH: ES = -0.26 [-1.03 ; 0.51], $p = 0.46$, electronic supplementary material S3 table S1A; figure 2a), despite a considerable early-life reduction in mtDNA copy number during the growth period (age: $p < 0.001$, equivalent to the reduction occurring over 3.5 years post-growth based on the age-related regression equation in figure 1a). Contrary to our predictions, increasing prenatal THs led to longer telomeres (measured as rTL) soon after hatching (day 2), and this effect was maintained at the end of the growth period (day 12) (TH: ES = 1.11 [0.03 ; 2.19], $p = 0.036$, electronic supplementary material, S3 table S1B, figure 2b). This is confirmed by the analysis of absolute telomere length (TL) at day 12, showing longer telomeres in birds hatched from TH-injected eggs (TH: ES = 1.91 [0.01 ; 3.81], $p = 0.044$, electronic supplementary material, S3 table S1C,

figure 2c) compared with control eggs. The effect of increasing prenatal TH on telomere length was substantial (i.e. large 'biological' effect size), being equivalent to offsetting *ca* 4.3 years (rTL) and 3.6 years (TL) of post-growth telomere shortening (based on age-related regression equations in figure 1b,c). It was unfortunately not possible to evaluate any long-lasting impact of prenatal TH supplementation on adult telomere length. Yet, considering that most telomere dynamics occur during the growth period and that telomere length is a repeatable trait [13,14], it is likely that the effects of prenatal THs observed here on early-life telomere length would be carried over into the adult stage.

The positive effect of prenatal THs on telomere length is unlikely related to oxidative stress prevention, since we previously found no differences in oxidative stress markers in these experimental birds [6], or in a similar experiment in a closely-related species [22]. The selective disappearance of TH-embryos with short telomeres could potentially explain why chicks hatched from TH-eggs have longer telomeres than controls. Yet, this seems unlikely to explain our results since we found a higher hatching success of TH-eggs [6]. One previous study in humans reported that the promoter of *hTERT* (the catalytic subunit of the enzyme telomerase, responsible for elongating telomeres) contains a binding site for THs [23]. Consequently, one hypothesis would be that prenatal THs could elongate telomeres early in life through the activation of the telomerase enzyme. Yet, we are currently lacking both *in vitro* and *in vivo* studies testing such a hypothesis.

While an evolutionary trade-off is generally expected to occur between fast growth and telomere shortening [7], prenatal TH supplementation both enhances early-life growth [6] and leads to longer early-life telomeres in our study system. Egg THs could be a potential signal transferred from the mother to the embryo to adjust offspring phenotype to the expected environmental conditions [4]. Yet, if raising egg TH levels only had benefits, natural selection would have favoured higher maternal transfer of THs into eggs, and/or offspring maximizing growth and telomere length without such a maternal signal. Consequently, it is likely that high egg TH levels have costs that we have not been able to detect here (e.g. reduced immunity) or that could only become visible later in life (e.g. altered reproductive potential), which clearly deserves further investigation. Importantly, we can expect the potential cost-benefit balance of low or high egg TH levels to depend on environmental conditions [4]. Such potential context-dependent effects will require experimental manipulations of both egg THs and environmental conditions in order to be revealed (e.g. [22]).

While the mechanisms remain to be identified, our study demonstrates that prenatal TH levels have the potential to modulate telomere length in early life, and thus to influence the 'biological' age at birth. It has previously been shown that prenatal exposure to glucocorticoids could shorten telomeres [24], but our study is the first to show that telomere length at birth could be increased by modulating the prenatal hormonal environment. Thyroid function is known to influence cardiovascular disease risk and life expectancy in adult humans [3], but no information is currently available regarding the impact of prenatal TH exposure on adult health and lifespan. Epidemiological and long-term experimental studies investigating the impact of prenatal THs on adult phenotype, health and lifespan are now required to establish if the effect observed here on early-life telomere length could be translated into a longevity gain, but also to identify potential costs of elevated prenatal exposure to THs.

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Ethics. The study was conducted in the long-term monitored population of collared flycatchers on Gotland, Sweden (Jordbruksverkets permit no. ID 872).

Data accessibility. Data [25] used in this article are publicly available at: <https://doi.org/10.6084/m9.figshare.11688504.v1>.

Authors' contributions. A.S., B.-Y.H. and S.R. designed the study. B.-Y.H. and S.R. conducted the fieldwork, A.S. and C.M. conducted laboratory work. B.D., L.G. and P.B. contributed to data collection. A.S. analysed the data and wrote the manuscript with input from all authors. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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