Born to be young: prenatal thyroid hormones increase early-life telomere length

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Keywords

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Abstract

Prenatal environmental conditions can have lifelong consequences on health and aging. The underlying mechanisms remain nonetheless little understood. Thyroid hormones (THs) are important regulators of embryogenesis transferred from the mother to the embryo. In an avian model, we manipulated embryo exposure to maternal THs through egg injection and investigated the consequences on postnatal growth and ageing. We first report that mitochondrial DNA (mtDNA) copy number and telomere length significantly decrease from early-life to late adulthood, thus confirming that these two molecular markers are hallmarks of ageing in our wild bird model. The experimental elevation of prenatal THs levels had a transient positive effect on postnatal growth. Elevated prenatal THs had no effect on mtDNA copy number but significantly 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 have a key role in setting the 'biological' age at birth, and thus might influence longevity.

Introduction

Prenatal environmental conditions can have lifelong consequences on health and ageing, but most 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 human [5]. Surprisingly, there is currently no experimental data on the effects of prenatal THs on postnatal health and ageing.

Two challenges must be overcome when testing for long-term consequences of maternally-transmitted hormones. First, manipulating the prenatal hormonal environment in mammalian models is usually problematic since treatments are applied to the mother and can have indirect effects on the embryo. Avian models offer an ideal alternative since prenatal conditions can be directly manipulated through hormonal injection in the egg [4]. The second challenge is to be able to measure long-term effects using health and ageing markers that are accurately and adequately mirroring age-related health impairments. Two promising markers are mitochondrial DNA (mtDNA) copy number and telomere length since both markers are decreasing with age and have been associated with increased mortality risks [6-8]. Telomere length is considered as a proxy of 'biological' age, with more relevance to mortality risk than chronological age [9]. Interestingly, most of the inter-individual variation in telomere length is already set at birth, and thus may be caused by different exposure to maternal hormones [10].

In this study, we aim to validate the use of mtDNA copy number and telomere length as ageing markers in a free-living bird population of collared flycatchers (*Ficedula albicollis*), and to test the effects of prenatal THs on postnatal health and ageing hallmarks. To this aim, we first investigated age-related changes in telomere length and mtDNA copy number using data covering the entire lifespan spectrum for this population. 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 [11], we predicted that increasing prenatal THs should increase early-

life mtDNA copy number, which could be a cellular pathway supporting the transient growth-enhancing effect previously demonstrated in this species [12]. Conversely, since THs are known to increase oxidative stress and enhance growth (two pathways accelerating telomere shortening [10]), we predicted that increasing prenatal TH levels should shorten telomere length at birth, and/or increase early postnatal telomere shortening.

Materials & Methods

This study was conducted in the long-term monitored population of collared flycatchers on Gotland, Sweden. We selected 44 adult birds of known-age (1 to 7 years old, *i.e.* cross-sectional data; maximum lifespan = 9.8 years) from the long-term monitoring program. Thirty-two nests were used for the prenatal manipulation of THs, 16 *Control* (vehicle-injected) and 16 *TH* nests in which eggs were injected with *ca.* a 2SD increase of TH egg content based on natural range, following the procedure described in [12]. Two to three chicks per nest were weighed and blood sampled as soon as possible after hatching (day 2, < 10µL of blood) and at the end of growth (*i.e.* day 12; < 50µL of blood). Relative mtDNA copy number of blood cells has been measured as described in [13]. qPCR efficiencies of mitochondrial gene and control nuclear gene were 90.7 $\pm$ 0.6% and 92.8 $\pm$ 1.0% respectively. Technical repeatability of mtDNAcn was high, namely R = 0.95 ([0.94-0.96], $p < 0.001$) at the intra-plate level and R = 0.93 ([0.90-0.96], $p < 0.001$) at the inter-plate level. Both relative telomere length (*rTL* measured using qPCR) and absolute telomere length (*absTL*, measured using *in-gel* TRF) have been measured as described in [14]. qPCR efficiencies of telomere and control gene were 99.6 $\pm$ 3.2% and 102.4 $\pm$ 2.3% respectively. Technical repeatability of rTL was high, namely R = 0.95 ([0.94-0.96], $p < 0.001$) at the intra-plate level and R = 0.90 ([0.76-0.96], $p < 0.001$) at the inter-plate level. Technical repeatability of absTL was high at the intra-gel level: R = 0.97 ([0.94-0.99], $p < 0.001$). DNA integrity has been evaluated on 20 samples chosen randomly and deemed satisfactory as described in [15]. Age-related variations in mtDNA copy number and telomere length were tested using parametric correlation tests. The effects of prenatal TH elevation and age on body mass, mtDNA copy number and

telomere length (*rTL* and *absTL*) were tested using linear mixed models, with nest identity as a random effect (to control for multiple birds per nest), bird ID as the repeated effect, and age, treatment and their interaction as fixed effects. Non-significant interactions were removed from final models. Sex was excluded from final analyses since it was never significant.

Results and Discussion

We found strong evidence that both mtDNA copy number (r = 0.57, p < 0.001, Fig. 1A) and telomere length measured either using a relative qPCR method (*rTL*, r = 0.30, p = 0.022, Fig. 1B) or an absolute *in-gel* quantification (*absTL*, r = 0.48, p < 0.001, Fig. 1C) significantly decrease from growth completion to late-adulthood. These findings are in accordance with reports from the human literature [6,7] and validate their use as hallmarks of ageing in our avian model. Since individuals exhibiting short telomeres or low mtDNAcn have been shown to disappear earlier from the population [6,8], the age-related slopes from our cross-sectional sampling presented in Fig. 1 are likely to underestimate the real age-related decline in both telomere length and mitochondrial density.

We confirmed the growth-enhancing effect of prenatal THs (Age*TH: p = 0.033, Fig. 2A) in our subsample of individuals from [12] but found no significant impact of prenatal THs on mtDNA copy number (TH: p = 0.44, Fig. 2B), 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 in individuals post-growth based on Fig. 1A). Contrary to our predictions, increasing prenatal THs led to longer telomeres (measured as *rTL*) soon after hatching, and this effect was maintained at the end of the growth (day 12) period (TH: p = 0.035, Fig. 2C). This is confirmed by the analysis of absolute telomere length (*absTL*) at day 12, showing longer telomeres in birds hatched from TH-injected eggs (TH: p = 0.044, Fig. 2D). The effect of increasing prenatal THs on telomere length was substantial (*i.e.* large biological effect size), being equivalent to offsetting *ca.* 4.3 years (*rTL*) and 3.6 years (*absTL*) of telomere shortening (based on Fig. 1B and 1C). It was unfortunately not possible to evaluate the effects of prenatal TH supplementation on adult telomere length or post-fledging survival considering the very

low recruitment rate in the study population from 2017 to 2018 (2.7% instead of *ca.* 12-15% usually from 1985-2015). Yet, considering that most of the telomere dynamics occurs before reaching adulthood and that telomere length is a very repeatable trait when measured with an appropriate methodology (*e.g.* within-individual consistency/repeatability in wild jackdaws: $R = 0.95$ [16] or captive Japanese quails: $R = 0.93$ [17]), it is likely that the effects of prenatal TH observed here on early-life telomere length would be carried over the adult stage.

The beneficial 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 [12], or in a similar experiment in a closely-related species [18]. One previous study reported that the promoter of *hTERT* (the catalytic subunit of the enzyme telomerase, responsible for elongating telomeres) contains a binding site for THs [19]. Consequently, one hypothesis would be that prenatal THs could elongate telomeres early in life through the activation of the telomerase enzyme. The positive correlation found between the mRNA expression of the thyroid-stimulating hormone and telomere length in human adipose tissue could support such an hypothesis [20].

While the exact 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 set the 'biological' age at birth. This is the first study 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 lifespan are now required to establish if the effect observed here on telomeres translates into a longevity gain.

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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 availability

Data used in this article is publicly available at: https://figshare.com/s/be8dca3133cc1db8af90.

Author contribution: AS, BYH & SR designed the study. BYH and SR conducted the fieldwork, AS and CM conducted laboratory work. BD, LG & PB contributed to data collection. AS analyzed the data and wrote the manuscript with input from all authors.

Competing interest statement: the authors declare having no competing interests

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Figure captions

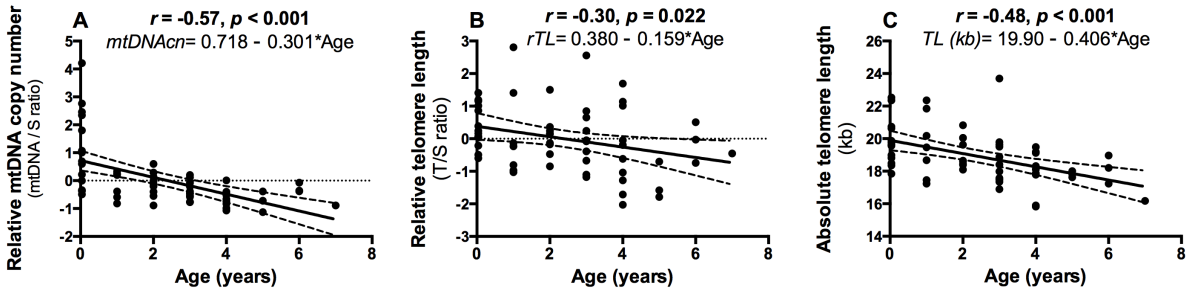

Fig. 1: **Age-related variation in potential key hallmarks of ageing in wild collared flycatchers**: **(A)** decrease in relative **mtDNA copy number**, **(B)** decrease in **relative telomere length** measured with qPCR, and **(C)** decrease in **absolute telomere length** measured with *in-gel* TRF. Data is cross-sectional, adult birds were of known-age and chicks were 12 days old (from control group only, 1 chick per nest). Regression lines are plotted ± 95% C.I., N = 58 (44 adults, 14 nestlings).

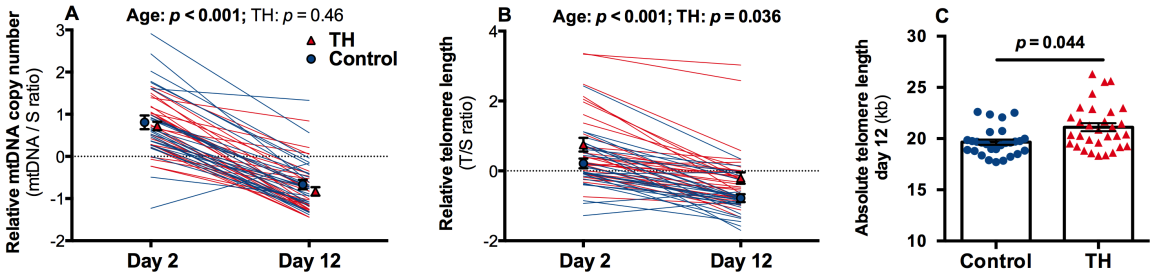

Fig. 2: **Effects of experimental prenatal thyroid hormone elevation** on: **(A) body mass** growth, **(B)** early-life dynamics of **mtDNA copy number**, **(C)** early-life dynamics of **relative telomere length**, and **(D) absolute telomere length** at the end of growth (day 12). Means are plotted ± SE, *p*-values and sample sizes are indicated within each panel.