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Cycloastragenol Increase in Telomere Length Leads to Faster Feather Regeneration*

ABSTRACT

Telomeres - the protective ends of linear chromosomes - reveal themselves not only as a good proxy in terms of longevity, but more recently also as a marker of healthy ageing in laboratory rodents. Telomere erosion is prevented by the activation of telomerase, an enzyme suspected to be also vital for tissue regeneration and which experimental activation improves health state in mice. One emerging hypothesis is that telomerase activity accounts for the frequently reported positive links between telomere lengths and individual quality in a wide range of organisms. Still, we lack an experimental approach testing the exact impact of inter-individual differences in telomere length on individual trait variability. In a first step study, we tested the impact of the cycloastragenol, a plant-derived product stimulating the expression and the activity of telomerase, on telomere lengths and flight feather renewal capacity of captive zebra finches (Taenopygia guttata). Telomere length was longer in cycloastragenol treated finches while their feather grew faster than in controls. Our data support the idea that long telomeres could reflect high telomerase activity, and in so doing be a good predictor of greater telomerase-dependent tissue regeneration, which may ultimately explain variation in organism quality and longevity.

1. INTRODUCTION

The very end of linear chromosomes is progressively losing DNA sequences because of the inability of DNA polymerase to replicate the whole DNA double-strand. In the same time, the DNA repairing mechanisms must be able to distinguish real chromosome-ends from DNA breaks. By doing so, wrong end-joining recombination or end-to-end fusion between different chromosomes will be avoided, preserving the cell genome integrity (De Lange, 2009). In both of these end-replication and end-protection problems, telomeres are playing a crucial role (Blackburn, 2000). Telomeres consist of noncoding DNA in association with proteins of the shelter in complex which forms a specific structure that caps the end of the chromosomes (Blackburn, 1991; De Lange, 2005), hiding them from general DNA-damage response pathways. Telomere length is dynamic and results from a balance between erosion (end replication—Blackburn, 1991, oxidative stress—Von Zglinicki, 2000) and restoration (telomerase activity, Greider and Blackburn, 1985) factors. So, telomere length remains a key feature that indicates the viability of a cell (Blackburn, 2000). The utilisation of telomere dynamics as a proxy of lifespan has been widely and successfully extended to the organism level (Cawthon et al., 2003). In particular, evolutionary ecology studies took rapidly advantage of such a lifespan proxy, which provides them indication not only about survival rates (Bize et al., 2009; Heidinger et al., 2012) but also about the costs of life history tradeoffs (Heidinger et al., 2012). For instance, in the common terns (Sterna hirundo), telomere erosion was enhanced by reproduction but highly successful individuals were also those losing less telomere length (Bauch et al., 2013), suggesting that individual quality may be indicated by long telomeres.

* Excerpt from: Sophie Reichert, et al(2014): Experimental increase in telomere length leads to faster feather regeneration

Still, a question remains: how can telomere length be related to individual quality? At first glance, this relationship can result from up-stream mechanisms that both affect telomere length and the overall performances of the organism. As such, oxidative stress may be a major factor that can accelerate telomere erosion but also damage vital cellular functions (Von Zglinicki, 2000). A premature loss in telomere length may also be induced by low quality growing conditions that have been previously associated with shortened telomeres at adulthood (Tarry-Adkins et al., 2008). The set-up of the organism as a “functioning nexus” during growth will be of tremendous importance for the future performances of the adult. Then, if growth is disturbed, a bad set-up may lead to the concordance of short telomeres and low individual performances at adulthood (reviewed in Metcalfe and Monaghan, 2001), but without any direct functional link. Recent major advances in biomedicine are pointing toward a central role of “telomerase activity as a rate-limiting factor for organism ageing” (de Jesus et al., 2011). Experimental activation of telomerase activity in laboratory mice has demonstrated that this enzyme can rescue telomere length with concomitant positive impact on health (de Jesus et al., 2011), ageing and lifespan (Bernardes de Jesus et al., 2012). Of note, in mice long telomeres per se were also found to enhance cell viability and/or allow higher rate of cell renewal (Hao et al., 2005), thereby permitting a better functioning of the organism. Because most of our knowledge comes from laboratory mice, we still know very little about the generality of telomerase-increased activity on the phenotype of non-mammalian species, especially on its potential positive effects on telomere length and individual quality.

As a first attempt to fill this gap, we performed an experiment in a small bird species, the zebra finch, where captive adult males were treated with a stimulator of the telomerase activity (cycloastragenol) (de Jesus et al., 2011). We then investigated the effects of our treatment both on telomere lengths in red blood cells (RBCs) and on the renewal of flight feathers. In birds, RBC telomere length has been found to predict life expectancy (Heidinger et al., 2012), and the maintenance of flight feathers is paramount for bird individual performances, self-maintenance and survival (Lindhe Norberg, 2002).

2. METHODS

2.1.Experimental design

Twenty-eight adult male zebra finches were randomly assigned to two experimental groups: a control group treated with sterilized water (100 ÿl) and a group treated with cycloastragenol(0.5 mg diluted in 100 ÿl of sterilized water per day). Cycloastragenol is a small-molecule purified from the root of Astragalus membranaceus that stimulates telomerase activity (de Jesus et al., 2011).

Flight feather growth was used as a proxy for individual tissue regeneration capacity. This was achieved by plucking one feather and measuring its speed of re-growth with an electronic calliper every 2 days until it recovered its initial size. For each individual, we assessed feather growth capacity twice. During the first phase of our experiment, we removed the 7th primary feather on the right wing of each individual without treating them. It allowed us to obtain a baseline speed of feather regeneration. When the first bout of feather regeneration was over, individuals were then treated daily (using a pipette) either with sterilized water (n = 14 individuals) or with cycloastragenol (n = 14) for a month before undergoing a second phase of feather regeneration by plucking the same feather again. The treatment phase was terminated when the second bout of feather growth was over.

RBC telomere length was assessed by qPCR methodology as previously conducted in the same species (Criscuolo et al., 2009). Telomere lengths were measured before the plucking of the feather (Time 1), at the end of the first phase of feather regeneration (just before the start of treatment with water or cycloastragenol) (Time 2), a month after the beginning of the treatment (just before the second phase of feather regeneration started) (Time 3), and at the end of the second phase of feather growth (Time 4). Details on qPCR conditions are provided in the ESM.

2.2.Data analysis

Effects of the treatment on telomere length and feather growth were analyzed using general linear mixed models (SPSS 18.0), using Bird Identity as random factor, sampling Time as repeated factor, Treatment and the interaction between Treatment and Timeas fixed factors. When the Treatment by Time interaction was significant, each Time point was subsequently analyzed separately (using t-tests) to assess the Treatment effect.

3. RESULTS

There were no significant differences in age, wing length and body mass between the two experimental groups (see ESM).

The significant interaction Treatment × Time showed that telomere lengths were affected by the experimental treatment and by time (Table 1). Tests for each time showed that telomere lengths were not significantly different between control and cycloastragenol treated birds at Times 1 and 2 (i.e. before treatment, Time 1: p = 0.28; Time 2: p = 0.18; Fig. 1.). However, cycloastragenol treated birds were characterized by longer telomeres than control during Phase 2 (i.e. during treatment, Time 3: p = 0.004; Time 4: p< 0.001).

Table 1

Linear mixed model analysis explaining the impact of cycloastragenol treatment on (a) telomere length and (b) the rate of wing feather renewal of zebra finches.

	ddl	F	*p*
a) Telomere length			
Bird Identity 1.077 ± 0.362			
Treatment	1, 26	4.93	0.035
Time	3, 78	5.58	0.002
Treatment × Time	3, 78	24.31	<0.001
b) Feather renewal rate			
Bird Identity 0.0001 ± 0.0004			
Treatment	1, 26.3	132.61	<0.001
Phase	1, 25.7	542.38	<0.001
Treatment × Phase	1, 25.7	360.13	<0.001

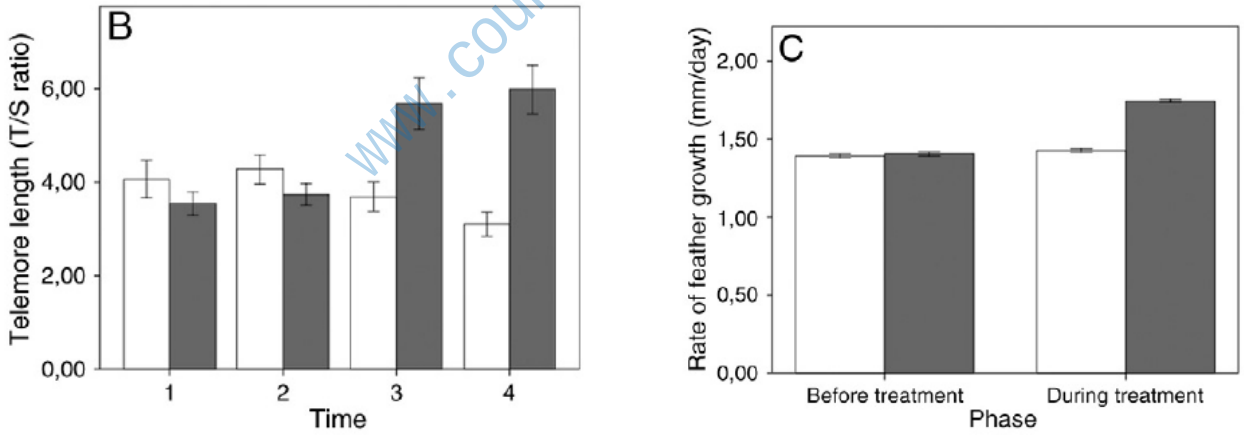

Fig. 1. Mean?àSE telomere length and rate of feather re-growth in cycloastragenol (grey) or control birds (white) over the different times or phases of the experiment.

The experimental treatment also had a significant impact on wing feather renewal rate (Table 1, Fig. 1.). The significant Treatment × Phase interaction indicate that cycloastragenol birds exhibited a faster feather growth than control ones after the treatment (Phase 2: p < 0.001) while no differences were detected before the treatment (Phase 1: p = 0.45). The rate of feather re-growth (Phase 2) was significantly correlated with telomere at the end of the treatment (Time 4) in cycloastragenol birds (Pearson's correlation, r = 0.63, p = 0.015) but not in control birds (r = 0.16, p = 0.58; Fig. 2).

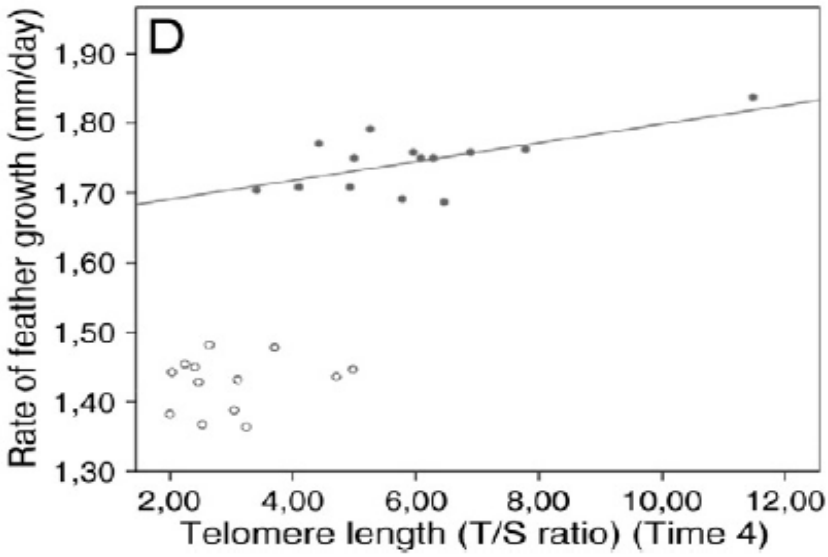

Fig. 2 Correlation plots between the individual rates of feather growth with telomere length in treated and control birds.

4.Discussion

We found that male zebra finches treated during 1 month with cycloastragenol presented longer RBC telomere lengths and higher rates of feather renewal than control birds treated only with water. In addition, there was a direct relationship between telomere length and feather re-growth rate in cycloastragenol birds, the longer the telomere, the faster the feather growth. Our data therefore supports the idea that cycloastragenol can rescue telomere length (de Jesus et al., 2011; Harley et al., 2011) with a global positive effect on the proliferating capacity of cells (i.e. epidermal in our case).

Enhanced telomerase activity and/or preservation of telomere length may favour a higher rate of cell renewal. This has been previously observed in mouse skin and subcutaneous adipose tissue, bone, lung or neuromuscular tissue (Bernardes de Jesus et al., 2012), thus being associated with an improvement of the global health status of adult mice (de Jesus et al., 2011). Indeed, telomerase gene therapy has recently emerged as a potential mean to counteract organism malfunction linked to ageing (Bernardes de Jesus et al., 2012). Premature loss of telomere length associated with mutation of telomerase protein component (TERT) is responsible of multiple pathologies (Aubert and Lansdorp, 2008) that are likely to affect the future survival and reproduction of those individuals. Overall, the enhanced tissue fitness in cycloastragenol mice led to an extension of median lifespan by 24 to 40% (Bernardes de Jesus et al., 2012). Interestingly, cycloastragenol treatment in mice is also favouring hair re-growth (de Jesus et al., 2011). The authors proposed that the preservation of the skin layer and of the subcutaneous adipose tissue is important to delay the multiple skin lesion associated with ageing in this species.

Our study extends those findings from mice to birds. Our results provide encouraging support to the general idea that long telomeres might reflect high telomerase activity, and in so doing be good predictors of greater telomerase -dependent tissue regeneration and organism health and longevity. Still, we need to be prudent in our conclusions because, despite the fact that flight feather maintenance is paramount for bird survival, a higher rate of growth of feather is not always synonym of better plumage quality (Dawson et al., 2000). Studies are now required to determine whether stimulation of the telomerase activity has positive effects on bird fitness-related traits such as flying capacity, resilience to environmental stress, reproduction or lifespan as it has (at least for health and lifespan) in mice.

5.CONCLUSION

we tested the impact of the cycloastragenol, a plant-derived product stimulating the expression and the activity of telomerase, on telomere lengths and flight feather renewal capacity of captive zebra finches (Taenopygia guttata). Telomere length was longer in cycloastragenol treated finches while their feather grew faster than in controls.

Our data support the idea that long telomeres could reflect high telomerase activity, and in so doing be a good predictor of greater telomerase-dependent tissue regeneration, which may ultimately explain variation in organism quality and longevity.

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