

## Review



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# Does oxidative stress shorten telomeres *in vivo*? A review

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The length of telomeres, the protective caps of chromosomes, is increasingly used as a biomarker of individual health state because it has been shown to predict chances of survival in a range of endothermic species including humans. Oxidative stress is presumed to be a major cause of telomere shortening, but most evidence to date comes from *in vitro* cultured cells. The importance of oxidative stress as a determinant of telomere shortening *in vivo* remains less clear and has recently been questioned. We, therefore, reviewed correlative and experimental studies investigating the links between oxidative stress and telomere shortening *in vivo*. While correlative studies provide equivocal support for a connection between oxidative stress and telomere attrition (10 of 18 studies), most experimental studies published so far (seven of eight studies) partially or fully support this hypothesis. Yet, this link seems to be tissue-dependent in some cases, or restricted to particular categories of individual (e.g. sex-dependent) in other cases. More experimental studies, especially those decreasing antioxidant protection or increasing pro-oxidant generation, are required to further our understanding of the importance of oxidative stress in determining telomere length *in vivo*. Studies comparing growing versus adult individuals, or proliferative versus non-proliferative tissues would provide particularly important insights.

## 1. Introduction

Because of the central function of telomeres in protecting chromosome ends and genome integrity, their study has gained interest in different domains of biology, ranging from cellular biology and epidemiology to ecology and evolutionary biology [1,2]. It has been shown that telomeres shorten with age in a broad range of organisms [3,4], and more importantly that telomere length and/or shortening rate could predict subsequent survival [4,5]. Consequently, telomere length and/or attrition has been suggested to act as a biomarker of individual 'biological age'. Telomere dynamics has been linked not only to individual survival prospects, early-life growth conditions and reproductive success, but also to various physiological and psychological stressors. Telomeres are thus thought to be a biomarker of exposure to environmental challenges and individual lifestyle [1,2,6].

Although the pivotal role of telomeres in health and ageing biology is well recognized, our understanding of the physiological determinants of telomere dynamics *in vivo* is still imperfect. For instance, information regarding the *in vivo* effects of oxidative stress on telomere length and/or shortening rate remain limited because most studies conducted so far have used an *in vitro* approach. Yet, most studies on telomere dynamics make the assumption that, because there is an *in vitro* effect of oxidative damage on telomeres, it is also the case *in vivo*. The recent paper by Boonekamp *et al.* [7] highlights this limitation and the gap that exists in the literature on these *in vivo* effects.

With this review, we aim to provide a clearer picture of the situation by focusing on what we do and do not know about the *in vivo* links between

oxidative stress and telomeres. We provide a brief summary of telomere structure and main mechanisms by which telomere length is regulated. We then cover the *in vivo* aspects of the impact of oxidative stress on telomere dynamics. We survey the literature and critically evaluate *in vivo* correlative and experimental studies investigating the link between oxidative stress and telomere length and/or shortening. Finally, we highlight several key parameters likely to contribute to the mixed results published so far, and propose different experimental approaches that should help to provide robust data in future studies.

## 2. Telomeres structure and shortening

Telomeres, protective DNA–protein complexes situated at the end of eukaryotic chromosomes, are made of non-coding DNA sequences that consist of tandem repeats of a simple sequence of nucleotides which is rich in guanine (G) [8]. While the length of telomeres varies between chromosomes and species, the sequence is similar in all eukaryotes, indicating that telomeres are a highly conserved and ancient structure with a significant evolutionary role in protecting genome integrity [9].

The length of telomeres is dynamic and results from a balance between restoration and loss processes. Because DNA replication is a partially incomplete process, each time a cell divides telomeric DNA sequences of the chromosomes are lost, a phenomenon known as the ‘end replication problem’ [10]. Telomeres can shorten by 30 to 200 bp per cell division, but only 10 bp loss is thought to be due to the end replication problem in human cultured cells [11]. Oxidative stress leading to DNA damage is thought to be the main factor responsible for the remaining loss [12].

Oxidative stress can arise from the reactive oxygen species (ROS) generated from exogenous sources (UV radiation and pollutants), but the majority of intracellular ROS are thought to arise as a by-product of aerobic metabolism and ATP production in the mitochondria [13]. ROS are highly reactive and will cause oxidative damage to various biomolecules. Such damage can either be prevented by defence mechanisms known as antioxidant defences, or repaired in some cases after they occur. Oxidative stress is thus the result of an imbalance between antioxidant defences and ROS production. Owing to their high guanine content, telomeres are thought to be especially sensitive to oxidative damage [14]. If not prevented, the oxidative damage of telomere regions will lead to an accumulation of damage to DNA and exacerbate telomere loss. Although oxidative damage can cause telomere shortening through double stranded breaks to DNA, most telomere loss due to oxidative stress occurs during DNA replication as a result of single-strand DNA damage [12]. As telomeric regions have a low efficiency of single-strand DNA damage repair, telomeres containing such single-strand DNA damage will not be fully replicated at the next cellular division. Therefore, telomeres containing such DNA damage will shorten more following the next cellular division because the sequence beyond the damage will be lost [15]. Different mechanisms exist to maintain or restore telomere length, and the main one is the activity of telomerase, a ribonucleoprotein able to elongate telomeres [16]. In the absence of restoration, telomere length shortens with each cell division; when the telomeres reach a critical length threshold, they induce a permanent arrest in the cell cycle known as cellular senescence, which

may be followed by cell death. Given their role in cellular senescence, telomeres are thought to be also implicated in organismal senescence and ageing [1].

Most of the work looking at the effects of oxidative stress on telomere dynamics has been conducted *in vitro*. Except for a couple of studies [17,18], most *in vitro* experiments have shown that oxidative stress accelerates telomere shortening [12,15,19]. Oxidative stress is, therefore, thought to mediate the effects of several environmental factors on telomere dynamics at the organismal level, but surprisingly *in vivo* effects of oxidative stress on telomere dynamics have been relatively poorly investigated, as highlighted in a recent publication [7].

## 3. What is the current evidence showing that oxidative stress shortens telomeres *in vivo*?

We searched the published literature using the Web of Science search engine in May 2017, using combinations of the following terms: telomere\*, oxidative stress, antioxidant\*, oxidative damage, correlation\*, experiment\*. We identified studies of interest reporting either correlations between oxidative stress markers (without restriction on the nature of the markers) and telomere length and/or shortening, or experimental manipulations of oxidative stress (antioxidant depletion/supplementation) and subsequent measures of telomere length and/or shortening.

### (a) Correlative studies

Eighteen studies reported correlative information on the links between oxidative stress and telomeres (table 1); eight in humans and 10 in avian species. Overall, 10 of 18 studies report significant correlations between a variety of oxidative stress marker(s) and telomere length and/or attrition. Studies in humans (six of eight) were slightly more likely to report significant results than studies in birds (four of 10). The methodology used for telomere measurement had no major effect on the outcome, with two of five studies using terminal restriction fragment (TRF) and seven of 12 studies using quantitative PCR (qPCR) reporting significant results. Surprisingly, markers of oxidative damage were not more likely (six of 14) to be associated with telomere length than markers of antioxidant defences (five of 12). In birds, studies looking at telomere shortening were slightly more likely to find significant results than those looking at telomere length *per se* (four of eight versus one of eight). Overall, the correlative evidence remains equivocal in supporting the assumption that oxidative stress contributes to telomere shortening *in vivo*.

### (b) Experimental studies

In total, eight studies used a controlled experimental approach (i.e. manipulation of oxidative stress) to investigate the links between oxidative stress and telomeres (table 2). Two studies used L-buthionine sulfoximine (BSO) treatment to selectively reduce the endogenous levels of glutathione, an important intracellular antioxidant. The six other studies used supplementation with various antioxidants either alone or in combination, such as vitamins C and E, coenzyme Q10 or methionine. Overall, seven of eight studies provide partial or total support for a significant effect of oxidative stress on telomere length and/or shortening rate. The only study not supporting this hypothesis [45] was conducted during embryonic

**Table 1.** Summary of correlative studies conducted *in vivo* and testing the relationships between oxidative stress markers and telomere length (TL) and/or telomere shortening ( $\Delta$ TL). The directions of the correlations are not presented in the table, because they were always in the predicted direction, namely that high oxidative damage were associated with shorter telomeres or faster telomere shortening, while high antioxidant levels were associated with longer telomeres or reduced telomere shortening. Method of telomere length measurement is indicated as quantitative polymerase chain reaction (qPCR), terminal restriction fragment (TRF) or quantitative fluorescence *in situ* hybridization (qFISH). RBCs, red blood cells; TAC, total antioxidant capacity; SOD, antioxidant enzyme superoxide dismutase; glutathione, a major intracellular antioxidant; ROMs, reactive oxygen metabolites, a marker of overall oxidative damage.

species	sample type (TL versus OS)	oxidative stress (OS) markers	TL method	significant link between OS and TL/ $\Delta$ TL	references
human	<i>Homo sapiens</i>	urinary lipid damage	TRF	TL: yes	[20]
human	<i>Homo sapiens</i>	plasma TAC	TRF	TL: yes	[21]
human	<i>Homo sapiens</i>	plasma protein damage, glutathione and SOD	TRF	TL: no overall <sup>a</sup>	[22]
human	<i>Homo sapiens</i>	DNA damage	qFISH	TL: yes	[23]
human	<i>Homo sapiens</i>	oxidative stress genes polymorphism	qPCR	TL: yes	[24]
human	<i>Homo sapiens</i>	plasma non-enzymatic antioxidants	qPCR	TL: yes	[25]
human	<i>Homo sapiens</i>	plasma vitamins C and E	qPCR	TL: yes	[26]
human	<i>Homo sapiens</i>	urinary lipid and DNA damage	qPCR	TL: no	[27]
great tit	<i>Parus major</i>	plasma ROMs and TAC	qPCR	TL: no $\Delta$ TL: yes (ROMs)	[28]
common yellowthroat	<i>Geothlypis trichas</i>	plasma TAC, RBC DNA damage	qPCR	TL: not reported $\Delta$ TL: yes (TAC)	[29]
king penguin	<i>Aptenodytes patagonicus</i>	plasma ROMs and TAC	qPCR	TL: yes (TAC+ROMS) $\Delta$ TL: yes (ROMs)	[30]
king penguin	<i>Aptenodytes patagonicus</i>	ROMs, TAC and DNA damage	qPCR	TL: no	[31]
coal tit	<i>Periparus ater</i>	RBC DNA damage and plasma TAC	qPCR	TL: no $\Delta$ TL: yes (DNA damage)	[32,33]; see electronic supplementary material
European starling	<i>Sturnus vulgaris</i>	plasma lipid damage	qPCR	TL: no $\Delta$ TL: no	[34]
European starling	<i>Sturnus vulgaris</i>	plasma DNA damage	qPCR	TL: no $\Delta$ TL: no	[35]
zebra finch	<i>Taeniopygia guttata</i>	plasma ROMs+TAC, and RBC DNA damage	qPCR	TL: no $\Delta$ TL: no	[36]
tree swallow	<i>Tachycineta bicolor</i>	plasma ROMs + TAC	TRF	TL: no	[37]
jackdaw	<i>Corvus monedula</i>	plasma ROMs + lipid damage, RBC glutathione	TRF	TL: not reported $\Delta$ TL: no	[7]

<sup>a</sup>Except for a significant correlation between protein damage and TL in Parkinson's disease patients only.

**Table 2.** Summary of experimental studies conducted *in vivo* and testing the effects of antioxidant depletion or supplementation on telomere length (TL) and/or telomere shortening ( $\Delta$ TL). Method of telomere length measurement is indicated as quantitative PCR (qPCR), terminal restriction fragment (TRF) or quantitative fluorescence *in situ* hybridization (qFISH) and arrows describe decrease ( $\searrow$ ), increase ( $\nearrow$ ) or non-significant ( $\leftrightarrow$ ) effects.

species	type of study	tissue type	oxidative damage	TL method	telomere length (TL)/telomere shortening ( $\Delta$ TL)	significant link between OS and TL/ $\Delta$ TL	reference
CAST/Ei mouse	antioxidant depletion	12 tissues	$\nearrow$ protein damage	TRF	$\searrow$ TL in 5/12 tissues	yes, but tissue-dependent	[38]
New Zealand white rabbit	antioxidant depletion	contralateral arteries	$\nearrow$ oxidized glutathione	TRF	$\searrow$ TL	yes	[39]
Wistar rat	antioxidant supplementation	heart	$\leftrightarrow$ lipid damage $\searrow$ protein damage <sup>a</sup>	TRF	$\searrow$ $\Delta$ TL and $\nearrow$ TL	yes	[40]
broiler chicken	antioxidant supplementation	lymphocytes	$\searrow$ DNA damage for vit C and E	qFISH	$\searrow$ $\Delta$ TL for vit E but not vit C	yes, but for vit E only	[41]
blue tit	antioxidant supplementation	RBCs	not measured	qPCR	$\searrow$ $\Delta$ TL	yes	[42]
zebra finch	antioxidant supplementation	RBCs versus plasma	$\leftrightarrow$ lipid damage <sup>b</sup>	qPCR	$\searrow$ $\Delta$ TL and $\nearrow$ TL in females $\leftrightarrow$ TL and $\leftrightarrow$ $\Delta$ TL in males	yes in females	[43]
yellow-legged gull	antioxidant supplementation	RBCs	not measured	qPCR	$\nearrow$ TL in 'bold' chicks $\leftrightarrow$ TL in 'featherful' chicks	yes in 'bold' chicks	[44]
yellow-legged gull	antioxidant supplementation	RBCs versus plasma	$\leftrightarrow$ lipid and protein damage	qPCR	$\leftrightarrow$ TL	no	[45]

<sup>a</sup>In the 'recuperated' group at three months of age only.

<sup>b</sup>Presented in [46].

development, when telomerase activity is supposed to be high, and did not show a clear effect on oxidative damage levels either. Still, it is worth noting that the effects of oxidative stress on telomere length are likely to be tissue dependent [38], and in some cases, restricted to particular groups of animals that might be more sensitive to changes in antioxidant defences than others [43,44]. Among the six studies measuring the impact of the treatment on oxidative damage levels, five of them obtained results that were mostly consistent between the effects of the treatment on oxidative damage on the one hand, and on telomere length and/or shortening on the other hand. Overall, the experimental evidence gathered so far mostly support the assumption that oxidative stress contributes to telomere shortening *in vivo*.

#### 4. Limitations of the current correlative and experimental evidence

Several experimental aspects could explain the heterogeneity of results we found in studies looking at *in vivo* relationships between oxidative stress and telomere length. First of all, the tissues sampled as well as the timing of sampling are key parameters to consider. Indeed, it was shown that increased telomere shortening in response to oxidative stress is likely to be tissue dependent [38]. However, most correlative studies (13 of 18) measured oxidative stress markers and telomere length in different tissue types (e.g. oxidative stress in plasma and telomere length in DNA isolated from blood cells). This probably precludes obtaining robust information because both variations in telomere length and oxidative stress markers can be tissue dependent (e.g. [47,48]). Similarly, measuring oxidative damage to lipids/proteins but not to DNA is not ideal when testing the effect of oxidative stress on telomere length, because oxidative damage levels to different biomolecules are not necessarily correlated (e.g. [49,50]).

The timing of sampling to measure both oxidative stress and telomere length is also a key parameter to take into account. Indeed, oxidative stress levels are likely to vary much more quickly than telomere length. Moreover, most of the effects of oxidative stress on telomere length are supposed to be visible only after the next cellular replication, because single-strand damage is more likely to occur than double-strand breaks, and such single-strand damage will only shorten telomeres during replication [12]. Therefore, the effects on telomere shortening of a rise in oxidative stress at a given time point might only be visible later on. This implies not only that experimental studies should look at telomere length long enough for replication to happen after the manipulation occurs, but also that correlative studies should wisely choose their sampling timing. For instance, one potential sampling strategy could be to measure 'initial' telomere length and oxidative stress, measure 'final' telomere length later on (ideally considering the timing of cellular division in the target tissue), and then correlate telomere shortening to initial oxidative stress levels. Indeed, because telomere length is likely to be largely determined by inheritance and early-life conditions [51,52], using the rate of telomere shortening will avoid this 'background noise' in a correlation with oxidative stress levels. Accordingly, we show in electronic supplementary material S1 and S2, using one of our own datasets (data available in electronic supplementary material, S3), that such an approach was the only one revealing a significant relationship between oxidative

damage to DNA and telomeres in coal tit (*Parus ater*) nestlings (information on oxidative stress and telomere measurements were previously published separately in [32,33]).

The life stage at which animals are sampled is also a paramount aspect to consider. For instance, telomerase is likely to be active during embryo development, and potentially at later life stages in particular tissues in some taxa [3]. This is important to consider, because it could mask the true relationship between oxidative stress and telomere shortening *in vivo*. In addition, during the growth period, the end replication problem during cellular division is likely to be one key driver of telomere shortening, which can have different consequences that researchers should consider. Indeed, the rapid cellular division and the associated end replication problem during growth could reduce the likelihood of finding significant results in correlative studies, because it will decrease the relative proportion of telomere shortening being linked to oxidative stress. Alternatively, rapid cellular division linked to growth could increase the likelihood of detecting significant results in experimental studies by converting rapidly single-strand damage into actual telomere shortening.

The nature of the experimental manipulation should also be carefully considered. Indeed, while antioxidant supplementation studies detailed in table 2 were quite successful in finding significant beneficial effects on telomere length, any non-significant result of such supplementation would be unsurprising in our opinion. Indeed, such antioxidant supplementation is likely to be beneficial only if there is a need for extra antioxidants, and not if animals are not naturally resource-limited [53]. This could explain why in some cases antioxidant supplementation was only beneficial for some specific groups of animals [43,44].

Finally, other types of biases, such as statistical bias or publication bias could also skew our understanding of the effects of oxidative stress on telomeres. Indeed, keeping in mind that 'correlation is not causation', the lack of significant correlation is definitively not good support against causation either. Importantly, the type II statistical error (i.e. 'false-negative') thus has to be carefully considered before drawing conclusions about non-significant relationships (as done by [7]), and sample sizes have generally to be very large to limit type II error. The potential bias toward the publication of only significant results is also likely to alter the overall picture found in the scientific literature so far. This is likely to be especially true in experimental studies as their main focus is on the links between oxidative stress and telomere shortening; correlative studies are probably less sensitive to this bias because they are often reporting the correlation between oxidative stress and telomeres as part of other biological information.

#### 5. What should we do to move the field forward?

We believe that only carefully designed experiments will provide a robust answer to the question of the importance of oxidative stress for telomere shortening *in vivo*. Direct manipulation of ROS production or downregulation of antioxidant defences is undoubtedly a more powerful approach than antioxidant supplementation, because supplementation is only efficient in response to a natural limitation in antioxidant defences. However, manipulating ROS *in vivo* is very challenging as highlighted in a recent review [54]. Still, some



experiments using pro-oxidant molecules have been successful in inducing moderate oxidative damage (e.g. [55]), and measuring telomere length in such context should provide useful information. The selective downregulation of the endogenous antioxidant glutathione using BSO is undoubtedly one of the most powerful tools available to researchers [38]. This manipulation is highly selective because BSO only inhibits glutathione synthesis and does not affect other cellular pathways. It is also worth mentioning that experimental studies are more likely to reveal a significant impact of oxidative stress on telomeres than correlative studies. Indeed, it is possible that organisms under natural conditions are able in most cases to maintain oxidative stress at a threshold level that does not impact telomeres, while experimentally manipulating oxidative stress could disrupt such balance.

Regardless of the kind of experimental manipulation employed, it is important to validate the impact of the treatment on oxidative damage (preferably on DNA) before examining the impact on telomere length and/or shortening. If possible, oxidative damage and telomere length should be measured in the exact same sample type. Investigating the impact of the treatment should be done in several tissues because the most convincing study to date [38] found tissue-specific effects of BSO on telomere length. As mentioned in the previous paragraph, life stage as well as tissue type could constrain the effects of oxidative stress on telomere dynamics. Conducting the same experiment in both growing and adult individuals and comparing proliferative versus non-proliferative tissues will thus be important, in order to assess the sensitivity of telomeres to oxidative stress at different life stages as well as the importance of cellular division in revealing the impact of oxidative stress on telomere length. Finally, given the various experimental constraints (e.g. repeated injections or continuous supplementation in water/food, close monitoring of health

state) and ethical considerations, we suggest that such studies should be conducted in captive animals.

## 6. Conclusion

The limited number of studies investigating the *in vivo* connection between oxidative stress and telomere dynamics highlights that our understanding of this link still remains incomplete. Although the correlative studies display equivocal results, findings from the limited number of experimental studies conducted so far seem to indicate that oxidative stress affects telomere shortening *in vivo*. Yet, experimental studies are more likely to be susceptible to publication bias as mentioned above. The key to a better understanding of the impact of oxidative stress on telomere shortening *in vivo* will undoubtedly come from robust experimental studies, especially if conducted in a broad range of organisms because between-taxa differences in telomere biology do exist. Finally, when the number of published studies is sufficient to overcome limitations linked to data heterogeneity, it will be of utmost importance to conduct a quantitative meta-analysis of the relationships between oxidative stress and telomere length *in vivo*.

**Data accessibility.** Data are accessible as an excel file in electronic supplementary material S3.

**Authors' contributions.** A.S. and S.R. had the original ideas and wrote the paper.

**Competing interests.** We declare we have no competing interests.

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