Growing Up or Growing Old? Cellular Aging Linked With Testosterone Reactivity to Stress in Youth

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Background: Given the established relation between testosterone and aging in older adults, we tested whether buccal telomere length (TL), an established cellular biomarker of aging, was associated with testosterone levels in youth. *Methods:* Children, mean age 10.2 years, were recruited from the greater New Orleans area, and salivary testosterone was measured diurnally and during an acute stressor. Buccal TL was measured using monochrome multiplex quantitative real-time polymerase chain reaction. Testosterone and TL data were available on 77 individuals. The association between buccal TL and testosterone was tested using multivariate generalized estimating equations to account for clustering of children within families. *Results:* Greater peak testosterone levels ($\beta = -0.87$, $P < 0.01$) and slower recovery ($\beta = -0.56$, $P < 0.01$) and reactivity ($\beta = -1.22$, $P < 0.01$) following a social stressor were significantly associated with shorter buccal TL after controlling for parental age at conception, child age, sex, sociodemographic factors and puberty. No association was initially present between diurnal measurements of testosterone or morning basal testosterone levels and buccal TL. Sex significantly moderated the relation between testosterone reactivity and buccal TL. *Conclusions:* The association between testosterone and buccal TL supports gonadal maturation as a developmentally sensitive biomarker of aging within youth. As stress levels of testosterone were significantly associated with buccal TL, these findings are consistent with the growing literature linking stress exposure and accelerated maturation. The lack of association of diurnal testosterone or morning basal levels with buccal TL bolsters the notion of a shared stress-related maturational mechanism between cellular stress and the hypothalamic pituitary gonadal axis. These data provide novel evidence supporting the interaction of aging, physiologic stress and cellular processes as an underlying mechanism linking negative health outcomes and early life stress.

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"Getting older" is considered a negative process for adults. The link between advanced aging and negative health outcomes is well established. A significant body of recent research suggests that the divergence between chronological and biological aging may underlie the negative health outcomes associated with early adversity and stress, thus highlighting the need for greater examination of the underlying biological processes. More recently, a downward extension of the aging process suggests that the biological trajectories of accelerated aging are established during early developmental windows typically characterized by greatest health, that is, childhood. The ability to understand the aging process from a life course perspective and measure the impact of stress on maturational processes is critical as we seek to disentangle the factors contributing to accelerated aging early in development when maturation likely has different implications and associations. As such, the impetus for this present study is to expand the number of biological indicators of aging with applicability across the life course and explore the relationship among them. The long-term goal of this line of research is to inform novel intervention and prevention efforts directed at ameliorating the negative effect of stress as early as possible in development.

Telomere Length as a Marker of Aging Across the Life Course

Telomere length (TL) is an established cellular marker of aging that is gaining validity as a marker of stress and cumulative adversity.[1,2] Shorter TL, in adults, is associated with a range of aging-related negative health outcomes, including cardiovascular disease, cancer, cognitive decline, diabetes and psychopathology.[3,4] TL serves as a biological cellular clock and figures prominently in cellular differentiation and senescence.[5] Factors that trigger DNA damage, such as toxins, oxidative stress and radiation, preferentially affect telomeres, thereby accelerating TL decline.[6] In youth, shorter TL has been associated with early life adversity, socioeconomic levels, prenatal exposure to tobacco and neighborhood and community violence in multiple studies.[2,7–9]

Testosterone and Aging

Testosterone has historically been thought of as a masculinizing hormone that rises (especially in boys) and triggers secondary sexual characteristics related to pubertal maturation and behavioral changes more commonly seen after puberty. As a lipid-soluble steroid hormone, testosterone easily crosses through cell membranes, travels into the nucleus of the cell to directly alter gene expression, passes through the acinar cells and into saliva by passive diffusion and crosses the blood-brain barrier to influence neural function.[10] Receptors for testosterone are found throughout the brain,[11,12] particularly within limbic structures. Acute changes in testosterone modifies an individual's responses to salient emotional stimuli involving largely fear,[13] anger[14] and reward cues,[11,15] suggesting that acute testosterone enhances an individual's reactivity, both physiologically and behaviorally, to emotional and stressful stimuli within a brief time course. Most previous studies have examined the impact of exogenously administered testosterone on behavior and physiology to understand acute testosterone changes, yet

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this is challenging in pediatric populations. Endogenous testosterone release can be triggered by exposure to stressful challenges,[16] such as the Trier Social Stress Test for Children (TSST-C),[17] which takes advantage of testosterone's response to social evaluative threat to acutely alter testosterone release within 20 to 60 minutes.[13,18] To more closely capture maturational processes, morning testosterone levels are often used as an index of basal testosterone levels[19] because testosterone values are typically at their highest in the morning. In addition, testosterone's diurnal change across the day is expected to capture the flexibility or change in this axis from nadir to zenith and has been suggested to be a useful index of the range of testosterone to which the child must adjust.[20,21] Although all of these measure the hypothalamic pituitary gonadal (HPG) axis, these measurements each capture unique aspects of HPG development, including acute stress responsivity, level of development and changing maturation processes.[22] All 3 aspects are measured in the present study.

Testosterone, similar to TL, is also related to aging. For example, testosterone levels decline in older adults, and testosterone replacement therapy has been thought to slow down the aging process.[23] In youth, who represent the other end of the lifespan, elevated levels of testosterone advance maturational processes.[19,24] Puberty and testosterone are highly correlated, especially within boys,[20] and testosterone is responsible for many secondary sexual characteristics,[25,26] including physical growth spurts.[27] Administration of testosterone results in rapid physical growth and pubertal advancement in boys with delayed puberty or constitutional growth delays.[28,29] In adolescence, testosterone rise associated with stress and competition is especially robust in box sexes; however, sex differences in the behavioral patterns associated with testosterone reactivity are notable.[30] Together, these findings indicate that testosterone, similar to TL, is reflective of aging and maturational processes across the life course. Concurrent exploration of both testosterone reactivity and diurnal measurements is expected to provide novel insight into the links between stress, aging and gonadal maturation.

Linking Testosterone and TL

Testosterone, similar to TL, is associated with a range of health problems,[31] even within young populations and indirect evidence of their association exists.[20] Studies have demonstrated an association between testosterone and telomerase activity in spermatogonia that develop adjacent to testosterone-producing Leydig cells.[32,33] Elevated levels of reactive oxygen species and oxidative stress result in decreased testosterone and decreased telomerase with subsequent decreased TL.[34] Oocyte TL shortening has also been related to ovarian testosterone levels, suggesting that the relationship between testosterone and TL may be evident in both males and females.[35] One previous study in elderly males showed an association between aging and both leukocyte TL and basal testosterone levels. However, in this study, testosterone basal levels were not directly associated with TL, suggesting that if a direct relationship exists between TL and testosterone, testosterone stress reactivity may better capture this association.[36] No previous study, to our knowledge, has examined the relation between TL and testosterone in youth.

Capitalizing on an existing cohort of children recruited from the greater New Orleans area, the association between TL and testosterone reactivity during a social stressor, morning basal levels and diurnal testosterone measures were tested. We hypothesized that elevated testosterone would be associated with shorter TL. However, to better refine our competing hypotheses related to the potential association of testosterone and TL, we tested the association of TL with 3 overarching testosterone indices in the same child: basal, diurnal and stress-reactive testosterone. Demonstration of an association between TL and testosterone would increase the utility of both biomarkers and provide additional evidence that the divergence of biological from chronological aging as a result of stress underlies the lasting negative health outcomes associated with early adversity.

METHODS

Subjects

Children, aged 5 to 15 years, were recruited from the greater New Orleans area. Families were recruited using street outreach techniques, including ethnographic mapping and targeted sampling and through schools in these communities. Recruitment neighborhoods were identified using the community identification process, a mapping method to record epidemiological indicators of the prevalence and incidence of community violence and other selected social and health conditions. Interested families contacted the research site to schedule an appointment. Transportation was provided, and families were compensated. This study was approved by the Tulane University Institutional Review Board.

Data

Parental caregivers provided information about multiple levels of the child's social ecology (ie, household and neighborhood) using an interview-assisted computer survey administered face-to-face at the research site (Questionnaire Development System; Nova Research, Bethesda, MD). Oral responses were recorded by trained interviewers on the computer. Buccal swabs were collected from the child for DNA analysis of TL. Body mass index (BMI) was measured at the research site.

Telomere Length

DNA was collected using Isohelix SK1 buccal swabs (Cell Projects, Kent, United Kingdom) and extracted using the QIAamp DNA Mini Kit protocol (Qiagen, Valencia, CA). Concentration of extracted DNA was quantified with a Qubit dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA), purity of the DNA was determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and DNA integrity was confirmed by gel electrophoresis. DNA was stored at −80°C. The average relative buccal cell TL was determined from the telomere repeat copy number to single gene (albumin) copy number (T/S) ratio using an adapted monochrome multiplex quantitative real-time polymerase chain reaction (PCR) and a BioRad CFX96.[37] Ten microliters of DNA sample, containing ~0.1 to 0.5 ng of DNA, was combined with 15 μL of PCR mixture, for a final volume of 25 μL per reaction. The PCR consisted of 0.75X Sybr Green I (Invitrogen), 1X Gene Amp Buffer II (Applied Biosystems, Foster City, CA), 0.8 mM dexoynucleotide triphosphates (dNPTs), 10 mM $MgCl_2$, 3 mM dithiothreitol, 1 M betaine, 2.5 U AmpliTaq Gold polymerase (Applied Biosystems), 0.9 μM telg primer (ACACTAAGG TTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT), 0.9 μM telc primer (TGTTAGGTATCCCTATCCCTATCCCTATCCCTAT CCCTAACA), 0.6 μM albd2 primer (GCGGGCCCGCGT GGCGGAGCGAGGCCGGAAAAGCATGGTCGCCTGT) and 0.6 μM albu2 primer (GCCTCGCTCCGGGAGCGCCGC GCGGCCAAATGCTGCACAGAATCCTTG). The reaction proceeded for 1 cycle at 95°C for 15 minutes, 2 cycles at 94°C for 15 seconds and 49°C for 1 minute, 4 cycles at 94°C for 15 seconds