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The Effects Of HIV Disease And Lifestyle Factors On Cellular Aging In Trangender Women

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ACCEPTANCE

This thesis, THE EFFECTS OF HIV DISEASE AND LIFESTYLE FACTORS ON CELLULAR AGING IN TRANSGENDER WOMEN by Scott Sohn was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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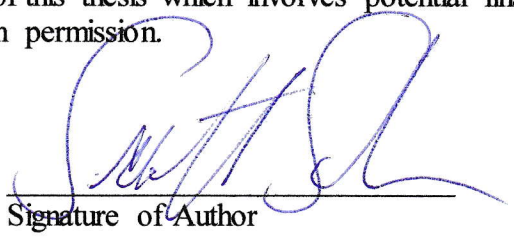

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ABSTRACT

THE EFFECTS OF HIV DISEASE AND LIFESTYLE FACTORS ON CELLULAR AGING IN TRANSGENDER WOMEN

by

Scott Stephen Sohn

Background: Telomeres are short tandem repeats of nucleotides at the ends of chromosomes. These specialized structures serve as caps on the end of the chromosomes, which protect DNA integrity. Telomeres get shorter each time a cell replicates, but the DNA remains intact as long as the telomere caps are a sufficient length. In time, telomeres become too short to protect DNA, which leads to cellular death. Previous research has shown that disease and negative lifestyle factors play a role in accelerated telomere attrition throughout the cellular lifecycle.

Objective: The purpose of this study was to determine if HIV infection and lifestyle factors in a transgender population living in Atlanta Georgia are associated with telomere length reduction.

Participants/setting: This study is a secondary analysis of data provided by a Georgia State University study entitled "Telomere Length, Environmental Stressors and Health Related Outcomes among Transgender Women". The study included 92 transgender women from Atlanta, Georgia with 49 reporting HIV infection. Two sources of data were collected, survey responses collected during face to face interviews and a saliva sample for DNA analysis.

Statistical analysis: Frequency statistics were used to describe the sample population. A Mann Whitney U was used to evaluate telomere length using the T/S ratio by HIV status, by physical activity level (healthy active or low active) and by fruit and vegetable intake category (Don't eat, 1-2 servings/day, 3-4 servings/day vs. $\geq$5 servings/day) in the total

population. Multiple regression analysis was used to examine the association between independent variables (activity level, body mass index, fruit and vegetable intake, hormone use, race, HIV status and age) and telomere length.

Results: The majority of the population was Black (84%) with a median age of 33 years (range, 18 to 65 years). No significant association was observed between HIV infection and T/S ratio. The vast majority of the population reported low activity level and only 9% reported consuming ≥5 servings of fruits and vegetables daily. No significant association was found between fruit and vegetable intake or physical activity level and T/S ratio in this population.

Conclusion: HIV infection**,** Fruit and vegetable intake, and physical activity were not found to impact telomere length in an urban population of transgender women. Future research is needed to further understand the mechanisms that impact telomere length throughout the cellular lifecycle within the transgender population.

THE EFFECTS OF HIV DISEASE AND LIFESTYLE FACTORS ON CELLULAR AGING IN TRANSGENDER WOMEN

by
Scott Stephen Sohn

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ABBREVIATIONS

ADP	Adenosine Diphosphate
AIDS	Acquired Immunodeficiency Syndrome
aTL	Absolute Telomere Length
BMI	Body Mass Index
cART	Combination Antiretroviral Therapy
cm	Centimeters
CRP	C-Reactive Protein
DHA	Docosahexaenoic acid
DII	Dietary Inflammatory Index
DNA	Deoxyribonucleic acid
EPH	Eicosapentaenoic acid
GA	Georgia
Hcy	Homocysteine
HIV	Human Immunodeficiency Virus
HIV-	HIV Negative
HIV+	HIV Positive
IQR	Interquartile Range
kb	Kilo-base Pairs
kg	Kilograms
m	Meters
MACS	Multicenter AIDS Cohort Study
MD	Mediterranean Diet

miRNA	Micro-Ribonucleic acids
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
SAM	S-adenosyl-methionine
SN	Seronegative
SP	Seropositive
TERT	Telomerase Reverse Transcriptase
TERC	Telomerase RNA component
THF	Tetrahydrofolate
TNF	Tumor Necrosis Factor
TRF	Terminal Restriction Fragment
T/S	Telomere-to-Single Copy Gene

CHAPTER I

THE EFFECTS OF HIV DISEASE AND LIFESTYLE FACTORS ON CELLULAR AGING IN TRANSGENDER WOMEN

INTRODUCTION

The World Health Organization estimates that approximately 40 million people worldwide are living with the Human Immunodeficiency Virus (HIV), which is the virus that causes Acquired Immunodeficiency Syndrome (AIDS), the most advanced stage of HIV infection.[1] Additionally, another 2 million are infected throughout the world annually, and in 2014 roughly 1.2 million people died from AIDS. HIV seeks out and destroys the infection-fighting CD4 cells of the immune system. Depletion of CD4 cells caused by HIV infection inhibits the body's ability to fight off infections and other neoplasms. Without treatment, HIV slowly destroys the immune system and advances into full blown AIDS. Current treatment for HIV includes a variety of drugs referred to as combination antiretroviral therapy (cART), which can effectively control the virus and stop the progression to AIDS.[2] The prevention of AIDS related complications has been extremely successful through the use of Antiretroviral therapy. However, patients using cART are still at a much higher risk for many diseases that are typically seen within older age groups. Cardiovascular disease, neurocognitive impairment, osteoporosis, cancer, and frailty are common within the HIV population. The incidence of HIV-associated non-AIDS defining conditions is increasing within the aging and HIV infected population.[2]

It is thought that disease and lifestyle factors, including diet, and physical activity play important roles in the various aspects of aging. Those who eat healthy diets with plenty of anti-oxidant rich foods, while engaging in regular physical activity, have increased life spans and fewer incidences of several diseases[3]. Increased life spans, and healthy aging have been attributed to chromosomal preservation of telomeres. Telomeres are the caps on the ends of chromosomes that progressively shorten throughout life due to aging, disease, and environmental factors. When telomere length decreases to a critical point, cellular death (senescence) ensues.

Several studies have shown that the length of telomeres is influenced by a combination of disease and lifestyle factors. The length of telomeres decreases by approximately 100 bases per cell division. After a child is born it is thought that the length of telomeres has already been reduced to 10,000 base pairs.[3] Throughout a lifetime, the length of telomeres decreases to around 5000 base pairs, at which time the cell can no longer divide. Chromosome telomeres are important in regulating the lifecycle of human cells and therefore, telomere length is a marker of biological aging. Evidence suggests that when the telomere shortens to a critical length, cell senescence is triggered. Telomere degradation has been attributed to age-related conditions such as cardiovascular disease, Parkinson's disease, Alzheimer disease and cancer. Decreased telomere length is also seen in age-related nutritional diseases, such as obesity, diabetes, and atherosclerosis. In addition to age related conditions, decreased telomere length has been associated with HIV infection, low physical activity and poor sleeping patterns. Studies suggest that telomere length is influenced by inflammation and increased levels of reactive oxygen species (ROS). Data from studies have supported the role of lifestyle factors in inhibiting the expression of risk alleles.[3,5] A risk allele is a variant or alternative form of a gene that is present

on a chromosome. Alleles determine certain gene expressions that affect the prevalence of disease onset and influence inherited traits such as eye color and blood type.

The associations between telomere length, HIV infection, environmental factors, nutrition, and physical activity are unknown. Continued research elucidating the processes involved in cellular aging is important for understanding and managing HIV worldwide. Knowledge gained from these type of studies could help people living HIV make positive lifestyle choices that may impact longevity and healthy aging. The purpose of this project is to identify correlations between telomere length and independent variables including HIV infection, diet and physical activity in a population of transgender women who participated in the Telomere Length, Environmental Stressors and Health-related Outcomes among Transgender Women study at Georgia State University. This study utilizes T/S ratio for determining relative telomere lengths by quantitative polymerases chain reaction (PCR). Each DNA sample is measured, by which the sample differed from a reference DNA sample, in its ratio of telomere repeat copy number to a single gene copy number. This ratio should be proportional to the average telomere length. This secondary analysis used demographic and environmental data collected during face-to-face interview, and telomere length data that were collected using saliva sample for DNA analysis.

Specific Aim 1: To examine the association between HIV status and T/S ratio in a population of transgender women.

Research Hypothesis 1: T/S ratio will be smaller in transgender women with vs. without HIV infection.

Specific Aim 2: To examine the association between dietary patterns and T/S ratio by HIV status.

Research Hypothesis 2: Transgender women with or without HIV who consume a greater number of servings of anti-oxidant rich foods will have a higher T/S ratio than those who consume fewer servings.

Specific Aim 3: To examine the association between physical activity level and T/S ratio by HIV status.

Research Hypothesis 3: Transgender women with or without HIV who report greater physical activity levels will have a higher T/S ratio than those who report a lower level of physical activity.

CHAPTER II

Literature Review

Telomeres

The first identification of telomeres came from the discovery of the telomerec DNA sequence in tetrahymena thermophile, a single celled animal with 40,000 chromosomes. The first paper was published in 1978 by Elizabeth H. Blackburn and Joseph G. Gall, which showed the existence of several copies of a repeated DNA sequence at the ends of chromosomes identified as telomeres.[4] Further research also revealed the existence of several accessory proteins, as well as the enzyme telomerase, which is used for elongation of eukaryotic telomerec DNA.[4, 5] In addition to telomerase, it was shown that chromosomes also use a recombination mechanism that facilitates elongation in an alternative pathway.

The nucleus of a cell contains the DNA, which contains genetic information that is assembled into structures called chromosomes. Each successive cell division duplicates the chromosomes. However, the proteins that are necessary for copying the DNA may not reach the end of the DNA strand, leading to progressively shorter chromosomes.[5] It is thought that telomeres have several functions within the chromosome. They may protect against attrition, aid in reconstruction, and mitigate loss of DNA material. Additionally, telomeres prevent chromosomes from fusing with each other and from being identified as double-strand breaks by DNA repair proteins.

Due of the limited capacity of DNA polymerase to replicate chromosomes, telomeric DNA consists of noncoding double-stranded repeats of G-rich tandem DNA sequences

(TTAGGG) which function as caps at the ends of chromosomes. With successive cell divisions, telomeres shorten until they reach a critical length. Telomeres, in fact act as a mitotic clock for cellular life. Following a finite number of divisions, eukaryotic cells experience a growth arrest in the G1 phase of the cell cycle referred to as "cellular senescence". This termination of growth is understood to be linked to the shortening of telomeres. Therefore, the length of telomeres is considered to be a marker that indicates the number of cell divisions in conjunction with environmental stressors.[3]

Upon conception, the length of telomeres in a human averages roughly 15,000 base pairs or 2,500 (TTAGGG) repeats. These repeats are referred to as a G-rich overhang denoted as a G-tail. While the ends of telomeres are shortened with each cell division, most eukaryotes use the telomerase enzyme to extend telomerec DNA. Telomerase counteracts the effects of successive shortening due to cell division and stressors.[3] Telomerase is a ribonucleoprotein structure that utilizes its RNA as a template to extend the 3" overhang of the telomere. The catalytic center of telomerase is comprised of the telomerase reverses transcriptase (TERT) and the telomerase RNA (TERC). The structure includes a reverse transcriptase (Est2), a template RNA (TLC1) and two additional proteins (Est1 and Est3) that are required for telomerase activity.[3]

The usual method for determining telomere length in DNA samples identifies a mean terminal restriction fragment (TRF) length. This procedure requires large amounts of DNA and 3-5 days to process. Additionally, TFR lengths may vary by 5% or more depending on the restriction enzyme used. The method designed by Cawthon and used in this analysis determines mean telomere length across all chromosomes for all cells sampled. Real-time PCR assay includes identifying a Telomere-to-Single Copy Gene (T/S) ratio, which is shown to be proportional to telomere length in cells. This method includes two quantitative PCR reactions for

each subject. One reaction for a single copy gene (S) and one reaction in the telomerec repeat region (T). Both reactions are compared to a standard reference sample prior to T/S ratio calculation. The T/S ratio is equivalent to 1 if the sample is identical to the reference. The T/S ratio of one sample compared to another should approximate the telomere length in DNA.[6]

HIV-1 Infection and Aging

Rickabaugh et al. (2011) estimated that the lifespan of HIV-infected subjects is reduced by an average of 10 years despite cART, (combination anti-retrovirus treatment).[7] Disease and mortality within the HIV infected population is comparable with older uninfected populations aged 50 and up. Diseases such as cancer, osteoporosis, cardiovascular disease, diabetes and hypertension are common within HIV infected groups. This cross-sectional study included twenty-eight HIV-1 SN (seronegative) participants aged 19-30 years, nineteen SN participants aged 47-60, nine HIV-1 SP (Seropositive) participants 20-32, and ten SP participants 39-58 years old.

Decreased lifespan and higher instances of various diseases has led researchers to estimate that HIV infection causes accelerated aging throughout the body.[7] Normal aging is usually associated with qualitative changes within the naïve CD4+ T-Cell compartment. Shorter telomeres, decreased capacity, hyporesponsiveness, reduced IL-2 production, and changes in cell surface phenotype are usually seen in aging adults. It is thought that HIV-infected populations may experience similar age related illnesses at earlier chronological periods. The results of the Rickabaugh et al. (2011) study also suggests that HIV infection has a cumulative effect on the naïve CD4+ T cell compartment. While drug therapy controls the HIV, damage to the immune system may not be restored to normal levels with cART.[7]

In a separate study conducted by Rickabaugh et al. (2015) on the effects of HIV infection and age, the researchers observed that similar methylation sites are influenced by both age and HIV infection.[8] Participants in this study were recruited from the Multicenter AIDS Cohort Study (MACS), a study of HIV infection in men who have sex with men. Two groups participated in the data set. In the first group, twenty-four of the participants were 20-24 years of age and twenty-four were 48-56 years old. Each age group consisted of twelve HIV-1 SP men and twelve SN men. Data set two consisted of twenty-four individuals 27-35 years old and twenty-four participants, 36-56 years old. Each age group included twelve HIV-1 SP men and twelve HIV-1 SN men. Each HIV-1 SP sample was matched to a SN control according to selection criteria. Methylation patterns were calculated at over 450,000 sites using Infinium methylation arrays. The correlation between age and HIV infection on methylation levels was examined and showed a strong association for both data sets. Sites that are normally hypomethylated with age are comparable with the HIV infected population, while sites normally hypermethylated with age showed further methylation in the HIV infected participants. This study examined the associations between methylation patterns, age and HIV status. Strong correlations were found with respect to age, HIV status and methylation patterns. The study hypothesized that HIV infection accelerates age related methylation patterns in peripheral blood mononuclear cells by an average of 13.7 years in data set one, and 14.7 years in data set two.[8]

HIV-1 Infection and Telomere Length

Current knowledge regarding telomere attrition has conclusively shown the correlation between shorter telomeres and chronic disease states. HIV infection is one such condition that has been of particular interest among scientists due to system wide evidence of shorter telomeres compared to un-infected populations. In a study by Liu et al. (2015), absolute telomere lengths

(aTL) in peripheral leukocytes were measured from 231 HIV-infected adults and 691 HIV-uninfected adults. When compared to HIV-uninfected participants, the mean aTL in HIV-infected subjects was noticeably shorter by 27 kbp/genome ($p<0.001$). The slopes of aTL vs. age were not however different ($p=0.469$). Subjects with longer known durations of HIV infection and lower CD4 counts had shorter aTLs, which was also found in older age, and smoking.

It is thought that HIV infection accelerates the aging process through several mechanisms. Leukocyte telomere lengths are markers of cellular aging which have been shown to be considerably shorter with HIV infection when compared to uninfected populations.[9] Telomeres become shorter with each successive cell cycle until a critical length is reached which triggers cell senescence. Immune system activation and microbial translocation may be responsible for telomere attrition, but the extent of shortening that results from the initial infection is still unknown. While it's been shown that accelerated telomere attrition occurs with HIV infection, it is thought that initiation of treatment may stabilize telomere loss. This study hypothesized that early intervention with cART therapy may slow the rapid aging process experienced by HIV-infected patients. However, it appears that the initial infection with HIV causes a system wide shortening of telomeres which cannot be reversed. After treatment begins, telomere attrition stabilizes and normal age related telomere degradation ensues, which resembles an un-infected population.

Environmental Factors and Telomere Length

Diet

Many external factors play a role in telomere attrition throughout the cell cycle. It is well known that telomeres become shorter with age, stress, infection and disease.[7] Inflammation has been shown to cause cell proliferation during infection and disease which results in loss of

telomerec DNA material. Additionally, elevated C-reactive protein (CRP) levels have been associated with shorter telomere length. Inflammation is also known to create oxidative stress which causes base modifications and breaks in DNA. Telomeres are highly susceptible to oxidative damage which causes a loss of functionality, leaving them vulnerable to fusion with other chromosomes and double stranded breaks. Fusion with other chromosomes causes alterations of DNA material which destabilizes the gemome.[10] Diet and nutritional status may be associated with telomere length, with anti-oxidant rich diets and healthy lifestyles shown to increase telomerase activity in mononuclear cells.[10]

A variety of vitamins and minerals are necessary for cellular maintenance and may mitigate damage to telomerec DNA induced by oxidative stress. Folate is required for methylation and DNA integrity.[10] Tetrahydrofolate (THF) is necessary as precursor for DNA synthesis as a component in the production of pyrimidine thymidylate and of purines. Additionally, Methyl-THF provides methyl groups for the conversion of homocysteine (Hcy) to methionine, which is the precursor of S-adenosyl-methionine (SAM). Lack of folate may create imbalances in the nucleotide pool, which may disrupt replication and cause telomere attrition.[10]

Vitamin A may also protect against telomere attrition. Tumor necrosis factor (TNF) is thought to be reduced by increased plasma levels of vitamin A, while higher intake of the vitamin may increase concentrations of the anti-inflammatory cytokine interleukin-10.[10] Decreased vitamin D levels have been associated with high concentrations of (CRP) which may cause attrition of telomeres. Additionally, vitamins C and E are thought to aid in telomere stability by reducing plasma levels of ROS which mitigates oxidative stress.[10] Similarly, plasma magnesium levels are believed to be positively correlated with telomere length in two ways. Low magnesium levels inhibit DNA repair capacity, and are linked to elevated (CRP) levels and

oxidative stress.[10] Zinc may also play a protective role against telomere loss through many different pathways. Reverse transcriptase, RNA polymerase, and DNA polymerase enzymes within the cells are all dependent on zinc for functionality. The enzyme telomerase is a reverse transcriptase that depends on zinc for functionality as well. Poly (ADP-ribose) also requires zinc for activation and is involved in DNA repair. It is thought that zinc may compete with prooxidant metals for cysteine binding sites which could prevent the formation of free radicals. Furthermore, low levels of zinc are associated with higher infection rates, which cause inflammation that is linked to telomere attrition.[10]

Omega-3 fatty acids have also been associated with decelerated telomere attrition due to their anti-oxidative properties. Eicosapentaenoic acid (EPH) and docosahexaenoic acid (DHA) inhibit the formation of (ROS), which target telomeric DNA and result in telomere attrition.[10] Omega-3s have cardioprotective properties including reducing arrhythmias and interfering with the production of prostaglandins, thereby reducing inflammation and supporting platelet and endothelial function.[11] Current research elucidates the importance of these components in human diets.

In a prospective cohort study of 608 ambulatory outpatients with stable coronary artery disease, Farzaneh-Far et al. (2010) measured leukocyte telomere length at baseline and again after 5 years.[12] Results showed those patients with lowest concentrations of plasma DHA/EPA exhibited the fastest rates of telomere shortening, while those with the highest concentrations experienced the least amount of attrition. The study found each 1-standard deviation increase in DHA/EPA levels was associated with a 32% reduction in the odds of telomere shortening.

The Nurses' Health Study, Crous-Bou et al. (2014) sought to examine whether consumption of a Mediterranean diet was associated with longer telomere length.[13] The study is

an ongoing prospective cohort of 121,700 nurses enrolled in 1976, with 32,825 providing blood samples and 4676 participants completing a food frequency questionnaire. This study found that greater adherence to a Mediterranean style diet was associated with longer telomeres. The Mediterranean Diet includes a high intake of fruits, vegetables, legumes and whole grains, as well as a high intake of olive oil. Additionally, the diet contains a moderate intake of fish with limited intake of meats, poultry and dairy, and moderate intake of alcohol. This diet is linked to lowered mortality, less chronic disease, and healthier aging. Studies have shown that telomere attrition can be modified through diet, as considerable variability exists in the pace of telomere reduction, which is independent of chronological age. The study proposes that variability in telomere length may be partly due to lifestyle factors, including dietary patterns. High anti-oxidant and anti-inflammatory components of the diet are thought to limit telomere attrition and reduce disease onset.[13]

In a prospective study conducted by Garcia-Calzone et al. (2015), five hundred twenty participants at high cardiovascular risk were recruited into the PREDIMED-NAVARRA (Prevencion con Dieta Mediterranea-NAVARRA) trial.[14] Telomere length was measured at baseline and after 5 years of follow-up. Those patients who consumed a Mediterranean diet (MD) consisting of a low dietary inflammatory index (DII) were observed to have longer telomeres. Additional analysis revealed that increased anti-inflammatory content of this diet may reduce the rate of telomere attrition. Conversely, diets with the highest pro-inflammatory values after a 5-year follow-up were associated with nearly a 2-fold increased risk of accelerated telomere loss as compared to diets with low DII values. This study suggests that telomere length may be linked to the pro and anti-inflammatory components of diets. It is hypothesized that

adherence to a Mediterranean diet is related to a slowing of age related telomere shortening, and may decrease the cumulative inflammatory burden on human cells.[14]

Recent data on life expectancy in Mediterranean countries show male life expectancy close to 80 years, and female life expectancy close to 85 years.[15] These findings suggest that a Mediterranean diet may have a protective factor against telomere attrition and correlates to lowered risk for several chronic diseases such as cardiovascular disease, hypertension, diabetes mellitus, and cancer. Consumption of fresh fruits, vegetables, cereals, red wine, nuts and legumes is considered to be the main factor for protection from several diseases.[16] The beneficial effects of the Mediterranean diet has been attributed to compounds such as polyphenols (catechins, flavonols, flavones, anthocyanins, proanthocyanidins and phenolic acids) and antioxidant vitamins, which have been shown to reduce oxidative stress.

Polyphenols are considered as secondary metabolites of plants that provide protection from ultra-violate rays and pathogens. Over 8000 polyphenolic structures have been identified in several plant species. Phenolic acids, flavonoids, stilbenes, and ligands have been shown to protect against several diseases such as cardio-vascular disease, diabetes, neuro-degenerative disease, and cancer.[17]

Shorter telomere length has been linked to age-related diseases and decreased life expectancy while telomere maintenance is thought to be a major determinant of good health in aging populations.[15,16] It is thought that telomere length could be a more accurate indicator of life expectancy than chronological age.

Physical Activity

Regular physical activity has several positive metabolic outcomes and is a key factor in human health and longevity.[18] The Physical Activity Guidelines for Americans, released by the U.S. Department of Health and Human Services recommends that adults have at least 150 minutes of moderate intensity physical activity per week. For substantial health benefits, adults should perform at least 150 minutes per week of moderate intensity, or 75 minutes per week of vigorous-intensity aerobic physical activity.[19]

Chilton et al. (2014) used a sample of twenty-two, non-smoking males with a mean age of 24 years. Subjects engaged in a 30 minutes of continuous treadmill running at 80% of previously determined $O_{2peak.}$[18] Blood samples were taken at three intervals: prior to exercise; immediately following; and 60 minutes after completion. Pooled white blood cell RNA was analyzed to test the effects of intense physical activity on a variety of telomeric genes. This study showed that acute exercise could lead to transcriptional regulation of several telomeric genes in immune cells. Both Telomerase Reverse Transcriptase (TERT) and micro-RNAs (miRNA) were shown to be regulated by physical activity. TERT is a major component involved in telomere maintenance, while miRNAs are thought to control expression of certain genes responsible for telomere stability. The study concluded that 30 minutes of intense physical exercise is sufficient to up regulate the telomeric gene TERT mRNA. Additionally, the study showed the regulation of 56 miRNAs which may have a possible influence on telomeric gene transcripts. Peripheral blood mononuclear cells were analyzed and showed that 34 mRNAs were significantly regulated by physical activity. These mRNAs influence genes associated with various signaling pathways which include cytokine regulation, cell communication and lymphocyte activation. This study

shows a clear association between TERT mRNA expression and telomerase activation in several tissues.[18]

The positive effects of physical activity on health are well documented, while sedentary lifestyles are associated with higher mortality and increased risk for many diseases.[18] Physical activity has been shown to reduce mortality rates even when exercise starts later in life.[20] Melk et al. (2014) examined the association between telomerase activity and physical activity in 59 middle-aged men 45-65 years of age with a previously sedentary lifestyle. A mean training duration of 214 minutes per week was maintained with a measurable increase in cardiorespiratory fitness over a six-month period. Increased telomerase activity was observed throughout the duration of the exercise intervention, as evidenced by longer telomere length in $CD8^+$ T-cells. This longitudinal study showed that physical activity improves biological markers of age in a group of middle-aged men despite previously sedentary lifestyle patterns.

Body Composition and Telomere Length

It is thought that telomere shortening may be accelerated by both ROS and obesity.[21] Genome association studies have concluded the role between lifestyle factors in mitigating the expression of risk alleles. Increased waist circumference and higher body mass index (BMI) is thought to be linked to telomere attrition.[21] However, dietary changes that effect anthropometric measurements can be easily obtained through caloric restriction and increased physical activity levels. In a study conducted by Rode et al. (2014), it was found that high BMI was associated with short telomere length observationally. It was thought that elevated CRP levels, responsible for telomere attrition, are common in those with a high BMI. Elevated BMI contributes to both oxidative stress and system wide inflammation, which may increase cell division thereby

accelerating telomere attrition. However, this study did not show an association between elevated BMI and shorter telomere length.[21]

In a separate study conducted by Zannolli et al. (2008), telomere length was examined in 53 children and 23 adults. Healthy, normal weight and obese adults and children were included in this study. The telomere lengths were not shown to differ between the two groups of obese and normal weight children. Interestingly though, variations in telomere length between obese and normal weight adults was significant. The reason for these differences between adolescent comparisons and adult comparisons is unknown. Persistent chronic obesity is an unproven cause, but it is likely to be a contributor.[22]

CHAPTER III

Methods

This study is a secondary analysis of data provided by the Georgia State University IRB approved "Telomere Length, Environmental Stressors and Health-related Outcomes among Transgender Women" study conducted by Dr. Laura Salazar in the School of Public Health at Georgia State University. The study included 92 transgender women with 49 (54%) of the participants reporting HIV infection. The original study was an anonymous, cross-sectional study design. Two sources of data were collected: survey responses collected during a face-to-face interview and a saliva sample for DNA analysis of telomere length. The targeted population were individuals who identify as transgender (male to female), between the ages of 18-65 years, born biologically male (born as a male), English proficient, and who indicated that they have had sex with a man in the past 6 months.

The qualtrics survey used in this study was a comprehensive questionnaire covering a wide range of areas involving mental health, physical health, disease history and consumption patterns. Specific sections of the questionnaire regarding HIV status, dietary patterns, and physical activity were used for the purpose of this study. Questions pertaining to physical activity levels, and a fruit and vegetable consumption frequency, determined levels of physical activity and dietary patterns among participants. The questions were obtained from the American College Health Association National College Health Assessment.[23] Physical activity was defined as low active for participants who reported <150 minutes of moderate-intensity aerobic physical activity or <100 minutes of vigorous-intensity aerobic activity throughout the

week. The survey question asked if the subject participated in vigorous exercise for at least 20 minutes or moderate exercise for at least 30 minutes. The answer was scored from 0 through 7 days per week. Subjects who scored from 0 through 4 were considered low active while those scoring 5 through 7 days were considered to be within healthy active guidelines. Fruit and vegetable intake was determined using four categories representing consumption patterns. Respondents were asked to report average daily fruit and vegetable intake by selecting one of four choices; Don't eat, 1-2 servings, 3-4 servings, or 5 or more servings. Fruit and vegetable intake were categorized into low intake (don't eat, 1-2 servings/day or 3-4 servings/day) or high intake (5 or more servings/day).

Genotec performed the analysis of telomere length. Technologies at Genotec are comprised of different established protocols that allow the determination of telomere length at the individual level, both from cellular (blood) and tissue samples. For this study, telomere length was determined in DNA extracted from saliva samples by the Terminal Restriction Fragment analysis (TRF), in which telomere length parameters were obtained using Southern blots of terminal restriction fragments. After DNA isolation and extraction, DNA was inspected for integrity, digested, resolved by gel electrophoresis, transferred to a membrane, hybridized with labeled probes and exposed to X-ray film using chemiluminescence. Telomere length was recorded as the number of kilo-base (kb) pairs. For the current study T/S ratio was used as the measure of telomere length (kb pairs x 92).

Data management

Data collected for this study were kept anonymous and housed in the School of Public Health at Georgia State University under the supervision of Dr. Laura Salazar. The only personal identifier collected was zip code. Expedited IRB approval was given for this secondary analysis.

Statistical Analyses

Frequency statistics were used to describe the study population. The Mann Whitney U test was used to evaluate T/S ratio by HIV status, by physical activity level and by fruit and vegetable intake category in the total population. Multiple regression analysis was used to determine which variables significantly contributed to attrition or elongation of telomeres. Telomere length (as indicated by T/S ratio) was the dependent variable. HIV infection, reported dietary consumption of fruits and vegetables, and reported physical activity were independent variables. The interaction of fruit and vegetable intake category and HIV status as well as physical activity level and HIV status was evaluated using moderation analysis. This analysis will determine whether fruit and vegetable intake or physical activity moderate the association between HIV status and T/S ratio. All statistical analyses were performed using SPSS (version 23.0, SPSS, Inc., Chicago, IL).

CHAPTER IV

Results

The demographic and anthropometric characteristics and telomere length of the population are shown in Table 1. Normality testing revealed that the age, anthropometric measures and T/S ratio variables were not normally distributed. Therefore, non-parametric statistics were used in the analyses. The median age of the study population (n=92) was 33.5 years (range, 18-65 years). The majority of the population was Black (83.7%) with 6.5% reporting Hispanic ethnicity (Figure 1). Infection with HIV was reported in just over half of the study population (54.3%) (Figure 2). Approximately half of the total population (45.7%) reported current treatment with combination cART.

Table 1. Demographic, Anthropometric and Telomere Length of the Total Population Data (N=92)

	Median	**IQR**	**Range**
Age (years)	33	(24, 42)	18-65
Height (cm)	172.7	(166.37, 179.07)	154.9-198.1
Weight (kg)	75.0	(62.5, 87.5)	51.4-147.7
BMI (kg/m^2)	24.6	(21.11, 28.09)	16.5-45.3
T/S Ratio	2173.88	(1728.39, 2619.37)	398.78-5697.28

cm – centimeters; kg – kilograms; BMI – body mass index; m – meters; IQR – interquartile range (25%,75%)

Figure 1. Race Distribution of the Total Population

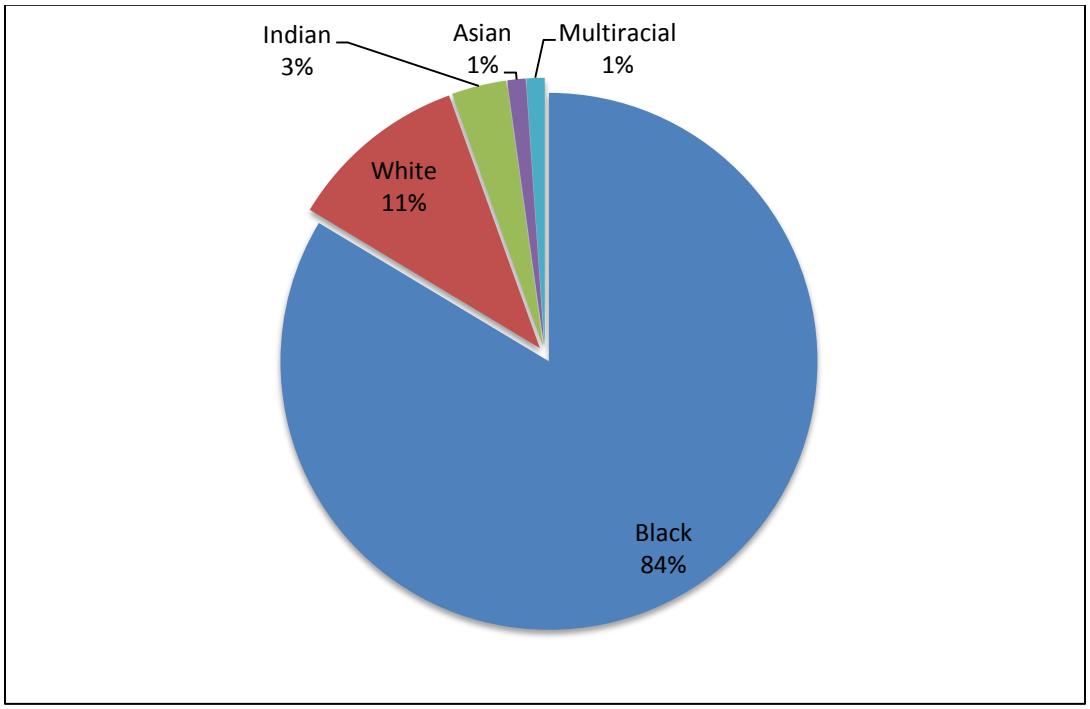
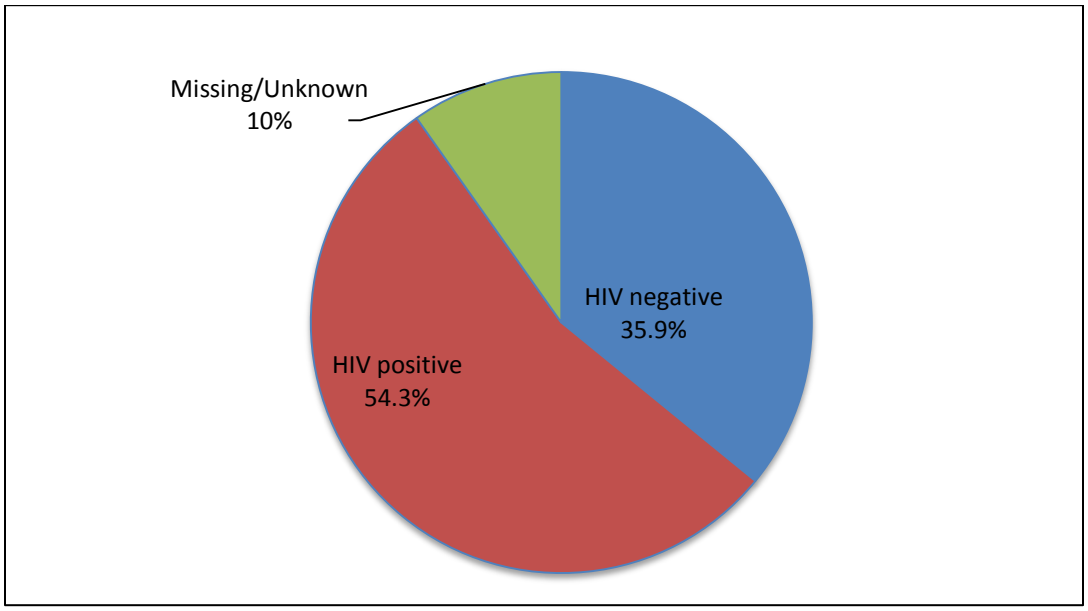


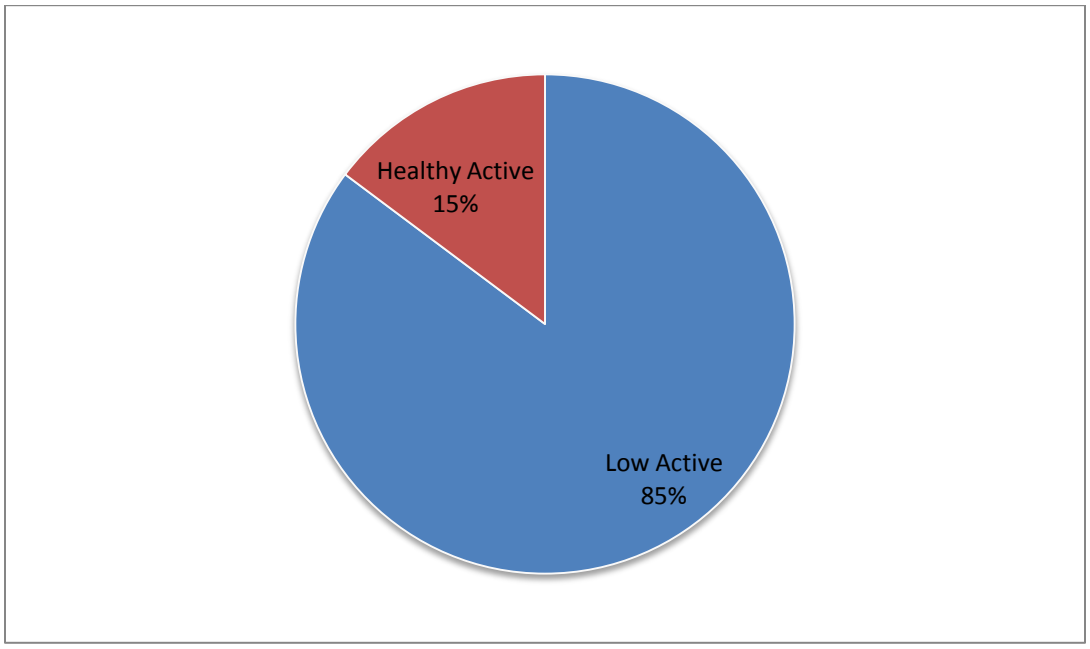
Figure 2. HIV Status of the Total Population



HIV – human immunodeficiency virus

The vast majority of the total population reported a low activity level while 15% reported activity within healthy active recommended guidelines (Figure 3). Approximately half of the participants (57%) reported consuming 1-2 servings of fruits and vegetables per day while only (9%) reported 5 or more servings (Figure 4).

Figure 3. Activity Level of the Total Population



Low Active: <150 minutes of moderate-intensity aerobic physical activity or <100 minutes of vigorous-intensity aerobic activity throughout the week.
Healthy Active: 150 minutes or greater of moderate-intensity aerobic physical activity or 100 minutes or greater of vigorous-intensity aerobic activity throughout the week.

Figure 4. Fruit and Vegetable Intake of the Total Population

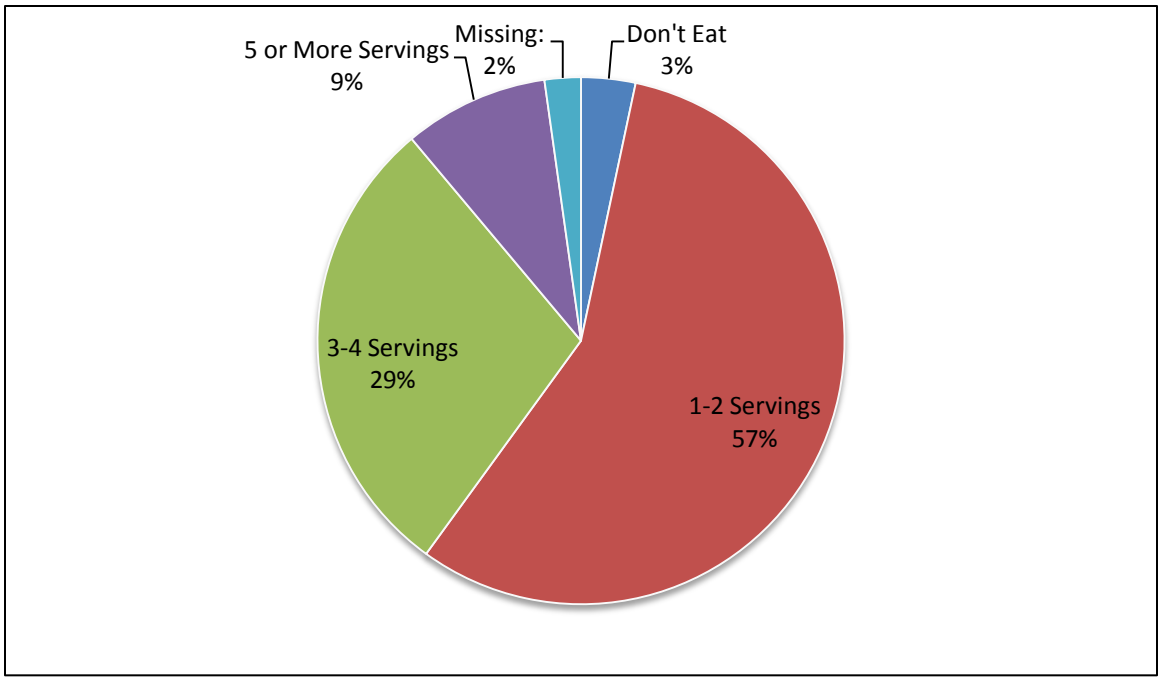


Table 2. T/S Ratio by HIV Status (n=81)

HIV Status	**HIV -** **(n=32)**	**HIV +** **(n=49)**	**P-Value**
T/S Ratio*	2214.28 (2007.34, 2756.22)	2106.12 (1564.06, 2534.49)	0.195

*Median (IQR; 25%, 75%)
HIV – human immunodeficiency virus; HIV - = HIV negative; HIV + = HIV positive

Research Hypothesis 2: Transgender women with or without HIV who consume a greater number of servings of anti-oxidant rich foods will have a higher T/S ratio than those who consume fewer servings.

No significant difference in T/S ratio was found by reported intake of fruits and vegetables (Table 3). The moderation analysis model using fruit and vegetable intake category and HIV status as a main effect and an interaction term of Fruit and Vegetable Intake x HIV status was not statistically significant (p=0.165). However, in the moderation analysis the fruit and vegetable category was independently significant (p=0.048).

Table 3. T/S Ratio by Fruit and Vegetable Intake in the Total Population

Fruit and Vegetable Intake	**Low Intake (n=80)**	**High Intake (n=8)**	**P-Value**
T/S Ratio*	2136.59 (1651.16, 2555.33)	2601.77 (1969.41, 3701.13)	0.082

*Median (IQR; 25%, 75%)
Low Intake – don't eat, 1-2 servings/day or 3-4 servings/day
High Intake – 5 or more servings/day

Research Hypothesis 3: Transgender women with or without HIV who report greater physical activity levels will have a higher T/S ratio than those who report a lower level of physical activity.

The median T/S ratio by physical activity level in the total population is shown in Table 4. No significant difference in telomere length was found between those with low vs. healthy active levels. The moderation analysis model using physical activity level and HIV status as a main effect and an interaction term of PA x HIV status was not statistically significant (p=0.510).

Table 4. T/S Ratio by Activity Level in the Total Population

Activity Level	**Low Active (n=75)**	**Healthy Active (n=13)**	**P-Value**
T/S Ratio*	2122.71 (1658.77, 2667.66)	2216.41 (2119.59, 2375.29)	0.668

*Median (IQR; 25%, 75%)

Multiple regression analysis was used to determine which variables significantly contributed to attrition or elongation of telomeres. Activity level, BMI, fruit and vegetable intake category, hormone use, race, HIV status and age were the independent variables used. The predictors explained only 6.1% of the variance in telomere length and the model was not statistically significant (p=0.690). In the multivariable model, none of the independent variables were significant.

CHAPTER V

Discussion and Conclusions

The purpose of this study was to examine the association between HIV disease and lifestyle factors on telomere attrition. Decreased telomere length has been shown to occur with HIV infection as well as other negative lifestyle factors such as poor diet, lack of substantial exercise and intake of toxic substances such as alcohol and tobacco. This study did not reveal any significant associations between telomere lengths and HIV infection in a population of transgender women from Atlanta, GA. Additionally, no associations were observed with respect to fruit and vegetable intake or exercise patterns on the telomere length. Therefore, we reject our hypotheses that the T/S ratio will be smaller in transgender women with HIV infection, and that transgender women who consume a greater quantity of fruits and vegetables and who report a greater physical activity level will have a higher T/S ratio than those who consume fewer antioxidant rich foods and who report a low level of activity.

Previous studies have reported a correlation between HIV infection and decreased telomere length. In a study by Liu et al. (2015), shortened lifespan and higher instances of several diseases in the HIV-infected population compared to similar aged uninfected controls led the researchers to hypothesize that HIV infection causes accelerated telomere loss. Compared to HIV-uninfected subjects, the mean aTL in HIV-infected patients was shown to be considerably shorter by 27 kbp/genome. The researchers measured absolute telomere length (aTL) in peripheral leukocytes from 231 HIV-infected adults. Comparisons were then made to 691 HIV-

uninfected individuals from a population-based sample. This study showed an association between HIV infection and telomere attrition within a broad population base. In contrast, our study included transgender females exclusively. The transgender population may experience higher levels of stress compared to other population samples, regardless of HIV status, due to social pressures and higher exposure to negative lifestyle factors. Increased telomere attrition associated with high stress levels has been previously reported. Using a bioassay, Shalev et al, (2012) found an association between stress and telomere degradation.

Exposure to stressors during childhood is thought to cause detrimental health outcomes later in life. Systems throughout the body including the immune system, the sympathetic nervous system, and the hypothalamus-pituitary-adrenal axis are all thought to be negatively impacted by stress. Additionally, early stress exposure has been associated with respiratory problems, cardiovascular disease, metabolic disorders, and neurological problems.[24] While stress is thought to be a factor in telomere attrition, previous studies have also observed a relationship between shorter telomere length and low fruit and vegetable intake as well as low exercise levels. Researchers with the Nurses' Health Study proposed that variability in telomere length might be partly due to lifestyle factors, including dietary patterns.[13] High anti-oxidant and anti-inflammatory components of the Mediterranean Diet are thought to limit telomere attrition and reduce disease onset. The Nurses' Health Study population consists of an ongoing cohort of 32,825 nurses, of which 825 submitted blood samples for analysis between 1989 and 1990. The women selected were healthy controls from nested case-control studies within the Nurses' Health Study. Each woman had a previously measured leukocyte telomere length and had completed a food frequency questionnaire at the time of the blood draw. The average relative leukocyte telomere length was calculated as the ratio of telomere repeat copy number to a single gene copy

number (T/S ratio). The researchers reported that Nurses who had longer telomeres reported greater adherence to the Mediterranean Diet. Our study sample was very small in comparison to the Nurses' Health Study subsample, and included only transgender women. In addition, the food frequency questionnaire used in the Nurses 'study was more comprehensive, whereas the diet questions used to assess fruit and vegetable intake in the current study were limited.

In addition to HIV infection and dietary patterns, exercise has been linked to changes in telomere length. A study conducted by Chilton et al. (2014) revealed that acute exercise could lead to the transcriptional regulation of several key telomeric genes in immune cells. Both Telomerase Reverse Transcriptase (TERT), and micro-RNAs (miRNA) were shown to be regulated by physical activity. Thus, it is thought that increased physical activity leads to stimulation of telomerase activity, which may lead to the elongation of telomeres, hence cellular longevity. Chilton used a sample of twenty-two non-smoking males with a mean age of 24 years. The mean age for this group was younger than the mean age in our study, and the Chilton study measured telomere length following high intensity aerobic activity sessions in a controlled setting. Our study used subjective recall data that approximated physical activity levels throughout the week.

Our study has several limitations. The population consisted of a small sample of transgender women with and without HIV. Therefore, our results may not be generalizable to the larger transgender population or to those who are not transgender. The dietary and exercise questions in the survey were limited and have not been validated in the transgender population. Anti-oxidant levels of fruits and vegetables consumed were not available, therefore the data has limited efficacy in terms of telomere attrition. Dietary questions asked for a recall estimation of

consumption patterns and exercise frequency throughout the week. Additionally, this study did not include alcohol and tobacco use as independent variables when analyzing telomere lengths.

Examination of T/S ratios between non-transgender groups including straight, gay, and bi-sexual orientation may reveal variations in T/S ratios based on sexual orientation and lifestyle factors. Additional studies are needed to further clarify the effects of HIV infection, diet, and physical activity level on telomere attrition in transgender women. Prospective study of the complex cascade leading from stress exposure and lifestyle factors during early life to cellular aging via telomere biology among transgender women will aid in the understanding of the aging process and the effects of diseases and lifestyle factors on those processes. Future research is needed to further understand the complexities and mechanisms that impact telomere length throughout the cellular lifecycle.

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