THE INFLUENCE OF IN UTERO MATERNAL AND CHILD FACTORS ON TELOMERE LENGTH AND POSSIBLE DIFFERENCES BY RACE: CLUES TO THE RACIAL DISPARITY IN PROSTATE CANCER

by
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The influence of in utero maternal and child factors on telomere length and possible differences by race: clues to the racial disparity in prostate cancer

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Abstract

There is a pronounced racial disparity in prostate cancer risk and mortality. Modifiable factors in adulthood that explain the disparity have not been identified. Whether racial differences in utero may account for this disparity is understudied. Shorter telomeres have been associated with cancer, and telomere length (TL) at birth is hypothesized to set the trajectory for lifetime telomere shortening and influence adult disease risk including prostate cancer. TL is heritable but telomeres also shorten with cell replication and oxidative damage, and thus may serve as an indicator of cumulative exposure. We investigated the association of in utero maternal and neonate factors with cord blood TL, and whether TL differences between black and white male neonates, as a marker for inherent and exposure racial differences in utero, may explain some of the prostate cancer disparity. We also addressed whether TL differs by race in adult males. We used the Hormones in Umbilical Cord Blood Study (HUB) and Expanded HUB (EHUB) to evaluate whether TL differs by race in neonates, and to estimate associations between maternal and neonate factors and TL. We used the Health Professionals Follow-up Study (HPFS) to evaluate midlife racial differences and associations. In HUB, no factors were associated with TL, and TL did not differ by race. In EHUB, higher pre-pregnancy

maternal BMI and less education were inversely associated with neonate TL. Black mothers and neonates had shorter telomeres compared to whites but adjustment for BMI and education eliminated this difference. Maternal and neonate TL were moderately positively correlated regardless of race and after adjustment for confounders. In HPFS, waist circumference, physical inactivity, and smoking were inversely associated with TL but TL did not differ by race before or after adjusting for these factors. Our hypotheses were not supported in HUB or HPFS but were in EHUB. In EHUB the maternal-neonate TL correlation suggests that TL may differ by race at birth due in part to inherent and, in part, racial differences in maternal factors. These findings inform the prostate cancer racial disparity and the influence of the in utero environment on offspring adult disease risk.

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computer along.

I would like to dedicate this to my grandfather, Henry Addante, who died of prostate cancer much too young, and to the rest of my friends and family who fought, or continue to fight, cancer.

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Chapter 1: Introduction

Many cancers, including prostate, show a racial disparity in incidence and mortality rate (1). In the US, black men have a 60% higher risk of prostate cancer and have about two and a half times the risk of dying of prostate cancer compared to white men (1). Black men are also more likely to be diagnosed with prostate cancer that has a more aggressive phenotype (2-5). While a risk factor for many cancers, race is not modifiable, so is not a direct target for cancer prevention. Thus, the identification of other factors that may explain the racial disparity and that are modifiable is necessary for public health prevention and intervention. For prostate cancer, modifiable factors measured in adulthood that may explain the racial disparity have not been found despite the extensive study. Whether racial differences early in life, including in utero, may account for this disparity is understudied.

The fetal origins hypothesis posits that fetal environmental stressors influence risk of diseases in later life through fetal programming (6). Fetal programming of prostate cancer, specifically differential fetal programming between blacks and whites, beginning in utero and continuing to midlife, merits evaluation. Early life exposures, including in utero exposures and their relationship to prostate cancer have been explored in previous studies with conflicting results (7-13). After the discoveries of the relationship between diethylstilbesterol and vaginal cancer and the role of hormones in the developing prostate and prostate cell division, some researchers hypothesized that the origins of prostate cancer could be in utero and related to hormones (14-16). Studies began evaluating pregnancy characteristics and birth outcomes and their association with future prostate

cancer. Birth outcomes, such as birth weight, could be an indirect measure of the hormonal milieu and could also be a measure of other specific in utero exposures, such as maternal weight gain.

Given the growing prevalence of obesity in the US and around the world, understanding the impact of exposure to maternal obesity on future disease risk is important. It is possible that fetal exposure to maternal obesity modifies fetal programming, and thus the development of diseases, including prostate cancer, later in life. Obese individuals are known to be under greater oxidative stress, and to have greater circulating levels of growth factors (17, 18), which may, in turn, act as fetal stressors. Maternal obesity has also been associated with higher levels of oxidative stress in newborns (19). Further, high pre-pregnancy body mass index (BMI) and increasing gestational weight gain have both been associated with large for gestational age babies (20). Associations between higher birth weight and increased cancer risk in adulthood have been reported in some but not all studies (21, 22), including advanced prostate cancer and mortality (7-9, 11-13). Other birth outcomes that may reflect the in utero environment have also been studied in relation to prostate cancer. A suggestive association between placental weight and prostate cancer has been reported (11) and in a related study, an association was reported between longer gestation and a lower risk of prostate cancer (10). Thus, evidence is beginning to accumulate in support of the hypothesis that the in utero environment influences later life risk of prostate cancer.

Another biomarker that may reflect exposures in the in utero environment, and also be relevant to future prostate cancer risk is telomere length. Telomere length is inherited (23, 24) and is known to shorten with cell replication (proliferation) and with greater oxidative stress (25). Shorter circulating white blood cell and buccal cell DNA telomere length has been associated with cancer in multiple studies (26, 27). In the study of prostate cancer, my colleagues previously observed that shorter prostate stromal cell telomere length is associated with a higher risk of prostate cancer (28) and that greater variation in telomere length in prostate cancer cells combined with shorter telomere length in cancer-associated stromal cells is associated with an increased risk of death from prostate cancer (29). This work supports the role of telomere length dysfunction in the etiology of prostate cancer, especially disease with an aggressive phenotype.

However, studies exploring white blood cell telomere length and risk of prostate cancer have been inconsistent. One study found no association between leukocyte telomere length and prostate cancer risk or advanced disease (30, 31) but a recent study by my colleagues in the Health Professionals Follow up Study (HPFS) found longer leukocyte telomere length to be associated with total and localized prostate cancer but not with high grade, advanced, or lethal prostate cancer (32). It is possible that different mechanisms underlie the association between leukocyte telomere length and prostate cancer risk and the association between telomere length in prostate stromal or cancer cell tissue and prostate cancer risk; and this may account for the discrepancies in findings between these studies. Though the relationship between telomere length and prostate cancer has not been fully explained, telomere length may still capture some of the influence of the in

utero environment's effect on the fetus, and may highlight potential racial differences that help to determine if differences in the in utero environment explain some of the racial disparity in prostate cancer.

In this dissertation, I aimed to examine whether aspects of the in utero environment, including maternal and child factors, differ between black and white males, and thus might account for the racial disparity in prostate cancer risk. Maternal factors evaluated included obesity and extent of weight gain during pregnancy as well other factors like smoking and extent of physical activity that are pro-proliferative and pro-oxidative stress. Child factors included birth weight, placental weight, and cord blood concentrations of hormones, growth factors, and vitamin D. I used telomere length in neonatal white blood cells isolated from venous umbilical cord blood as an indicator of the possible influence of the in utero environment on the fetus. After taking into account maternal and child factors that may differ by race, I assessed whether inherent differences in telomere length exist that may also explain the racial disparity in prostate cancer. I also assessed the correlation between maternal white blood cell telomere length and cord blood telomere length overall and by race, and the racial difference in telomere length later in life to further support my hypotheses. As part of this dissertation, I directed the measurement of telomere length in maternal circulating white blood cells and in umbilical cord blood white blood cells of their babies. I designed the quality control protocol for the telomere assay and performed analysis of quality control measures to determine both validity (internal standards) and reliability (replicates of study samples) of the assay.

Specific Aims

Aim 1. To determine whether white blood cell telomere length differs between black and white males at birth and mid-life, and whether the correlation between mother and child telomere length differs by race.

Aim 1a. Racial differences at birth. *We hypothesized that babies born to black mothers have shorter telomeres than those born to white mothers in the unadjusted analysis.* This aim was addressed in the Hormones in Umbilical Cord Blood (HUB) Study, a cross-sectional study, and the Expanded Hormones in Umbilical Cord Blood study (EHUB), a longitudinal study of pregnant women and their newborns, by race, in Baltimore, MD.

Aim 1b. Racial differences in mid-life. *We hypothesized that black middle-aged men have shorter telomeres than white middle-aged men in the unadjusted analysis, and that the difference is greater than the difference at birth.* This aim was addressed in a cross-sectional study in a subset of men in the Health Professionals Follow-up Study (HPFS), an ongoing cohort study of male health professionals in the US.

Aim 1c. Racial differences in the correlation between mother and child. *We hypothesized that there is a positive correlation between each newborn and his mother's telomere length, and that magnitude of the heritability of telomere length is different by race.* This aim was addressed in EHUB.

Aim 2. To assess the association of child factors with venous umbilical cord white blood cell telomere length overall and by race. *We hypothesized that higher birth weight, extreme placental size (smaller and larger), and higher umbilical cord blood concentrations of testosterone, estradiol, insulin-like growth factor-1, insulin-like growth factor-2, and leptin, and lower concentrations of sex hormone binding globulin, insulin-*

like growth factor binding protein-3, and vitamin D are associated with shorter telomere length. We hypothesized that the direction and magnitude of these associations do not differ by race, but will, in part, account for any racial differences in telomere length observed in Aim 1a because of racial differences in the prevalence of these child factors. This aim was addressed in the HUB (child birth characteristics and cord blood biomarker concentrations) and EHUB (child birth characteristics only) studies. The cord blood biomarker concentrations were previously measured in HUB and racial differences in levels were noted (see **Background**).

Aim 3. To assess the association of maternal factors with venous umbilical cord white blood cell telomere length overall and by race. *We hypothesized that maternal obesity at the start of pregnancy and excess weight gain during pregnancy, as well as other maternal characteristics, including higher parity (a risk factor for maternal obesity) is be associated with shorter umbilical cord white blood cell telomere length. We hypothesized that the direction and magnitude of these associations will not differ by race, but will, in part, account for any racial differences in telomere length observed in Aim 1a because of racial differences in the prevalence of these maternal factors. Further, we hypothesized that the association between maternal obesity/weight gain and neonate umbilical cord white blood cell telomere length will be mediated by the size of the baby (birth weight) and the placenta (placental weight).* This aim was addressed in HUB (parity) and EHUB (maternal obesity, weight gain).

In addressing these aims, we allowed for the possibility that the findings in the crude analysis in Aim1 and in the adjusted analyses in Aims 2 and 3 would differ. For example, if we had observed that telomeres are shorter in black than white neonates in the crude analysis but not in the adjusted, this pattern would have implied that the differences in the measured and unmeasured fetal environmental factors by race may account for this racial difference in telomere length, not the fact of race itself. If so, we would have concluded that known and unknown environmental and social factors are of most importance in explaining the racial difference in telomere length (as opposed to biological or innate factors). These in utero factors would hopefully be modifiable and assuming telomere length at birth is a significant biomarker of prostate cancer, enable prevention efforts. On the other hand, if we had observed that a racial difference in telomere length persists after adjustment for the maternal and child factors, this would have implied that a biological or innate factor that differs by race is of most importance in explaining the racial difference. As these factors are unlikely to be modifiable, they would not be direct targets for intervention. Before beginning this research, we expected that the first possibility would be more likely, and, as such, preventing and/or intervening on those prenatal factors that explain, at least in part, the racial difference in telomere length, would help reduce the racial disparity in prostate cancer risk and aggressiveness.

In the following sections of the Introduction, I provide background on the racial disparity in prostate cancer, fetal origins of adult disease, previous work on the relationship between the fetal environment and prostate cancer, potential fetal environmental exposures that may increase the risk of prostate cancer and explain some of the racial

disparity, the use of telomere length as a marker of cancer risk, and the utility of telomere length as a marker of fetal programming and potential differences by race.

Background

Race is a risk factor for prostate cancer

Racial differences are seen in the risk of many diseases, including colorectal, lung, and prostate cancers (1). While colorectal and lung cancer have known modifiable risk factors, prostate cancer has few known significant risk factors – older age, positive family history, and race (33), and none are modifiable. Racial variation in prostate cancer incidence and mortality in the United States is marked, with black men having a 1.56 times higher incidence and a 2.45 times higher mortality rate than white men (1). While race itself is not modifiable, factors correlated with race may be modifiable, and thus represent potential targets to decrease the racial disparity. To date, studies on differences in associations of modifiable factors measured in adulthood with prostate cancer between black and white men have yielded inconsistent or null results (34). Thus, to be able to prevent or intervene on the excess burden of prostate cancer in black men, we need to search for novel modifiable factors that may explain the racial disparity. Further, because little has been discovered based on measuring factors in mid and later-adulthood that might explain the racial differences in prostate cancer risk, we must now look at earlier points in the life course.

The in utero environment may influence cancer risk later in life and partially explain the racial disparity in prostate cancer rates

The fetal period is one point in the life course that should be explored. One potential mechanism that might explain the racial difference in prostate cancer is fetal programming, a theory that fetal exposures can lead to permanent alterations resulting in increased risk of disease later in life and an under-studied area for prostate cancer. In my colleagues' previous work, differences have been found between black and white neonates in various maternal and child factors such as younger maternal age in black mothers compared to white, lower birth weight and slightly lower placental weight in black neonates compared to white, slightly higher concentrations of sex steroid hormones, slightly lower insulin-like growth factor concentrations (35) and vitamin D (36), and slightly higher leptin concentrations in black male neonates in umbilical cord blood compared to white neonates (37). In some studies, birth weight has been positively associated with prostate cancer later in life, especially advanced stage disease and mortality (7, 8, 11-13). The lower birth weight in black compared with white neonates seen in our HUB study (35) is also well recognized nationally in the US (38). Lower birth weight among black neonates may seem contradictory to our hypotheses since a larger baby would seem to have more cell division and proliferation due to more growth and thus shorter telomeres and a higher prostate cancer risk. However, low birth weight black babies compared with white and Asian babies have been found to put on more weight in infancy (39). This rapid catch-up growth in children in developed countries is believed to disproportionately add to fat mass of the child causing negative permanent changes in body composition (40). Rapid catch-up growth may lead to accelerated cell aging and

thus DNA damage and telomere shortening (41) if higher birth weight does not result in shorter telomeres.

The combination of racial differences in size at birth and rate of growth may point to racial differences in the prevalence and/or extent of exposure to factors beginning during fetal and early life development that may account for some of the racial differences in prostate cancer incidence and aggressiveness. David Barker developed the "fetal origin hypothesis" in the 1980's and 90's, now widely called the "Barker hypothesis" (42, 43). This hypothesis is based on the idea that certain exposures in utero can "program" a fetus and permanently change its structure and metabolism, resulting in altered risk of chronic disease later in life (44). Barker developed the hypothesis beginning with his work in England, and later working with Finnish cohorts and the offspring of women living through the Dutch Hunger Winter, where following a railway strike during World War II, the German occupation limited food rations of everyone in the west region of the Netherlands to as little as 400-800 calories per day (42, 44, 45). The end of World War II and a return to more than adequate nutrition provided a natural experiment involving those adults who experienced extreme undernutrition in utero. The Dutch Hunger Winter, the most well studied example of "fetal programming", showed a strong association between being undernourished in utero and a higher risk of cardiovascular disease in later life (42). In this example, ability to adapt to undernourishment in utero and fully develop to survive at birth permanently programmed the fetus and resulted in metabolic and physiologic changes (42). What began as a hypothesis about the fetal origins of cardiovascular disease, Barker and others later expanded to include hypertension,

diabetes, and cancer, along with various other diseases (46, 47). This hypothesis has also been further expanded to explore the possibility that different programming factors may differ by race and possibly account for racial differences in development of these chronic diseases (48).

Fetal environmental stressors that may cause fetal programming

Not all exposures are harmful and some are only harmful at certain doses. Some exposures are innocuous and may have no effect and some are even beneficial. In the context of exposures, a stressor is an exposure that may put harmful strain on a body's equilibrium and potentially cause disease. Undernutrition was the first stressor hypothesized to cause fetal programming but other stressors have been proposed since the original studies. For example, while undernutrition was widely studied early in the "programming" work, overnutrition is also of concern.

High birth weight has been associated with DNA-methylation changes, which have been linked to multiple cancers (49). High birth weight has been associated with breast, ovarian, and lung cancer (50, 51). High birth weight has also been observed to be associated with prostate cancer in retrospective cohort studies (8, 9), and a twin study (12), especially advanced prostate cancer (8, 11, 13), and another retrospective cohort study found a suggestive but not significant association between high birth weight and prostate cancer (7).

Maternal weight may also be a stressor, either directly or through its effect on birth weight, and is particularly relevant given that obesity is a growing problem in the developed world (52). Several studies have found that pre-pregnancy weight and maternal weight gain are associated with birth weight, specifically that women who gain less than the recommended weight tend to have babies who are small for gestational age (SGA) and women who gain more than the recommended weight tend to have babies who are large for gestational age (LGA) (20, 53, 54). However, many studies investigating the fetal origins of disease have found that birth weight is not always a good indication of maternal nutrition since the fetus can adapt and use the mother's stored nutrients (44). Maternal obesity may also cause exposure to excess adiposity and its metabolic consequences in utero may program the fetus directly or through the child's obesity for chronic disease later in life (55). The timing of fetal stressors has also been found to be important. Children of the Dutch Hunger Winter who were exposed to undernutrition in the beginning of gestation were of normal birth weight, while those exposed later tended to be of lower birth weight (45). Thus, even if maternal weight is a stressor, birth weight may not be a perfect measure of it.

Because birth weight is an imperfect proxy of fetal nutrition, placental weight is often used since the placenta is responsible for the transfer of nutrients between a woman and her fetus. Very low and high placental weights result in decreased placental efficiency and are associated with future risk of lung cancer and coronary heart disease, independent of birth weight (56, 57). Studies have shown that maternal weight and weight gain may be a mediator between placental size and fetal size and that maternal fat mass may affect

placental growth and efficiency (55, 58, 59). Placental weight and size seem to be good markers of intrauterine environment and as noted, birth weight has been associated with cancer in other studies. These two measures provide complementary information about the fetal environment and when studied together, can help provide a more complete picture of the intrauterine environment.

Other stressors have been hypothesized to cause fetal programming as well. While not studied in this dissertation, most recently, maternal psychosocial stress has been hypothesized to lead to chromosomal changes in the fetus that increase the risk of cancer in adulthood, and stress hormones have been shown to impact the immune system in ways that are speculated to lead to progression of cancer (60, 61). Stressors like excess adiposity or psychosocial stress may cause chromosomal changes, which may lead to chromosomal instability and thus an increased risk of carcinogenesis, as genetic instability is a key underlying driver of malignant transformation (25). Thus, identifying associations between various stressors, or potentially harmful exposures, and cancer may help illuminate mechanisms of carcinogenesis.

Telomere length as a biomarker of cancer risk

Much work has been done in the field of cancer to develop biomarkers or surrogates that can provide information on an individual's future cancer risk and the mechanism of carcinogenesis. One such biomarker under study is telomere length. Telomeres are regions of repetitive DNA (TTAGGG) at the end of the chromosomes, which coupled with Shelterin proteins, protect the ends from deterioration. In the absence of

compensatory mechanisms, telomeres shorten with each cell division (proliferation), and thus with age, because DNA polymerase cannot fully replicate the ends of the lagging strand of DNA; known as the "end replication problem" (25, 62). When the telomeres become moderately short, normal cells should become senescent and stop dividing (63).

Shortening of telomeres has been shown to be greater for smokers, and shorten with increases in obesity and age (64). These observations have led to the proposal that telomeres can be thought of as a measure of biologic aging as well as chronological, influenced by both genes and environment (23, 24, 65, 66). By this reasoning, white blood cell telomeres in umbilical cord blood are a marker, then, of possibly both inherited telomere length and exposure to stressors while in utero. Data from longitudinal and cross-sectional studies have shown that on average, telomeres, as measured in circulating white blood cells, may shorten by as many as 270 base pairs (bp) per year until age 3, when the immune system matures (67), and then shorten by about 40 bp/year (range 30-60 bp/year) (64, 68).

If telomeres do not undergo senescence and shorten past the critical point, chromosomes become unstable, possibly leading to carcinogenesis. Telomere maintenance past this critical point is generally achieved through reactivation of telomerase. Telomerase allows cells to continue to divide and neoplasm formation, new and abnormal growth of tissue that can be malignant and may lead to metastasis. Telomerase has been observed to be upregulated in more than 85% of cancer cells and is associated with metastasis in breast and lung cancer (62, 69). Thus, short telomeres may be a marker of carcinogenesis when

combined with upregulation of telomerase or an alternative method of telomere maintenance. Shortened telomeres in peripheral blood (white blood cells) and buccal cell DNA have been associated with bladder, esophageal, gastric, and renal cancers in case-control studies (27) and overall and esophageal cancer in prospective studies (27, 70). Additional studies have also found shorter telomere length in stromal cells in benign prostate tissue to be associated with advanced prostate cancer (28) and greater variation in telomere length in prostate cancer cells to be associated with a higher risk of prostate cancer death (29). Thus, shortened telomeres, along with being a marker of cell proliferation and biological aging, may also represent the pathway of carcinogenesis and be a good biomarker of cancer risk.

Markers are needed to study the effects of the fetal environment on fetal programming of prostate cancer and possible racial differences in programming

The feasibility of enrolling a large cohort of pregnant women, assessing the in utero milieu, and then following their children into mid- and late life is low, so markers are needed to measure the influence of the in utero environment on the fetus. Measuring such markers could then potentially inform later life risk of prostate cancer and, in particular, the racial disparity in this cancer. Associations of the fetal environment and telomere length in combination with inherent telomere length differences by race need to be explored further as a potential marker of the fetal programming of cancer and the potential differences of relevant modifiable factors by race. Studies comparing circulating leukocyte telomere length in black and white adults have had inconsistent results, with some reporting longer telomeres in white adults (65) and some reporting longer telomeres

in black adults (64, 71, 72). One study of newborns found black neonates had longer telomeres on average (73). Faster telomere shortening with age in black compared to white adults has also been observed in some studies (64, 65, 73). More research is needed to explore the racial differences in telomeres starting at birth and their change throughout adulthood to assess the potential of short telomeres throughout the life course to act as a biomarker of cancer risk and as a possible contributor to racial disparities in cancer.

Significance and Innovation of this Dissertation

Differences in the prevalence and extent of hypothesized prostate cancer risk factors between black and white men have been explored as explanations for the racial disparity in prostate cancer risk and aggressiveness, such as sex steroid hormones, vitamin D, diet, BMI, and smoking (34, 74, 75). However, these factors do not fully explain the racial variation. In this dissertation, our hypothesis was that black male neonates would have shorter telomeres than white male neonates either due to innate differences or difference in the in utero environment. We further hypothesized that any racial difference in telomere length could be explained by either a racial difference in the association between fetal environmental factors and telomere length or a racial difference in the prevalence/extent of exposure to fetal environmental factors that influence telomere length. We used peripheral white blood cell telomere length as a marker of proliferation and oxidative stress, potentially as a result of the fetal environment. This marker is also on the pathway of carcinogenesis, the pathway to prostate cancer in our study, and thus would show the possible mechanistic effect such prenatal factors have on the pathway to prostate cancer. Research is needed to assess the possible effects of maternal factors, such

as maternal weight, on the fetus in utero and different child factors (e.g., birth weight), and their role in carcinogenesis through shortened telomeres. I hypothesized that differences in this mechanism may explain racial variation in prostate cancer since these changes during development may alter the risk of cancer during adulthood. Racial differences in telomere length at birth may persist or even increase throughout the life course. It is also possible that there is no racial difference in telomere length at birth, but that differential exposure to risk factors through adulthood, telomeres may still be shorter in black men compared to white. Such differences may help explain part of the racial disparity in prostate cancer.

Previous studies evaluating the association between prenatal factors and prostate cancer have been done (7-13) but more research is needed on prenatal differences by race that may account for racial differences in cancer incidence. Our work was important as it is necessary to understand etiology to find modifiable risk factors for cancer, especially prostate cancer, for which few possible modifiable factors have been identified. Currently, race is one of only three known significant risk factors for prostate cancer along with age and family history, and it is necessary to discover if there are other factors associated with race that can be modified. For example, maternal obesity is modifiable and may be associated with race. The effect of maternal obesity has not been studied extensively in the context of fetal programming of cancer. Since obesity is a growing problem it is important to assess the impact that maternal obesity may have not only on the health of a child at birth, but throughout the child's life as well. It is then important to determine biological mechanisms that may underlie the potential lifelong effects to the

fetus and possibly influence prostate cancer risk later in life. For example, if the mechanism is through direct fetal exposure to additional adiposity, increased risk of adulthood obesity, or if it is a combination of multiple pathways, prevention strategies can be specifically targeted.

This work is necessary to begin to combine various fields into one comprehensive hypothesis and to find an efficient way to measure effects of fetal programming and future risk of cancer. This work may also help prevention efforts by examining potential mechanisms for biological aspects of sociodemographic exposures (e.g., race and obesity) to affect later life illness and try to minimize the damage of such stressors, specifically those that disproportionally affect certain races and create or exacerbate health disparities.

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Chapter 1 Figures

Figure 1.1 Conceptual Framework: The path from the in utero environment, through midlife, to prostate cancer, measured through racial differences in telomere length.

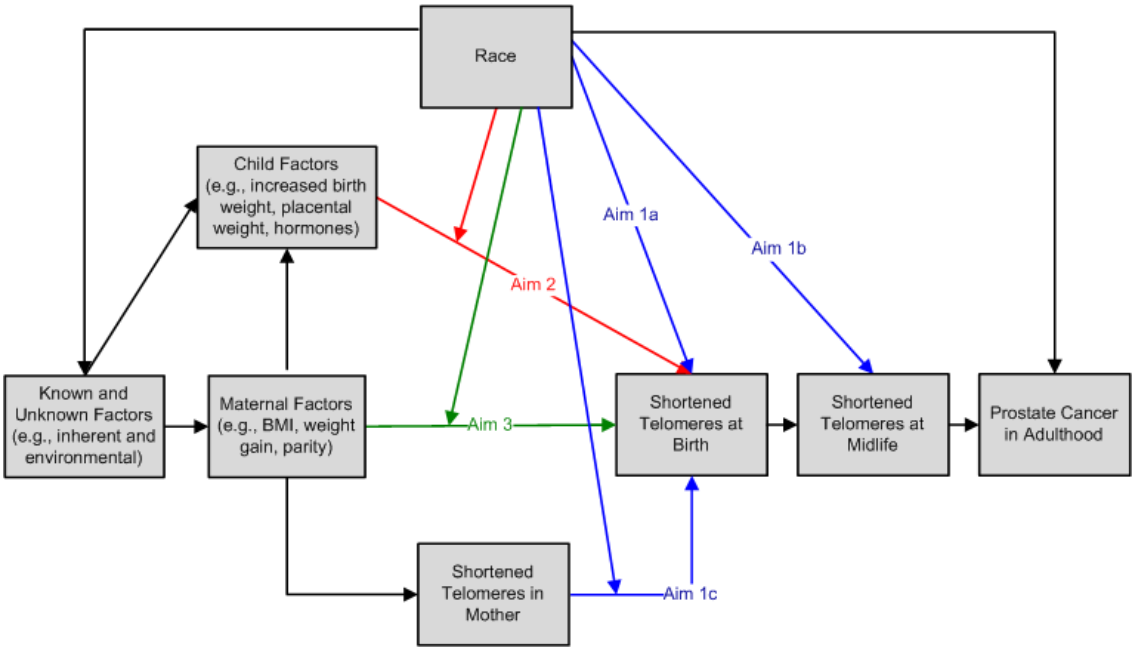

Figure 1.1 This conceptual framework is simplified and only reflects the pathways of interest for this study. The figure depicts the hypothesized path from exposures in utero to prostate cancer in adulthood. Both known and unknown factors, which may be influenced by race, cause differences in maternal and neonate factors. Higher prevalence of negative factors, or stressors, may cause shorter telomeres. Telomeres shorten with cell replication but also with greater oxidative stress, possibly caused by these stressors. Shorter telomeres at birth may persist or throughout life, leading to greater risk of adult diseases like prostate cancer. Racial differences in telomere length at birth due to a higher prevalence of stressors may also persist throughout life or even increase with greater exposure to relevant stressors later in life resulting in higher risk of adult diseases like prostate cancer. Telomeres measured in this study are peripheral white blood cell telomeres and not those of prostate tissue but telomeres in different organs and tissues in newborns have been shown to be highly correlated (76) and telomeres have been shown to shorten at similar rates in different tissues in adults (77). The telomere lengths of these samples are therefore, assumed to be surrogates for the length of telomeres in the prostate.

Chapter 2: Influence of in utero maternal and neonate factors on cord blood leukocyte telomere length: clues to the racial disparity in prostate cancer?

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Abstract

Modifiable factors in adulthood that explain the racial disparity in prostate cancer have not been identified. Whether racial differences in utero may account for this disparity is understudied. Shorter prostate cell telomeres, repetitive sequences that protect the ends of the chromosomes, are associated with a higher risk of aggressive prostate cancer and with poor prostate cancer outcomes. Telomeres shorten with each round of cell replication, and with oxidative damage, and thus, may serve as indicators of cell proliferation and cumulative exposures to oxidants. Using telomere length in umbilical cord blood leukocytes as a surrogate for prostate cell telomere length, we investigated the association of in utero maternal and child factors with umbilical cord blood telomere length, and whether telomere length differences between black and white males at birth, as a marker for exposure differences in utero, may explain some of the racial disparity in prostate cancer. We measured venous umbilical cord blood leukocyte relative telomere length by qPCR in 38 black and 38 white full-term male neonates. Compared with whites, black mothers were younger and had higher parity, and black neonates had lower birth and placental weights. However, none of these factors was associated with relative telomere length adjusting for or stratifying by race (all p-trend>0.4). Relative telomere length in

black (2.72, 95% CI 2.44-3.04) and white (2.73, 95% CI 2.45- 3.05) neonates did not differ, even after adjusting for maternal and neonate factors (all $p>0.9$). Maternal and neonate factors were not associated with cord blood leukocyte relative telomere length, and relative telomere length was not shorter in black than white male neonates. These findings do not support the hypothesis that shorter telomere length at birth, as a marker for in utero differences, explains the racial disparity in prostate cancer.

Introduction

The racial disparity in prostate cancer incidence and mortality rates is among the greatest across all cancer sites. US black men have a 60% higher risk of prostate cancer and greater than twice the risk of dying of prostate cancer (1). Further, black men tend to fair worse following surgical intervention of their primary prostate cancer and are more likely to die of their prostate cancer compared with white men (2-4). Despite extensive study, modifiable factors measured in adulthood that may explain this disparity have not been found (5, 6). However, early life exposures have not been systematically studied as an explanation for this racial disparity (7).

One potential early life mechanism that could influence the racial disparity in prostate cancer is fetal programming. The "fetal origin hypothesis" states that exposures in utero may "program" a fetus, that is, permanently change its structure and metabolism, resulting in altered chronic disease risk later in life (8, 9). If the prevalence or extent of such exposures differs by race, then the degree of fetal programming could account for racial differences in chronic diseases (e.g., prostate cancer).

Previous work suggests that birth characteristics, as indicators of the fetal environment, are associated with later life risk of prostate cancer (10-15). For example, positive associations (10, 11) and suggestive positive associations (12-14) have been found between higher birth weight and prostate cancer, especially advanced-stage disease and mortality in some studies, but not in others (15). Associations between other birth characteristics and aggressive prostate cancer have been reported including longer length

at birth and metastatic prostate cancer (14) and ponderal index and death from prostate cancer (15). There was also a suggestive association between higher placental weight and death from prostate cancer in one study (15). Additional studies have indirectly addressed the influence of the in utero environment on the prostate cancer racial disparity. For example, we previously reported slightly higher concentrations of testosterone, estradiol (16), and leptin (17) and slightly lower concentrations of insulin-like growth factors (16) and vitamin D (18) in the cord blood of black male neonates compared with white male neonates. In addition, higher testosterone and androstenedione concentrations in black compared to white mothers have been previously reported at the beginning of gestation (19, 20) or at time of delivery (21). These findings suggest that risk factors previously investigated for prostate cancer later in life, may differ in utero by race. However, these markers reflect differences at specific points during gestation, rather than the effect of differences in cumulative exposure in utero. Thus, markers of the cumulative influence of the in utero milieu on the fetus are needed.

Telomeres, repetitive DNA sequences that protect the ends of the chromosomes, may be such a marker. Telomere length is heritable, but for any given cell, telomeres shorten with each round of cell replication and with exposure to oxidative damage (22-24). Therefore, telomere length in leukocytes in cord blood may reflect an individual's starting point at birth, possibly integrating across cumulative proliferation and oxidative exposures during gestation. Further, telomere length is an attractive marker beyond reflecting cumulative exposure because telomere shortening contributes to carcinogenesis, including prostate cancer (25-28). Thus, telomere shortening may directly

link inheritance, cumulative in utero exposures, and prostate cancer. Here, we investigated the associations between race, maternal and neonate factors, and cord blood leukocyte telomere length in male neonates.

Methods

Study population and assessment of maternal and neonate factors

The Hormones in Umbilical Cord Blood Study (HUB) was a pilot study conducted by the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins and the Howard University Cancer Center Partnership and approved by the Institutional Review Boards of the Prince George's Hospital Center and the Johns Hopkins Bloomberg School of Public Health. In 2004–2005, venous umbilical cord blood samples (N=240) were collected from eligible neonates from the Johns Hopkins Hospital in Baltimore, MD and the Prince George's Hospital Center in Cheverly, MD. The eligibility criteria included: singleton, full-term birth (37-42 weeks), normal range birth weight (2500-4000 g), black or white, no pregnancy complications, and no maternal use of hormonal medications during pregnancy. Nurses completed a standardized study form on maternal age and parity (maternal factors), birth and placental weights, and time of birth (neonate factors). At delivery, nurses drew 15 mL samples of venous umbilical cord blood into 2 tubes containing sodium EDTA. As previously described, samples were stored in a refrigerator and processed, usually within 12 hours, into plasma, buffy coat, and red cells; aliquots were stored at -70°C in cryovials (16). Cord blood concentrations of steroid and peptide hormones, which reflect both maternal and neonate contributions, were previously measured for testosterone, androstanediol glucuronide (AAG), estradiol, sex hormone

binding globulin (SHBG), insulin-like growth factor (IGF) axis (IGF-1, IGF-2, IGF binding protein [IGFBP-3]) (16), 25-hydroxyvitamin D (18), and leptin (17). Bioavailable testosterone and estradiol were estimated as the molar ratio of each to SHBG (16). For this study, we used the 76 male samples collected at Johns Hopkins (38 white and 38 black).

Measurement of cord blood leukocyte relative telomere length

Leukocyte DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen, Venlo, Netherlands). Quantitative PCR was used to estimate the ratio of telomeric DNA to that of a single copy gene (β-globin) (29), with the following modifications (30). Briefly, 5 ng of genomic DNA was used in a 25 μL volume for either the telomere or β-globin reactions; each sample was run in triplicate. Each 96-well plate contained a no template negative control and two separate 5-point standard curves using leukocyte DNA; these standard curves allowed the PCR efficiency to be determined for each experimental run. Each plate also included three samples isolated from a series of cell lines with known telomere lengths, ranging from 3-15 kb, as determined independently by telomere restriction fragment analysis. Inclusion of these samples provided an additional quality control check. The mean telomere threshold (C_t) value and the β-globin C_t value were calculated from the telomere and the β-globin triplicate reactions, respectively. For each sample, the telomere of the experimental sample to the single copy gene (T/S) ratio ($-dC_t$) was calculated by subtracting the β-globin C_t value from the telomere C_t value. The relative ratio ($-ddC_t$) was determined by subtracting the $-dC_t$ from a 5 ng sample in the cell line series from the $-dC_t$ of each unknown sample. Across all samples, the mean CV

was 0.96% and 1.00% (maximum CVs were 4.38% and 2.70%) for the telomere and β-globin reactions, respectively.

Statistical analysis

Means of maternal and neonate factors were calculated by race and differences were assessed using t-tests. Using linear regression, we estimated geometric mean relative telomere length and 95% confidence intervals (CI) by the maternal and neonate factors and the cord blood steroid and peptide hormones, overall, after adjusting for race, and then stratified by race. We estimated the relative change in geometric mean relative telomere length per unit or per standard deviation of each maternal and neonate factor and tested for trend and interaction by race using the Wald test. Finally, we estimated geometric mean relative telomere length by race, overall and after adjusting for the maternal and neonate factors and for the cord blood steroid and peptide hormones. All analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

Results

The differences in maternal and neonate factors by race are shown in Table 2.1. Compared to white mothers, black mothers were younger (29 years vs 24 years; $p<0.005$) and had higher parity (0.6 vs 1.3; $p=0.03$). Compared to white neonates, black neonates had lower birth (3470.4 g vs 3207.2 g; $p=0.004$) and placental weights (701.7 g vs 640.9 g; $p=0.05$). Table 2.2 shows geometric mean relative telomere length in umbilical cord blood leukocytes by quartiles of maternal and neonate factors, overall, adjusting for race, and stratified by race. None of these maternal or neonate factors were associated with

relative telomere length before or after adjusting for race or when stratifying by race (all p-trend>0.4).

Relative change in geometric mean leukocyte relative telomere length per standard deviation change is shown for steroid and peptide hormone concentrations (Table 2.3). For the majority of the steroid and peptide hormones, we did not observe a statistically significant change in relation to the geometric mean leukocyte telomere length (all p-trend>0.1). However, for androstanediol glucuronide (AAG), relative telomere length was 11% longer per standard deviation (15.11 ng/mL) increase in AAG (p-trend=0.01), and remained statistically significant after adjusting for race (p-trend=0.01). After stratifying by race, the association between AAG and relative telomere length was similar in magnitude in black and white neonates (p-interaction=0.9), but was statistically significant only in black neonates (p-trend=0.01).

Finally, as shown in Table 2.4, cord blood leukocyte relative telomere length did not differ between black (2.72, 95% CI 2.44-3.04) and white (2.73, 95% CI 2.45-3.05) male neonates. These results were unchanged after adjusting for maternal factors (age and parity), neonate factors (birth weight and placental weight) or for the steroid and peptide hormones (data not shown).

Discussion

To address the role of early life factors that may contribute to the racial disparity of prostate cancer, we explored the association between maternal (mother's age and parity)

and neonate (birth and placental weights) factors with relative telomere length, a marker of the cumulative influence of the prenatal environment, in black and white male neonates. We hypothesized that maternal and neonate factors would be associated with relative telomere length, and would be a possible measure of differences in fetal programming between black and white neonates. Given the racial disparity in prostate cancer and our prior work on telomere length and prostate cancer risk and outcomes (1, 25, 26, 28), we hypothesized that relative telomere length would be shorter in black than white neonates. However, these maternal and neonate factors were not associated with cord blood leukocyte relative telomere length, and cord blood leukocyte relative telomere length did not differ between black and white male neonates.

While maternal age, parity, and birth weight were associated with race, none of the maternal or neonate factors were associated with relative telomere length. However, we did observe a positive association between AAG and relative telomere length. That association was present overall and within black neonates, although in our prior work AAG concentrations did not differ by race (16). Few prior studies in humans have assessed maternal and neonate determinants of newborn leukocyte telomere length. The most consistent findings to date are that older paternal age (31) and greater maternal psychosocial stress (32) are associated with shorter newborn leukocyte telomere length. Some studies report that adverse pregnancy complications, such as gestational diabetes and preeclampsia, are associated with shorter newborn telomere length (33). Murine studies showing the prenatal determinants of neonate telomere lengths are not available to

the best of our knowledge since inbred mouse strains have very long telomere lengths and thus are not directly comparable to human telomere lengths (34).

Although cord blood leukocyte relative telomere length did not differ between black and white neonates, our observations do not rule out early postnatal racial differences in telomere length. The black neonates were smaller on average than the white neonates, as is seen nationally (35). It is possible that since black neonates, on average, begin life smaller, they may experience compensatory catch-up growth that may, in turn, affect postnatal telomere lengths. In keeping with this idea, a longitudinal study found that black, low-birth weight neonates experienced greater catch-up growth and were close to the standard weight by age two while the white neonates were not standard weight (36). Rapid catch-up growth has been shown to affect programming and increase the risk of diseases such as cardiovascular disease and diabetes later in life (37) and obesity later in life (38). Some animal models have also shown that rapid postnatal growth following low-protein gestation was associated with accelerated telomere shortening in their aorta, pancreatic islets, and renal tissues (39). A recent longitudinal study measuring telomere length at birth and midlife showed that longer telomere length at birth was associated with a greater decrease in telomere length in adulthood overall (40). In that study, black neonates had longer telomere lengths at birth and therefore, greater telomere shortening to adulthood compared with whites; this relationship remained after adjustment for parental income and educational attainment. However, after stratification by gender, no association was present among males, even after adjustment for parental factors (40). Our findings are consistent with those observations. However, the overall findings from that

longitudinal study suggest that the combination of compensatory catch-up growth and greater telomere shortening may account for some of the racial disparity in later life chronic diseases, such as prostate cancer.

Some aspects of our study warrant discussion. Our study was designed specifically to investigate differences in the in utero environment that may account for racial disparities in prostate cancer later in life. As such, the race of the parents and neonate were documented to be the same. Due to the eligibility criteria, none of the mothers had pregnancy complications and all neonates were full-term with normal birth weight and thus, relevant to normal pregnancies rather than to extreme pregnancy settings. We did not collect paternal information; father's characteristics may also influence the gestational environmental (31). The steroid and peptide cord blood biomarkers of the in utero environment were previously measured, thus making our study efficient. However, our study is one of the first to evaluate the in utero environment associations with telomere length in cord blood leukocyte DNA at time of birth. We did not measure the change in relative telomere length across gestation in the neonate for feasibility reasons, thus we do not know if the rate of telomere shortening during gestation is the same by race. Although we did not directly assess whether telomere length in cord blood leukocytes is correlated to the various types of cells in the developing prostate, a recent study demonstrated a strong correlation between telomere lengths of different somatic tissues and leukocytes (41).

The lack of association of maternal and neonate factors with relative telomere length may have resulted from the use of crude measures of this environment. For example, we measured placental weight, but not other placental characteristics, such as width and shape, that may better capture the placenta's efficiency in transporting nutrients and oxygen from the mother to the fetus. Placental shape, but not placental weight, was associated with an increased risk of colorectal cancer (42), and small or large placental surface area was associated with an increased risk of lung cancer (43) in the Helsinki Birth Cohort studies. Finally, although our study sample size was small, it was powered to detect a minimum difference of 0.23 on the log scale. We hypothesized that the difference in geometric mean relative telomere length between black and white neonates would need to be large to account for the large disparity in prostate cancer risk (60% higher incidence).

Conclusion

In conclusion, these findings do not support the hypothesis that shorter telomere length at birth, as a marker for differences in fetal programming among black neonates compared to white neonates, explains the racial disparity in prostate cancer later in life. However, future investigations are warranted to explore whether other influences and consequences of the in utero environment may account for the racial disparities in prostate cancer outcomes observed later in life.

Chapter 2 References

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Chapter 2 Tables

Table 2.1 Maternal and neonate factors by race, males in the HUB Study

	Black	White	p-value
N	38	38	
Maternal Factors			
Mean Age (years)	24	29	0.005
Mean Parity	1.3	0.6	0.03
Neonate Factors			
Mean Birth Weight (g)	3,207.2	3,470.4	0.004
Mean Placental Weight (g)	640.9	701.7	0.05

Table 2.2 Geometric mean relative telomere length in umbilical cord blood leukocytes by maternal and neonate factors, overall and by race, males in the HUB Study

	Geometric Mean (95% CI)			
	Unadjusted	**Adjusted for race**	**Black**	**White**
Maternal Factors				
Age (years)				
≤19	2.60 (2.21-3.05)	2.58 (2.19-3.05)	2.70 (2.26-3.23)	2.35 (1.67-3.30)
20-25	2.72 (2.29-3.22)	2.70 (2.26-3.22)	2.73 (2.27-3.29)	2.67 (1.83-3.90)
26-31	3.01 (2.59-3.49)	3.03 (2.59-3.53)	3.23 (2.48-4.20)	2.93 (2.41-3.56)
≥32	2.58 (2.23-3.00)	2.59 (2.23-3.02)	2.37 (1.86-3.03)	2.70 (2.20-3.30)
Relative change per year	1.00	1.00	1.00	1.01
p-trend	*0.9*	*1.0*	*0.6*	*0.6*
Parity				
0	2.65 (2.38-2.96)	2.65 (2.37-2.96)	2.61 (2.20-3.11)	2.68 (2.30-3.11)
1	2.96 (2.52-3.48)	2.96 (2.52-3.48)	2.77 (2.24-3.42)	3.22 (2.48-4.18)
2	2.82 (2.27-3.50)	2.82 (2.27-3.51)	2.99 (2.28-3.93)	2.58 (1.78-3.73)
≥3	2.52 (2.00-3.16)	2.52 (2.00-3.19)	2.67 (2.07-3.44)	2.05 (1.21-3.46)
Relative change per birth	0.99	0.99	1.00	0.98
p-trend	*0.8*	*0.8*	*1.0*	*0.7*

Table 2.2 (continued) Geometric mean relative telomere length in umbilical cord blood leukocytes by maternal and neonate factors, overall and by race, males in the HUB Study

	Geometric Mean (95% CI)			
	Unadjusted	**Adjusted for race**	**Black**	**White**
Neonate Factors				
Birth Weight (g)				
≤3025	2.68 (2.30-3.14)	2.67 (2.28-3.13)	2.76 (2.29-3.32)	2.56 (1.96-3.35)
3026-3373	2.52 (2.16-2.95)	2.51 (2.14-2.95)	2.58 (2.16-3.09)	2.40 (1.80-3.21)
3374-3634	3.01 (2.58-3.52)	3.02 (2.58-3.54)	2.49 (1.95-3.18)	3.36 (2.74-4.13)
≥3635	2.71 (2.32-3.17)	2.73 (2.32-3.20)	3.29 (2.53-4.29)	2.48 (2.04-3.02)
Relative change per SD				
(399.99)	1.00	1.00	1.00	1.00
p-trend	*0.9*	*1.0*	*1.0*	*0.9*
Placental Weight (g)				
≤585	2.48 (2.12-2.90)	2.48 (2.12-2.90)	2.52 (2.06-3.09)	2.42 (1.85-3.16)
586-659	2.90 (2.48-3.40)	2.90 (2.48-3.40)	2.76 (2.23-3.41)	3.07 (2.39-3.95)
660-760	2.77 (2.37-3.24)	2.77 (2.37-3.25)	2.84 (2.33-3.48)	2.67 (2.05-3.49)
≥761	2.77 (2.37-3.24)	2.77 (2.36-3.25)	2.81 (2.14-3.69)	2.75 (2.23-3.39)
Relative change per SD				
(138.01)	1.02	1.02	1.06	1.00
p-trend	*0.6*	*0.6*	*0.4*	*1.0*

Table 2.3 Relative change in the geometric mean umbilical cord blood leukocyte telomere length per standard deviation change in umbilical cord blood hormone concentrations, overall and by race, males in the HUB Study

Hormone	Standard Deviation	Unadjusted	p	Adjusted for race	p	Black	p	White	p
Testosterone (ng/mL)	0.47	0.99	0.7	0.99	0.7	0.99	0.7	0.99	0.9
Estradiol (pg/mL)	5,464	0.97	0.4	0.97	0.4	0.98	0.8	0.96	0.5
SHBG (nmol/L	5.00	0.97	0.4	0.97	0.4	0.93	0.1	1.01	0.9
Androstanediol glucuronide (ng/mL)	15.11	1.11	**0.01**	1.11	**0.01**	1.12	**0.01**	1.08	0.3
IGF-1 (ng/mL)	51.76	1.02	0.6	1.02	0.6	1.05	0.6	1.02	0.8
IGF-2 (ng/mL	93.43	1.03	0.4	1.04	0.4	1.09	0.1	1.00	0.9
IGFBP-3 (ng/mL)	394.8	0.99	0.7	0.98	0.7	0.99	0.9	0.98	0.7
Leptin (pg/mL)	8,897	1.00	0.9	1.00	0.9	1.15	0.3	0.99	0.8
Vitamin D (ng/mL)	9.45	0.97	0.4	0.96	0.3	1.01	0.9	0.93	0.2
Molar ratios of:									
Testosterone/SHBG	0.13	1.03	0.5	1.03	0.5	1.04	0.4	1.01	0.9
Estradiol/SHBG	1.09	1.00	0.9	0.99	0.9	1.05	0.5	0.97	0.6
IGF-1/IGFBP-3	0.08	1.06	0.1	1.07	0.1	1.05	0.4	1.08	0.2
IGF-2/IGFBP-3	0.19	1.06	0.1	1.06	0.1	1.09	0.08	1.02	0.7

Table 2.4 Geometric mean telomere length in umbilical cord blood leukocytes by race, males in the HUB Study

	Geometric Mean Relative Telomere Length (95% CI)		
	Black	**White**	**p-value**
Unadjusted	2.72 (2.44-3.04)	2.73 (2.45-3.05)	0.96
Adjusted for Maternal Factors*	2.74 (2.43-3.09)	2.72 (2.41-3.07)	0.93
Adjusted for Neonate Factors**	2.73 (2.43-3.06)	2.73 (2.43-3.06)	0.99

*Mother's age and parity
**Birth weight and placental weight

Chapter 3: Midlife racial differences in peripheral blood leukocyte telomere length and in associations of modifiable factors with telomere length

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Abstract

We previously investigated whether cord blood leukocyte telomere length differs by race as a way to assess the role of early life factors in the prostate cancer racial disparity. We hypothesized that telomere length would be shorter in black neonates given their 60% higher adult prostate cancer risk, but did not observe a racial difference. Whether peripheral blood leukocyte telomere length differs by race in midlife – after experiencing decades of exposures that could shorten telomeres – is uncertain. Obesity, physical inactivity, and smoking have been associated with shorter leukocyte telomere length. These factors are among the few purported risk factors for lethal prostate cancer that are modifiable and for which prevalence differs by race. Thus, we examined whether telomere length is shorter in black compared with white men, and evaluated whether associations between these modifiable risk factors and telomere length differ by race. The Health Professionals Follow-up Study is a cohort of US men aged 40-75 years followed since 1986. At baseline and biennially men completed questionnaires on lifestyle and medical factors. Blood was collected in 1993-95. We measured relative telomere length (telomere/single copy gene) by quantitative PCR for a previously studied subset of the cohort without cancer. We estimated geometric mean relative telomere length and 95% confidence intervals (CI) for the 30 black and 41 white men with complete information

using linear regression adjusting for age and further for the modifiable risk factors. We estimated associations between the modifiable risk factors and the natural logarithm of relative telomere length by race using linear regression adjusting for age, and evaluated race interactions using the likelihood ratio test. Black and white men did not differ in age or waist circumference, but black men were less physically active and more likely to smoke. Geometric mean telomere length did not differ by race after adjusting for age (black: 1.44, 95% CI 1.28-1.63; white: 1.37, 95% CI 1.23-1.52). Waist circumference was possibly more strongly inversely associated with telomere length in black than white men (p-interaction=0.2). Physical activity was positively associated with telomere length only in black men (p-interaction=0.1). Smoking appeared to be inversely associated with telomere length in both black and white men (p-interaction=0.7). Telomere length also did not differ after further adjusting for these modifiable factors (black: 1.46, 95% CI 1.30-1.64; white: 1.36, 95% CI 1.23-1.50). Counter to the hypothesis, telomere length was not shorter in black compared to white men in midlife. We found possible racial differences midlife in the association of waist circumference and physical activity with telomere length, but these differences did not result in racial differences in telomere length. Smoking was associated with shorter telomeres in both black and white men.

Introduction

We previously investigated whether umbilical cord blood leukocyte telomere length, a possible cumulative marker of in utero exposures, differs by race as a way to assess the role of early life factors in the racial disparity in the incidence and aggressiveness of prostate cancer. We hypothesized that telomere length would be shorter in black neonates given their 60% higher adult risk of prostate cancer (1) and our recent findings that telomere length in prostate tissue of men without prostate cancer is associated with prostate cancer risk (2), and in prostate tissue of men with prostate cancer is prognostic for death from their cancer (3). However, we did not observe a racial difference in umbilical cord blood leukocyte telomere length, consistent with Okuda et al. who studied 163 US black, white, and Hispanic neonates (4).

Whether peripheral blood leukocyte telomere length differs by race in midlife – after experiencing decades of proliferative and oxidative exposures that influence telomere shortening including modifiable factors – has previously been addressed with contradictory results (5-8). The modifiable factors, obesity, physical inactivity (9), and cigarette smoking (10, 11) have been associated with peripheral blood leukocyte telomere length in adulthood and these three factors are among the few purported modifiable risk factors for lethal prostate cancer (12, 13), and for which the prevalence may differ by race (14). We hypothesize that associations between these modifiable factors and leukocyte telomere length could translate to racial differences in lethal prostate cancer risk through either racial differences in the prevalence of these risk factors or racial differences in the strength of association with leukocyte telomere length.

In this study, we therefore examined whether peripheral blood leukocyte telomere length differs between a subset of cancer-free black and white middle-aged and older men participating in the Health Professionals Follow-up Study. We also determined whether associations of modifiable risk factors for lethal prostate cancer with telomere length differ by race, and whether associations of markers of the hormonal and growth factor system that may underlie the influence of those modifiable factors with telomere length differ by race.

Methods

Study population

The Health Professionals Follow-up Study (HPFS) is an ongoing prospective cohort study of US male health professionals (dentists, pharmacists, optometrists, osteopath physicians, podiatrists, and veterinarians) that began in 1986. 51,529 men aged 40-75 years completed the baseline mailed questionnaire, which included questions on major ancestry and a semi-quantitative food frequency questionnaire (FFQ). Updated medical and lifestyle information was collected on biennial mailed follow-up questionnaires. Between 1993 and 1995, more than 18,000 of the HPFS participants provided a blood specimen (sodium EDTA tubes), which was returned by overnight courier and chilled on dry ice during transport. On receipt, the samples were centrifuged, aliquotted into plasma, buffy coat, and red blood cells, and stored in liquid nitrogen freezers at -70°C. As described previously (15, 16), after excluding men who had a cancer diagnosis, those who indicated more than one major ancestry, and those who did not live in the

continental US (for feasibility of blood specimen shipment), in 1996 we invited all 63 eligible black men and a random sample of 75 white men (of 14,665 eligible) to provide a second blood sample in 1996. Of those invited, 43 black and 56 white men returned the second blood sample using the previous protocol. For this study on telomeres, we restricted the analysis to men who returned the second blood sample with complete covariate data, thus the analytical cohort consisted of 30 black and 41 white men. The Institutional Review Boards at the Harvard School of Public Health and the Johns Hopkins Bloomberg School of Public Health approved studies on telomeres in the HPFS.

Assessment of race, modifiable risk factors for lethal prostate cancer, and hormones and growth factors

Race was assessed based on the men's response to questions about their major ancestry on the baseline questionnaire, with option to mark all that applied: southern European, Scandinavian, other Caucasian, Afro-American, Asian/Oriental, or other (unspecified) origin. Men who reported that their major ancestries were southern European, Scandinavian, and/or other Caucasian only were considered to be white. Men who reported that their major ancestry was Afro-American only were considered to be black.

Age at blood draw was calculated as the difference between date of blood draw and birth date. From the questionnaires, we selected modifiable and other factors that are purported risk factors for aggressive prostate cancer in the HPFS (12) and elsewhere (17, 18). Current height and weight, physical activity, and current cigarette smoking (yes/no) were obtained from the 1994 questionnaire, and waist circumference was obtained from the

1987 questionnaire. BMI was calculated as self-reported weight (kilograms) divided by the square of height (meters). Total physical activity (in MET-hours) was calculated as the sum of the product of the frequency of the specific activities and their metabolic equivalents of task (METs).

Plasma concentrations of sex steroid hormones and growth factors thought to underlie some of the associations between the modifiable risk factors for lethal prostate cancer and that have also been hypothesized to differ in concentration by race were previously measured: testosterone (radioimmunoassay), dihydrotestosterone (radioimmunoassay after celite column chromatography), androstanediol glucuronide (radioimmunoassay), estradiol (radioimmunoassay), sex hormone-binding globulin (radioimmunometric assay), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$; radioimmunoassay), 25-hydroxyvitamin D (25(OH)D; radioimmunoassay), insulin-like growth factor-1 (IGF-1; ELISA), and insulin-like growth factor binding protein-3 (IGFBP-3; ELISA). Mean intrapair coefficients of variation for blinded quality control samples were as follows: testosterone 4.3%, dihydrotestosterone 14.5%, androstanediol glucuronide 8.5%, estradiol 19.5%, sex hormone-binding globulin 12.9%, $1,25(OH)_2D$ 5.6%, 25(OH)D 6.7% (9), IGF-1 13.2%, and IGFBP-3 11.6%. Length of the androgen receptor gene CAG repeat, which is known to differ by race (16, 19), was also previously measured by polymerase chain reaction amplification of the region surrounding the repeat and sizing by automated fluorescence detection (15, 16).

Measurement of peripheral blood leukocyte telomere length

Leukocyte DNA was isolated from the stored buffy coat using the DNeasy Blood and Tissue kit (Qiagen). Quantitative PCR was used to estimate the ratio of telomeric DNA to that of a single copy gene (β-globin) as previously described (20), with the following modifications (21). Briefly, 5 ng of genomic DNA was used in a 25 μL volume for either the telomere or β-globin reactions; each sample was run in triplicate. Each 96-well plate contained a no template negative control and two separate 5-point standard curves using leukocyte DNA, thus allowing for determination of plate-specific PCR efficiencies. Each plate also included a series of cell lines with known telomere lengths, ranging from 3-15 kb, as determined independently by telomere terminal restriction fragment analysis, thereby providing an additional quality control check. The mean coefficient of variation was 0.98% for the telomere reaction and 1.22% for the β-globin triplicate reactions. The mean telomere threshold (C_t) value and the β-globin C_t value were calculated from the telomere and the β-globin triplicate reactions, respectively. For each sample, the ratio (-dC_t) of the telomere value for the experimental sample to the value for the single copy gene (T/S) was calculated by subtracting the β-globin C_t value from the telomere C_t value. The relative T/S ratio (-ddC_t) was determined by subtracting the -dC_t from a 5 ng sample in the cell line series from the -dC_t of each unknown sample. These relative T/S ratios were used in the statistical analysis and are referred to as relative telomere length.

Statistical analysis

We calculated means, medians, or prevalences of participants' modifiable and other factors by race (Table 3.1). Differences by race in these factors were assessed using the t-test for continuous factors that were normally distributed, the Wilcoxon rank sum test for continuous factors that were not normally distributed (i.e., waist circumference and physical activity), and the Fisher's exact test for current smoking. Racial differences in concentrations of the hormones and growth factors, and in length of the androgen receptor gene CAG repeat were previously published in the full set of these men (15, 16).

Next, we calculated means and prevalence of the factors across tertiles of relative telomere length separately by race (Table 3.2). Tertile cut points were based on the combined distribution in white and black men. We tested for trend in the factors across tertiles of telomere length using Cuzick's non-parametric trend test for all factors except current smoking for which we used the Cochrane-Armitage trend test of proportions. Relative telomere length was not normally distributed. Thus, we used the natural logarithm transformation and calculated geometric mean relative telomere length and 95% confidence intervals (CI) for the black and white men from generalized linear models. Next, we estimated geometric mean relative telomere length by race using linear regression to adjust for: 1) age, 2) age and the modifiable and other factors for lethal prostate cancer that based on Tables 3.1 and 3.2 are possible confounders, and 3) age and individually for the hormones and growth factors that based on Tables 3.1 and 3.2 differ by race and/or telomere length. Sensitivity analyses were performed for all analyses dropping one black man who had a relative telomere length greater than two standard

deviations from the mean among blacks and whites combined. The results were very similar after dropping the outlier, and thus, he was retained in analyses.

We used linear regression to estimate age-adjusted associations between a one standard deviation change in the continuous factors and the natural logarithm of relative telomere length separately by race. For current smoking, we estimated the age-adjusted association between current smoking (versus not) and the natural logarithm of relative telomere length using linear regression. We evaluated whether these associations differed by race using the likelihood ratio test to compare models with and without an interaction term (product of race and factor).

For this dissertation, we also conducted the analysis among all men for whom telomere length was determined (43 black, 56 white men) irrespective of covariate data completeness (results shown in Tables 3.1b, 3.2b, 3.3b, 3.4b in Appendix A). We confirmed that the extent of missingness for each covariate did not differ by race. Because the main results on racial difference in telomere length were generally similar in the full and complete case analyses (see Appendix A for summaries), to be able to generate comparable results for the various multivariable-adjusted analyses, we present as the primary findings those from the complete case analyses.

All p-values are from two-sided tests. We considered $p<0.05$ to be statistically significant. All analyses were done using SAS 9.4 (SAS Institute, Cary, NC).

Results

To characterize the men in this study and to begin to identify factors that may confound the racial difference in relative telomere length, Table 3.1 shows the men's characteristics by race, and Table 3.2 shows the men's characteristics across tertiles of relative telomere length by race. Black and white men did not differ in age or anthropometric factors, but black men were possibly less physically active and more likely to smoke, although these differences were not statistically significant (Table 3.1). Black men possibly had higher plasma concentrations of estradiol ($p=0.09$), and lower concentrations of dihydrotestosterone (this difference was not seen in the full set - see Appendix Table 3.1b), androstanediol glucuronide, IGF-1, IGFBP-3, and 25(OH)D (all $p<0.05$). Black men had fewer androgen receptor gene CAG repeats compared with white men ($p=0.01$). Among black men, waist circumference decreased across increasing tertiles of relative telomere length (p-trend=0.02) and physical activity increased across increasing tertiles of relative telomere length (p-trend=0.047) (Table 3.2). The patterns were similar in white men but not statistically significant. In both black and white men, the prevalence of current smoking was highest in men in the shortest tertiles of relative telomere length. Among black men, plasma concentrations of dihydrotestosterone increased (p-trend=0.004), while androstanediol glucuronide (p-trend=0.1) and estradiol (p-trend=0.2) possibly decreased across increasing tertiles of relative telomere length. In white men, plasma concentration of testosterone (p-trend=0.03) and possibly dihydrotestosterone (p-trend=0.1) and $1,25(OH)_2D$ (p-trend=0.1) decreased across tertiles of relative telomere length.

Relative telomere length did not differ by race before (black: 1.44, white: 1.37, p=0.6) or after adjusting for age (p=0.5) (Table 3.3). After further adjusting simultaneously for the modifiable risk factors that differed by race (Table 3.1) and/or were associated with relative telomere length (Table 3.2) – waist circumference, physical activity, and smoking – relative telomere length still did not differ by race (p=0.3). After individually adjusting for plasma concentrations that appeared to differ by race (Table 3.1) and/or were associated with relative telomere length (Table 3.2) – testosterone, dihydrotestosterone, androstanediol glucuronide, estradiol, $1,25(OH)_2D$, 25(OH)D, IGF-1, IGFBP-3, and the androgen receptor gene CAG repeat length – relative telomere length did not differ by race (data not shown).

To address the secondary question of this study of the extent to which purported modifiable risk factors for prostate cancer and biomarkers that may underlie associations of these factors with prostate cancer are differentially associated with relative telomere length by race, we show in Table 3.4 the age-adjusted change in relative telomere length per standard deviation increase in continuously distributed factors or per change in smoking status (not current to current). With respect to anthropometric measures, the inverse association for waist circumference appeared to be stronger in black (β=-0.14; p=0.04) than white (β=-0.05; p=0.3) men (p-interaction=0.2). Other anthropometric measures, height and waist-to-hip ratio, were not statistically significantly associated with relative telomere length in either black or white men. Physical activity was possibly positively associated with relative telomere length in black, but not white men (p-interaction=0.1). Current smoking appeared to be inversely associated with relative

telomere length in both black and white men. With respect to the biomarkers, associations of dihydrotestosterone (p-interaction=0.0002), and possibly androstanediol glucuronide (p-interaction=0.1) and 1,25$(OH)_2$D (p-interaction=0.05) with relative telomere length appeared to differ by race; for each of these biomarkers, the direction of association was opposite in black and white men (Table 3.4). Additionally, estradiol (p=0.01) and bioavailable estradiol (p=0.03) were inversely associated with relative telomere length in black, but not white men. The other measured biomarkers were not associated with relative telomere length in either black or white men (Table 3.4).

Discussion

This cross-sectional study investigated whether peripheral blood leukocyte relative telomere length differs between black and white men in midlife, and further, whether the association between modifiable risk factors for lethal prostate cancer and relative telomere length differs by race. We hypothesized that factors associated lethal prostate cancer (12, 13) that are also associated with shorter telomere length (9, 10, 22) would be more prevalent or have a stronger association in black compared to white men. We hypothesized that these differences would result in black men having shorter telomeres than white men in midlife, and that this difference may help explain some of the racial disparity in prostate cancer. However, contrary to the hypothesis, among a subset of participants in the HPFS, black men did not have shorter relative telomere length than white men in midlife either before or after taking into account the purported risk factors, and if anything relative telomere length was about 5% longer in black men. While we did find possible midlife racial differences in the association of waist circumference (stronger

inverse association in black than white men) and physical activity (positive association in black men and no association in white men) with relative telomere length, these differences did not result in racial differences in relative telomere length. Current smoking was associated with shorter relative telomere length in both black and white men.

With respect to our primary research question of whether telomere length differs by race, our finding of 5% longer telomeres in black than white men, while not statistically significant, is consistent (6-8) with some studies, but not others (5). Hunt et al. (n=2,453) found that black men and women had longer peripheral leukocyte telomere length than white men and women in early (NHLBI Family Heart Study) and older (Bogalusa Heart Study) adulthood in cross-sectional studies (6). Telomere length was measured by Southern blot in the full study, and by both Southern blot and qPCR in a subset of 234 adults in the Bogalusa Heart Study. In that subset, relative telomere length was about 9% longer using Southern blot and 10% longer using qPCR in black compared with white men adjusting for age and BMI cross-sectionally, confirming that the racial difference was robust to method of telomere length determination. The only longitudinal study that we could identify, also drawn from the Bogalusa Heart Study (the same source population as the Hunt et al. study), included 635 men and women ages 24-44 followed from baseline in 1995-96 until 2001-06 and found that black adults had longer leukocyte telomere length (as measured by Southern blot) than white adults at baseline (7.7%) and at follow-up (7.4%) ten years later; these racial differences were present and statistically significant after adjusting for age, BMI, smoking, and sex (7).

Our cross-sectional findings are also consistent with those in the National Health and Nutrition Examination Survey 1999-2002 (n=5,360), which found that black adults had approximately 5% longer relative leukocyte telomere length using qPCR than white adults of the same, adjusting for age, income, health behaviors and BMI ($p<0.01$) (8). In contrast to these studies and ours, Diez-Roux et al., in a cross-sectional study of 981 men and women age 45-84, found that relative telomere length (measured by qPCR) was 1% shorter in blacks than whites, but the racial difference was only significant after adjustment for age, sex, smoking, BMI, leisure time, and processed meat intake ($p=0.02$) (5). It is unclear why the Diez-Roux et al. (5) results differ from the other studies (6-8) including ours.

Even after adjusting for our originally hypothesized modifiable risk factors – obesity, smoking, and physical inactivity – relative telomere length was not shorter in black than white men in this study, and relative telomere length possibly remained slightly longer in black men. Adjusting individually for the circulating hormonal and growth factors concentrations or for the androgen receptor gene CAG repeat length also did not explain the lack of finding of shorter telomere length in black men despite racial differences in the association of testosterone (inverse association in white men and no association in black men) and dihydrotestosterone (positive association in black men and no association in white men) with relative telomere length.

With respect to the secondary research question of whether the associations of modifiable risk factors for lethal prostate cancer and the biomarkers with relative telomere length

differ by race, we did find racial differences in the association with relative telomere length in some modifiable factors that were also assessed in the previous studies such as waist circumference as a measure of adiposity and physical activity (5-7). Most of these other studies (5-7), however, did not evaluate racial differences in the associations between these factors and telomere length. One study did find that education was positively associated with telomere length only in whites (8), an association we could not address because the men in the HPFS are similarly highly educated. In our study, the inverse associations for increased waist circumference and less physical activity were stronger among black men, which would in theory, produce greater telomere shortening in black men, but this is not what we observed. While the other studies found black men had longer telomeres than white men, studies by Hunt et al.(6), Aviv et al. (7), and Needham et al. (22) did not assess racial differences in the associations between modifiable factors and telomere length and thus we cannot directly compare these results. However, these studies and ours found similar paradoxical results: black men had a higher prevalence of modifiable risk factors (e.g., higher BMI, smoking) that are associated with shorter telomeres than white men, but black men had longer telomeres than white men.

It is possible that in these studies the associations of BMI and smoking with telomere length were stronger in white men and could result in shorter telomere length in white men compared to black men, however we are unable to conclude this based on their analyses. Another possible explanation is that black men may have substantially longer telomeres than white men in the absence of factors that shorten telomeres, and when in

the presence of these factors, telomere length is only slightly longer in black men. We cannot rule out this explanation based on studies on racial differences in umbilical cord blood telomere length (4), including our studies (23, 24), because of possible secular environment/in utero trends (e.g., changing in maternal weight pre-pregnancy) in birth cohorts. We also found racial differences in the association of the biomarkers dihydrotestosterone, estradiol, and $1,25(OH)_2D$, which were not measured in the other studies. Of these racial differences in the association of factors with telomere length, the strongest difference in association that we observed was for dihydrotestosterone (p-interaction=0.0002). We did not have an a priori hypothesis specifically about racial differences in the association between plasma concentration of this androgen and peripheral blood leukocyte telomere length, and do not have a biologically plausible explanation for this finding. We performed a large number of statistical tests (>100) using α=0.05, we expected to observe ~5 statistically significant results due to chance alone. Thus, at this time, we cannot rule in or out that the dihydrotestosterone interaction is a chance finding.

We focused our work on racial differences in telomere length within the context of prostate cancer specifically. The reason for the prostate cancer focus is that our previous work observed in a pilot study that shorter prostate stromal cell telomere length is associated with a higher risk of prostate cancer in the Prostate Cancer Prevention Trial (2) and that greater variation in telomere length in prostate cancer cells combined with shorter telomere length in cancer-associated stromal cells is associated with an increased risk of death from prostate cancer in the HPFS taking into account the currently used

prognostic factors (3). However, a subsequent study in HPFS observed that longer peripheral blood leukocyte telomere length was associated with a higher risk of total and localized prostate cancer, and that telomere length was not associated with risk of high grade, advanced stage, or lethal prostate cancer (25). Two other recent studies found no association between leukocyte telomere length and prostate cancer risk (26, 27) or death in men with prostate cancer (27). Whether telomere length in prostate tissue in men with and without prostate cancer differs by race has not been studied. At this time, it is not possible to reconcile the findings of the studies that measured telomere length in peripheral blood leukocytes and those that measured telomere length in prostate tissue, the target organ, or how telomere length may help explain why black men have a higher incidence and aggressiveness of prostate cancer compared with white men.

Various aspects of this study warrant discussion. Data for this analysis came from the HPFS, a well-characterized cohort of men of generally equally higher socioeconomic status irrespective of race. Thus, use of this cohort reduces the likelihood of confounding by unmeasured correlates of race. Our cohort subset was also designed to compare the men by race, and thus black and white men were included in roughly equal numbers and several biomarkers were already measured in this subset that we were able to investigate here. We restricted this analysis to men with complete covariate information, which reduced the sample size from that designed. However, we did not identify differences in the extent of missingness by race. After confirming that the characteristics of all of the black and white men and those with complete covariate data were similar, and after confirming that the unadjusted geometric mean telomere length in all men versus those

with complete covariate data were very similar, we presented here the results for the complete case analysis only. We also chose to use waist circumference as our measure of adiposity instead of BMI like the previous studies due to the stronger association between waist circumference and telomere length and the racial differences in this association that we did not find with BMI. There was one black man with telomere length greater than two standard deviations from the mean. The fact that the telomere length for this man was possibly a statistical outlier does not make the measurement invalid: the measurement passed the quality control checks for the telomere measurement and the length is not outside of biological plausibility. Nevertheless, we ran a sensitivity analysis dropping this potentially influential observation and the same patterns remained.

In summary, in this small cross-sectional study of male health professionals, peripheral blood leukocyte telomere length does not appear to be shorter in black compared with white men in midlife; instead telomere may be slightly longer in black compared to white men. The association of waist circumference and physical activity with telomere length may differ by race in midlife, but these differences do not appear explain our findings for racial differences in telomere length. Smoking was associated with shorter telomere length in both black and white men, and also did not explain our findings for racial differences in telomere length. In the context of the racial disparity in prostate cancer incidence and aggressiveness, midlife peripheral blood leukocyte telomere length does not appear to be etiologically informative.

Chapter 3 References

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Chapter 3 Tables

Table 3.1 Characteristics of a subset of white and black men, Health Professionals Follow-up Study, 1993-1995

	White	Black	p
No.	41	30	
Age at blood draw (yr)	61.2	62.8	0.6
Height (inches)	70.2	70.1	0.8
Waist circumference (inches)	37.4	37.4	0.9
Waist-to-hip ratio	0.94	0.93	0.2
Physical activity (MET-hours/week)	18.6	15.0	0.3
Current smoker (%)	4.9	10.0	0.6
Plasma concentration			
Total testosterone (ng/mL)	4.7	4.8	0.8
Dihydrotestosterone (ng/mL)	0.35	0.29	0.05
Androstanediol glucuronide (ng/mL)	6.4	4.7	0.03
Estradiol (pg/mL)	18.2	20.6	0.09
Sex hormone-binding globulin (nmol/L)	28.6	25.0	0.2
Estradiol/sex hormone-binding globulin, $x10^3$ (molar ratio)	0.76	1.0	0.03
Testosterone/sex hormone-binding globulin (molar ratio)	0.18	0.22	0.07
$1,25(OH)_2D$ (pg/mL)	32.8	33.7	0.7
25(OH)D (ng/mL)	24.3	15.9	<0.0001
IGF-1 (ng/mL)	241	190	0.01
IGFBP-3 (ng/mL)	4002	3442	0.03
IGF-1/IGFBP-3 (molar ratio)	0.25	0.24	0.3
Androgen receptor gene CAG repeat length	22.2	20.0	0.01

Table 3.2 Means and prevalence of participant characteristics by tertile of relative telomere length among a subset of white and black men, Health Professionals Follow-Up Study, 1993-1995

	Tertile of relative telomere length							
	White men				Black men			
	1 Shortest	2	3 Longest	p-trend*	1 Shortest	2	3 Longest	p-trend*
No.	14	15	12		9	11	10	
Age at blood draw (yr)	61.3	63.9	59.3	0.4	64.1	61.8	62.7	0.9
Height (inches)	70.4	69.7	70.8	0.6	70.2	70.3	69.7	0.6
Waist circumference (inches)	38.1	37.5	36.4	0.6	40.8	36.2	35.9	0.02
Waist-to-hip ratio	0.95	0.93	0.94	0.4	0.96	0.90	0.93	0.3
Physical activity (MET-hr/wk)	17.9	18.2	19.7	0.7	5.83	16.4	21.6	0.047
Current smoker (%)	14.3	0	0	0.2	22.2	9.1	0	0.1
Plasma concentration								
Total testosterone (ng/mL)	5.05	5.05	3.78	0.03	4.67	4.64	5.00	0.7
Dihydrotestosterone (ng/mL)	0.38	0.36	0.31	0.1	0.21	0.30	0.35	0.004
Androstanediol glucuronide (ng/mL)	5.77	6.40	7.03	0.5	5.53	4.69	4.03	0.1
Estradiol (pg/mL)	18.9	17.6	18.2	0.9	23.3	19.9	18.9	0.2
Sex hormone-binding globulin (nmol/L)	29.0	31.4	24.7	0.5	23.2	24.9	26.8	0.5
Estradiol/sex hormone-binding globulin, $x10^3$ (molar ratio)	0.79	0.67	0.83	0.9	1.23	1.03	0.87	0.2
Testosterone/sex hormone-binding globulin (molar ratio)	0.20	0.18	0.17	0.09	0.22	0.22	0.21	0.8
$1,25(OH)_2D$ (pg/mL)	34.6	32.0	32.0	0.1	32.6	36.0	32.0	0.9
25(OH)D (ng/mL)	22.5	26.7	23.3	0.6	15.4	17.8	14.3	0.5
IGF-1 (ng/mL)	241	228	256	0.9	211	145	220	0.7
IGFBP-3 (ng/mL)	4090	3917	4006	0.7	3317	3184	3838	0.4
IGF-1/IGFBP-3 (molar ratio)	0.25	0.24	0.26	0.7	0.27	0.19	0.25	0.8
Androgen receptor gene CAG repeat length	21.9	23.0	21.5	0.8	19.0	20.9	20.0	0.6

*Cuzick's non-parametric trend test, Cochrane-Armitage test for trend in proportion for current smoking

Table 3.3 Geometric mean relative telomere length in a subset of white and black men, Health Professionals Follow-up Study, 1993-1995

	Geometric mean telomere length				
	White	95% CI	Black	95% CI	p
Unadjusted	1.37	(1.24, 1.52)	1.44	(1.27, 1.62)	0.6
Adjusted for age	1.37	(1.23, 1.52)	1.44	(1.28, 1.63)	0.5
Adjusted for age, waist circumference, physical activity, and smoking	1.36	(1.23, 1.50)	1.46	(1.30, 1.64)	0.3

Table 3.4 Age-adjusted change in the natural logarithm of relative telomere length per standard deviation change in modifiable and other risk factors for lethal prostate cancer and hormones and growth factors, in a subset of white and black men, Health Professionals Follow-Up Study, 1993-1995

		Change in the natural logarithm of relative telomere length per standard deviation change in factor				
		White men		Black men		
Factors	Standard Deviation	Relative Change	p	Relative Change	p	p-interaction
Height (inches)	2.99	0.004	0.9	-0.05	0.5	0.6
Waist circumference (inches)	3.85	-0.05	0.3	-0.14	0.04	0.2
Waist-to-hip ratio	0.05	-0.05	0.4	-0.07	0.3	0.7
Physical activity (MET-hours/week)	17.8	-0.003	0.9	0.13	0.09	0.1
Current smoker (yes)	-------	-0.26	0.2	-0.40	0.08	0.7
Total testosterone (ng/mL)	1.42	-0.08	0.08	0.02	0.8	0.2
Dihydrotestosterone (ng/mL)	0.12	-0.05	0.2	0.26	0.001	0.0002
Androstanediol glucuronide (ng/mL)	3.17	0.04	0.3	-0.15	0.2	0.1
Estradiol (pg/mL)	5.97	-0.03	0.5	-0.16	0.01	0.2
Sex hormone-binding globulin (nmol/L)	12.2	-0.01	0.8	0.05	0.6	0.5
Estradiol/sex hormone-binding globulin, $x10^3$ (molar ratio)	0.54	0.003	0.9	-0.12	0.03	0.2
Testosterone/sex hormone-binding globulin (molar ratio)	0.08	-0.06	0.3	-0.05	0.4	0.9
$1,25(OH)_2D$ (pg/mL)	7.34	-0.10	0.07	0.05	0.4	0.05
25(OH)D (ng/mL)	7.51	0.04	0.4	-0.10	0.3	0.2
IGF-1 (ng/mL)	83.8	0.008	0.9	0.02	0.8	0.9
IGFBP-3 (ng/mL)	1048	0.02	0.8	0.05	0.5	0.7
IGF-1/IGFBP-3 (molar ratio)	0.07	-0.01	0.8	-0.03	0.7	0.8
Androgen receptor gene CAG repeat length	3.54	-0.02	0.7	0.02	0.8	0.7

Appendix A. Chapter 3 Subset of the Men in the Health Professionals Follow-up Study for Whom Telomere Length Was Determined

There were few differences between this original sample and the men used in our analytical sample. The level of dihydrotestosterone was lower in black men in the analytical sample, resulting in a statistically significant difference between black and white men that was not seen in the original sample. IGF-1 and IGFBP-3 both were slightly higher in white men and slightly lower in black men in our analytical sample. However, statistical significance was not affected and the molar ratio of IGF-1/IGFBP-3 was unaffected, as the differences offset each other (Table 3.1 vs. Table 3.1b.)

The men excluded from the original sample also created a trend in dihydrotestosterone that appeared stronger among the black men by tertile of telomere length in the analytical sample (Table 3.2 vs. Table 3.2b.) However, this trend is still strong in the original sample so does not change interpretation. The sample difference also showed a stronger p-interaction for dihydrotestosterone and race with telomere length (Table 3.3 vs Table 3.3b.) Again, the interpretation remains the same in both samples. The overall differences in relative telomere length between black and white men did not change when using the total sample for whom telomere length was measured versus our analytical sample (results not shown).

Table 3.1B Characteristics of a subset of white and black men, Health Professionals Follow-up Study, 1993-1995

	White	Black	P
No.	56	43	
Age at Blood Draw (yr)	61.6	62.0	0.8
Body Mass Index (kg/m^2)*	25.5	26.5	0.1
Height (inches)	70.4	69.8	0.3
Waist Circumference (inches)**	37.3	38.0	0.7
Waist/Hip Ratio**	0.94	0.94	0.9
Total Physical Activity (MET-hours/week)	18.6	14.7	0.2
Current Smoker (%)	5.4	9.3	0.5
Plasma concentration			
No.	54	43	
Total testosterone (ng/mL)	4.7	4.8	0.6
Dihydrotestosterone (ng/mL)	0.34	0.34	0.6
Androstanediol glucuronide (ng/mL)	6.3	4.9	0.05
Estradiol (pg/mL)	18.6	21.3	0.04
Sex hormone-binding globulin (nmol/L)	28.3	24.8	0.2
Estradiol/sex hormone-binding globulin, $x10^3$ (molar ratio)	0.83	1.1	0.09
Testosterone/sex hormone-binding globulin (molar ratio)	0.19	0.22	0.05
No.	53	41	
$1,25(OH)_2D$ (pg/mL)	32.4	33.3	0.6
25(OH)D (ng/mL)	23.2	15.8	<0.0001
No.	55	42	
IGF-1 (ng/mL)	239	200	0.03
IGFBP-3 (ng/mL)	3980	3479	0.02
IGF-1/IGFBP-3 (molar ratio)	0.25	0.24	0.7
Androgen receptor gene CAG (repeat length)***	22.1	20.1	0.008

*BMI missing for 12 white and 4 white men
**Waist measurement missing for 13 white and 9 black men
***AR gene CAG missing for 1 white man

Table 3.2B Means and prevalence of participant characteristics by tertile of relative telomere length among a subset of white and black men, Health Professionals Follow-Up Study, 1993-1995

	Tertile of relative telomere length							
	White men				Black men			
	1 Short	2	3 Long	p-trend*	1 Short	2	3 Long	p-trend*
No.	19	18	19		14	15	14	
Age at Blood Draw (yr)	60.8	63.3	60.8	0.9	63.4	60.9	61.8	0.9
Body Mass Index (kg/m^2)	25.2	25.9	25.5	0.7	27.9	25.4	26.3	0.2
Height (inches)	70.8	69.6	70.8	0.9	69.4	69.9	69.9	0.6
Waist Circumference (inches)	37.9	37.5	36.4	0.7	41.7	36.1	35.9	0.004
Waist/Hip Ratio	0.95	0.94	0.94	0.6	0.99	0.90	0.93	0.05
Physical Activity (MET-hr/wk)	15.7	18.0	21.9	0.5	5.74	17.4	20.0	0.03
Current Smoker (%)	10.5	5.56	0	0.3	21.4	6.67	0	0.1
Total testosterone (ng/mL)	5.19	4.94	3.98	0.02	4.74	4.76	4.96	0.8
Dihydrotestosterone (ng/mL)	0.38	0.36	0.29	0.06	0.31	0.34	0.36	0.04
Androstanediol glucuronide (ng/mL)	5.72	6.60	6.54	0.4	5.51	4.80	4.29	0.2
Estradiol (pg/mL)	18.6	19.2	18.1	0.8	23.7	19.6	20.6	0.2
Sex hormone-binding globulin (nmol/L)	30.0	31.1	24.1	0.3	24.6	24.3	25.5	0.9
Estradiol/sex hormone-binding globulin, x10^3 (molar ratio)	0.78	0.76	0.93	0.7	1.16	1.01	1.00	0.3
Testosterone/sex hormone-binding globulin (molar ratio)	0.20	0.18	0.18	0.2	0.22	0.22	0.22	0.8
1,25(OH)$_2$D (pg/mL)	34.3	31.3	31.4	0.07	31.5	35.9	32.2	0.8
25(OH)D (ng/mL)	21.7	25.6	22.4	0.6	15.2	17.9	14.1	0.5
IGF-1 (ng/mL)	248	216	249	0.9	215	155	230	0.6
IGFBP-3 (ng/mL)	4230	3814	3878	0.3	3395	3131	3912	0.3
IGF-1/IGFBP-3 (molar ratio)	0.25	0.23	0.26	0.4	0.26	0.21	0.25	0.9
Androgen receptor gene CAG (repeat length)	21.4	23.1	21.9	0.5	19.9	20.9	19.3	0.9

*Cuzick's non-parametric trend test, Cochrane-Armitage test for trend in proportion for current smoking

Table 3.3B Geometric mean relative telomere length in a subset of white and black men, Health Professionals Follow-Up Study, 1993-1995

	Geometric mean telomere length				
	White	95% CI	Black	95% CI	p
Unadjusted	1.38	(1.25, 1.51)	1.42	(1.28, 1.58)	0.7
Adjusted for age	1.38	(1.25, 1.51)	1.42	(1.28, 1.58)	0.6

Table 3.4B Age-adjusted change in the natural logarithm of relative telomere length per standard deviation change in modifiable and other risk factors for lethal prostate cancer and hormones and growth factors, in a subset of white and black men, Health Professionals Follow-Up Study, 1993-1995

		Change in the natural logarithm of relative telomere length per standard deviation change in factor				
		White men		Black men		
Factors	Standard Deviation	Relative Change	P	Relative Change	P	p-interaction
Body Mass Index (kg/m^2)	3.15	0.002	0.7	-0.08	0.3	0.3
Height (inches)	2.81	-0.008	0.8	-0.001	0.9	0.8
Waist Circumference (inches)	4.05	-0.05	0.3	-0.16	0.01	0.1
Waist/Hip Ratio	0.06	-0.05	0.4	-0.11	0.03	0.4
Total Physical Activity (MET-hours/week)	18.0	0.02	0.7	0.013	0.05	0.1
Current Smoker (yes)	-------	-0.14	0.5	-0.38	0.07	0.4
Total testosterone (ng/mL)	1.40	-0.10	0.01	0.005	0.9	0.2
Dihydrotestosterone (ng/mL)	0.16	-0.10	0.04	0.04	0.5	0.04
Androstanediol glucuronide (ng/mL)	2.96	0.04	0.3	-0.10	0.3	0.2
Estradiol (pg/mL)	6.23	-0.02	0.7	-0.13	0.03	0.3
Sex hormone-binding globulin (nmol/L)	12.0	-0.03	0.4	0.03	0.7	0.6
Estradiol/sex hormone-binding globulin, x10^3 (molar ratio)	0.58	0.04	0.5	-0.10	0.09	0.2
Testosterone/sex hormone-binding globulin (molar ratio)	0.08	-0.06	0.2	-0.05	0.4	0.7
1,25(OH)$_2$D (pg/mL)	7.46	-0.10	0.06	0.06	0.3	0.03
25(OH)D (ng/mL)	7.46	0.03	0.5	-0.10	0.2	0.1
IGF-1 (ng/mL)	85.9	0.004	0.9	0.06	0.4	0.4
IGFBP-3 (ng/mL)	1075	-0.02	0.6	0.08	0.2	0.1
IGF-1/IGFBP-3 (molar ratio)	0.07	0.02	0.7	-0.02	0.8	0.7
Androgen receptor gene CAG (repeat length)	3.38	0.03	0.5	0.01	0.8	0.9

Chapter 4: Racial differences in maternal and umbilical cord blood leukocyte telomere length and their correlations

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Abstract

Telomere length at birth is hypothesized to set the baseline for lifetime telomere shortening trajectory and influence adult disease risk including prostate cancer, which shows a pronounced racial disparity. Telomere length is heritable, but may also be a marker of exposures in utero, including those influencing adult cancer risk. Thus, we investigated racial differences in leukocyte telomere length in maternal peripheral blood and umbilical cord blood from their male neonates, and in telomere length correlations between mothers and neonates. The purpose of this work is to inform whether telomere length differences at birth may account for some of the racial disparity in adult disease risk. Black and white Baltimore women were recruited at the start of pregnancy in 2006-7 and followed to postpartum. They completed questionnaires at enrollment and at the first postpartum visit. Medical records were abstracted from each clinic visit, mothers provided blood each trimester, and cord blood was collected at birth. 55 mother-male neonate pairs with complete materials were included. Relative telomere length was measured by quantitative PCR in leukocyte DNA. We used linear regression to estimate mean telomere length in mothers and neonates before and after adjustment. We calculated the Spearman correlation between mother and cord blood telomere length overall and by race. Black mothers were younger, less likely to have a graduate degree and to be nulliparous, and more likely to

be overweight/obese than white mothers. Adjusting for mother's age and assay plate, black mothers had shorter mean telomere length (2.18, 95% CI 1.81-2.56) than white mothers (2.65, 95% CI 2.35-2.95) and black neonates had shorter mean telomere length (2.64, 95% CI 2.27-3.01) than white neonates (3.02, 95% CI 2.73-3.31), although these differences were not statistically significant (p=0.08, 0.1, respectively). Differences were attenuated in mothers and eliminated in neonates after further adjustment for mother's education and pre-pregnancy BMI. Adjusting for mother's age, mother and cord blood telomere lengths were highly correlated overall (r=0.73, p<0.0001), and did not differ between blacks (r=0.77) and whites (r=0.67; p-interaction=0.6). Individually adjusting for maternal or neonate factors in addition to maternal age did not substantially change the correlation overall or in blacks or in whites. Our results appear to support the hypothesis that telomere length differs by race at birth in part due to inherent racial differences and in part due to maternal factors that differ by race, and thus, may inform the racial disparity in risk of adult diseases like prostate cancer.

Introduction

In a pilot study (Chapter 2) (1), we explored racial differences at birth in umbilical cord blood leukocyte telomere length because telomere length may reflect both inheritance and cumulative in utero exposures and thus may be a marker for racial differences in fetal programming. Telomere shortening is associated with cancer (2), including prostate cancer (3, 4), which shows a racial disparity (5). Given that black men have a higher risk of prostate cancer (5), we hypothesized that cord blood telomere length would be shorter in black than white neonates, reflecting racial differences in prenatal exposures and influencing differences in cancer risk later in life. While we did not observe the hypothesized racial difference, our pilot study had limited information on maternal and child factors that may differ by race and influence telomere length. Thus, we performed a second, more comprehensive study of racial differences in cord blood telomere length.

Telomeres, repetitive sequences of DNA that protect the ends of chromosomes, shorten with cell replication and oxidative stress (6) and are shorter in peripheral blood leukocytes of older individuals (7), and in leukocytes (8, 9) and prostate tissue (10) of those exposed to modifiable factors like smoking and obesity. Telomere length has also been shown to be highly heritable (11-14). The mode of telomere length inheritance is still unclear (15) with some studies suggesting a stronger paternal influence (16, 17) and others, a stronger maternal influence (18, 19). Beyond inheritance, maternal modifiable factors may also influence fetal telomere length, and the risk of later life disease in their neonate.

Initial studies on the influence of the in utero environment on later life disease risk, the "fetal origins hypothesis", addressed links between maternal nutrition, specifically undernutrition, and cardiovascular disease risk (20), and more recently studies have expanded to evaluate other lifestyle factors that may cause oxidative stress such as smoking, physical activity, and psychosocial stress with a multitude of disease outcomes (21, 22). Contemporary concerns about the influence of maternal nutrition on the fetus, now also include overnutrition since the prevalence of obesity has increased over the past three decades (23). Greater maternal weight and weight gain during pregnancy are associated with higher birth weight and higher prevalence of macrosomia in their neonates (24-26). Excess maternal adiposity may also have a direct effect on neonates since obese individuals are also known to be under greater oxidative stress as measured by levels of reactive oxygen species in adipocytes (27) and maternal obesity has been associated with higher levels of oxidative stress in the umbilical cord blood of their newborns (28). Thus, maternal obesity, as a contributor to both proliferation and oxidation, could produce fetal telomere shortening.

We also investigated the association between telomere length and neonate factors, including birth weight, that have previously been used as proxies for the influence of the in utero environment on the fetus. However, birth weight is an imperfect marker of the in utero environment and may only reflect specific timing of exposure and not overall programming throughout gestation (29). Thus markers are needed that

adequately integrate across the entirety of the influence of the in utero environment on the fetus. We propose that telomere length may be such a marker.

Since telomere length at birth is the baseline for lifetime telomere trajectory, we investigated maternal telomere peripheral blood leukocyte telomere length and maternal and neonate factors as determinants of cord blood leukocyte telomere length, overall and by race. Our pilot study (1) was performed using cord blood leukocytes and included limited maternal information, so it was not possible to assess racial differences in the association between maternal and neonate telomere length or maternal factors associated with neonate telomere length beyond age and parity. Thus, in our current study in which we followed women throughout their pregnancy, we investigated racial differences in 1) leukocyte telomere length in maternal blood and cord blood of their male neonates, 2) correlations between maternal and cord blood telomere length, and 3) the associations of maternal pre-pregnancy and pregnancy factors and neonate factors with cord blood telomere length. The purpose of this work is to inform whether racial differences in telomere length at birth may account for some of the racial disparity in adult disease risk, such as prostate cancer, and to inform whether these racial differences in telomere length are inherent, due to modifiable factors in utero, or both.

Methods

Study population

The Expanded Hormones in Umbilical Cord Blood Study (EHUB) is a longitudinal study of pregnant women designed to investigate racial differences in the hormonal and growth factor milieu in utero that may contribute to the racial disparity in prostate and

breast cancers. Participants were aged 18 years or older, received prenatal care at the Johns Hopkins Outpatient Center, and delivered at the Johns Hopkins Hospital in Baltimore, MD, between 2006 and 2007. 185 women, either black or white, were recruited during the first 6-16 weeks of pregnancy and provided consent. Women were eligible for EHUB if the father was the same race as the woman, they were willing to complete the study protocol, were not planning to bank their umbilical cord blood, had not been pregnant for 12 months prior to their current pregnancy, did not use in vitro fertilization or hormonal medications to become pregnant or to maintain their pregnancy, and did not have diabetes mellitus, thyroid disease, or polycystic ovarian syndrome.

Of the 185 recruited women, 126 completed the study. A participant was considered to have completed the study if she provided maternal blood during the first trimester, completed the questionnaire at the first pre-natal obstetric clinic visit, completed the questionnaire at the first post-partum clinic visit, and cord blood was obtained at birth. For this study on racial differences in telomere length, neonates were eligible if they were a live, single birth, with no major birth defects, and full-term defined as the 37th-42nd week, and we restricted to male neonates. Of the 126, 59 neonates were full term, singleton males, and of these blood was located in the repository for all 59 mothers and 55 neonates, resulting in 55 maternal-neonate pairs. EHUB and this analysis were approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health.

Recruited women provided 15 mL of blood (sodium EDTA) at their first pre-natal obstetric clinic visit (6-16 weeks) and completed a standardized questionnaire pertaining to pre-pregnancy administered by a trained research assistant. They were then asked for the dates of their future obstetrics appointments and permission to consult with the obstetrician about a potential due date so administration of study materials could coincide with these appointments. The second and third study visits coincided with their 20th (±2) and 35th (±1) week of pregnancy obstetrics clinic visits. At delivery, 15 mL of venous umbilical cord blood (sodium EDTA) were collected. Maternal and cord blood specimens were refrigerated until they could be processed, usually within 24 hours. The samples were centrifuged at room temperature for 15 minutes at 2,400 rpm, then separated into plasma, buffy coat, and red blood cells and aliquotted in cryovials and stored at -70°C. At the first post-partum obstetric clinic visit, a research assistant administered a standardized questionnaire to the participants pertaining to their pregnancy. Pregnancy complications, weight, blood pressure, and urine glucose test were abstracted from medical records from each obstetric visit along with birth information, including birth weight and length and placental weight.

Assessment of maternal and neonate factors

Mother's age was assessed at time of enrollment. Pre-pregnancy maternal factors were obtained from the questionnaire administered at the first prenatal visit, and for the analysis categorized as follows: parity (0, 1, 2+), attained education (graduate degree, less than graduate degree), active smoking and secondhand smoke exposure history (exposed, not exposed), alcohol consumption history (ever drinker, never drinker),

leisure time physical activity (hours/week), sedentary time (hours/week), usual weight, and height. We calculated body mass index (BMI) from weight (kg) and height (m) and categorized the women using the standard categories (normal 18.5 to <25 kg/m^2, overweight 25 to <30 kg/m^2, obese ≥30 kg/m^2) (30). The following pregnancy-related maternal factors were obtained from the questionnaire administered at the first post-partum clinic visit and expressed as follows: alcohol use during pregnancy (never drank, quit before pregnancy, quit during pregnancy), leisure time physical activity during each trimester (hours/week), and sedentary time (hours/week). The following maternal and neonate factors were obtained from the abstracted medical records: maternal weight recorded at the last obstetrics clinic visit before delivery, birth weight (g), placental weight (g), and birth length (in). Pregnancy weight gain was calculated by subtracting the weight recorded at the last obstetrics clinic visit before delivery from the self-reported usual pre-pregnancy weight. We categorized the women's weight gain as being within or outside of the Institute of Medicine's guidelines for healthy weight gain (31). Neonate ponderal index (kg/m^3) was calculated from birth weight (kg) and length (m).

Measurement of telomere length

Buffy coat samples were pulled from the study freezers and transported to the laboratory for the telomere length assay on dry ice. Leukocyte DNA was isolated from buffy coat from maternal blood and the cord blood of their neonates and re-purified to remove potential residual PCR inhibitors using the DNeasy Blood and Tissue kit (Qiagen, Venlo, Netherlands). Quantitative PCR was used to estimate the ratio of

telomeric DNA to that of a single copy gene (β-globin) as previously described (32), with modifications (33). Briefly, 5 ng of genomic DNA was used in a 25 μL volume for either the telomere or β-globin reactions; each sample was run in triplicate. Each 96-well plate contained a no template negative control and two separate 5-point standard curves using leukocyte DNA; these standard curves allowed the PCR efficiency to be determined for each plate. Each plate also included three samples isolated from a series of cell lines with known telomere lengths, ranging from 3-15 kb, as determined independently by telomere terminal restriction fragment analysis. Inclusion of these samples provided an additional quality control check. Maternal-child pair samples were run on the same plate to limit plate-to-plate variation and the laboratory was blinded to sample type, maternal or umbilical cord blood (Appendix B for quality control plan developed as a component of the primary data collection requirement). Samples were re-run if the coefficient of variation (CV) of either the telomere or the β-globin reaction was greater than 5% or either the telomere or the β-globin values fell outside the range of the standard curve. The mean telomere threshold (Ct) value and the β-globin Ct value were calculated from the telomere and the β-globin triplicate reactions, respectively. For those samples that were re-run (N=8), the mean telomere Ct and β-globin Ct values were compared to the original means to assess validity of the measurements. Because the re-run means were similar to the original run means, data from the original run were used (which preserves the benefit of running the maternal-cord blood pair samples on the same plate). Across all samples, the mean CV was 1.44% and 0.99% for the telomere and β-globin reactions, respectively. For each sample, the telomere measurement of the maternal or cord blood sample to the single

copy gene (T/S) ratio (-dCt) was calculated by subtracting the β-globin Ct value from the telomere Ct value. The relative T/S ratio (-ddCt) was determined by subtracting the -dCt from a 5 ng sample in the cell line series from the -dCt of each unknown sample. These relative T/S ratios were used in the analysis (for simplicity called telomere length throughout). We also assessed plate-to-plate variation by comparison of the overall mean T/S ratio among the plates. Because we found evidence of plate-to-plate variation that was not due to differences in the characteristics of mothers and neonates among the plates, we adjusted for plate in the statistical analysis.

Statistical analysis

Means, medians, and prevalences of maternal and neonate factors were calculated by race and were compared using a t-test (means), Wilcoxon-Mann-Whitney test (medians), or the Cochran-Armitage test (prevalences). Associations of maternal and neonate factors with maternal and cord blood leukocyte telomere length were estimated as unit change using generalized linear regression; these analyses were repeated by race. We did not estimate associations for maternal factors during pregnancy and neonate factors with maternal telomere length because these subsequent factors cannot influence maternal leukocyte telomere length in early pregnancy. We calculated the mean or prevalence of maternal and neonate factors across tertiles of maternal and cord blood leukocyte telomere length and tested for trend using Cuzick's non-parametric trend test or the Cochran-Armitage trend test (prevalences); these analyses were repeated by race.

To address the primary research question, we calculated mean maternal and cord blood leukocyte telomere length and 95% confidence intervals in whites and blacks using linear regression and tested for racial differences using a Wald test and estimated marginal means. We assessed interaction between race and assay plate (given the observed plate-to-plate variability in mean T/S ratio) using the likelihood ratio test; no significant interaction was observed, so the models were run adjusting for plate without an interaction term. Each maternal and neonate factor was assessed as a potential confounder of the racial difference in cord blood telomere length. Then, the analyses were adjusted for assay plate, maternal age, and other suggested confounders. Final confounders were decided based on a priori hypotheses and additional models were selected based on previous statistical testing.

We calculated unadjusted and adjusted (partial) Spearman correlation coefficients to determine the correlation between maternal and cord blood leukocyte telomere length overall and by race. We adjusted the correlation coefficients for each maternal and neonate factor individually and included the factors determined to be confounders or potential confounders previously. We also calculated these correlations by strata of maternal and neonate factors overall, in whites, and in blacks. We tested for heterogeneity by maternal and neonate factors in whites and blacks combined by calculating a z-score using the method of Cohen and Cohen (34); sample sizes were too small to test the correlations for heterogeneity between strata of maternal and neonate factors separately by race. All P-values are from two-sided tests. We considered $P<0.05$

to be statistically significant. All analyses were done using STATA 11 (College Station, TX: StataCorp LP).

Results

Table 4.1 shows maternal and male neonate factors by race. Black mothers were younger, less likely to have a graduate degree, were more likely to be overweight or obese, and less likely to be nulliparous than white mothers. Black and white mothers had different patterns of quitting the consumption of alcohol drinking relative to timing of the current pregnancy. Black neonates tended to have lower birth weight and length than white neonates.

Tables 4.2A and 4.2B presents non-parametric trends in maternal and neonate factors across tertiles of leukocyte telomere length in maternal blood and in cord blood, respectively, overall and by race. Overall, maternal factors tended not to change across tertiles of maternal telomere length, except possibly physical activity (P-trend=0.1), which was lowest in the bottom tertile and equally higher in the middle and top tertiles of maternal telomere length (Table 4.2A). In black mothers, age tended to be highest in the lowest tertile of maternal telomere length and equally lowest in the middle and top tertiles. No trends were observed in maternal factors across tertiles of maternal telomere length in white mothers. Overall, maternal pre-pregnancy BMI and neonate ponderal index decreased across tertiles of cord blood telomere length, whereas maternal education, pre-pregnancy physical activity, and birth length tended to increase across tertiles of cord blood telomere length (Table 4.2B). These patterns were similar in white

and black neonates, with the exception of ponderal index, where in black neonates, the pattern was similar to overall, but in white neonate there appeared to be no difference in ponderal index across tertiles of cord blood telomere length.

The age-adjusted associations of maternal and neonate factors with maternal and cord blood telomere length are shown in Tables 4.3A and 4.3B, respectively, overall and by race. Maternal age was inversely associated with maternal telomere length overall (β=-0.05, P=0.04), in blacks, and possibly in whites (4.3A). Maternal education was positively associated with maternal telomere length overall (β=0.59, P=0.05); associations were positive in both black and white mothers, but were not statistically significant. None of the other maternal factors was clearly associated with maternal telomere length (4.3A). Maternal education was positively associated with cord blood telomere length overall (β=0.78, P=0.02); associations were positive in both black and white neonates, but were not statistically significant (4.3B). Pre-pregnancy BMI was inversely associated with cord blood telomere length overall (β=-0.05, P=0.05); associations were inverse in both black and white neonates, but were not statistically significant (4.3B). Placental weight was positively associated with cord blood telomere length in white neonates (β=0.002, p=0.03), but not in black neonates (P-interaction=0.05; 4.3B). None of the other factors was associated with either maternal or cord blood telomere length.

Racial differences in telomere length in the mothers and their neonates are presented in Table 4.4. Adjusting for assay plate and maternal age, black mothers appeared to have

shorter (P=0.08) mean telomere length (2.18, 95% CI 1.81-2.56) than white mothers (2.65, 95% CI 2.35-2.95). Further adjustment for maternal education and pre-pregnancy BMI attenuated this racial difference (P=0.6). Adjusting for assay plate and maternal age, black neonates appeared to have shorter (P=0.1) mean telomere length (2.64, 95% CI 2.27-3.01) than white neonates (3.02, 95% CI 2.73-3.31). Further adjustment for maternal education and pre-pregnancy BMI eliminated this racial difference (P=0.9).

The Spearman correlation between maternal and cord blood telomere length was 0.63 overall (P<0.0001), and was similar in blacks and whites (P-heterogeneity=0.5) (Table 4.5). After adjusting for maternal age, and additionally individually adjusting for select maternal factors and neonate factors, the correlation between maternal and cord blood telomere length did not substantially change overall or in whites and in blacks. Table 4.6 shows the correlations between maternal and cord blood telomere length stratified by maternal and neonate factors; sample size was insufficient to show these results further stratified by race. The correlation appeared to be stronger (P-heterogeneity=0.08) among parous (r=0.78) than nulliparous (r=0.49) women. The correlation did not clearly appear to differ by strata of the other maternal and neonate factors.

Discussion

This study addressed the potential fetal origins of racial disparity in adult diseases, specifically prostate cancer by assessing racial differences in leukocyte telomere length in mothers and in their male neonates. We found that leukocyte telomere length was

shorter in both black mothers (age-adjusted) and their male neonate's cord blood than in white mothers (age-adjusted) and their male neonate's cord blood. However, these racial differences were attenuated (mother) or eliminated (cord blood) after adjusting for the maternal factors age, education, and pre-pregnancy BMI. We also examined the correlation between maternal and cord blood leukocyte telomere length overall and by race. We found a positive correlation and this correlation was the same in blacks and whites. The positive correlation did not substantially change after further adjusting for maternal or neonate factors. Our results appear to support the hypothesis that telomere length in males differs by race at birth in part due to inherent racial differences and in part due to maternal factors that differ by race, and thus, may inform the racial disparity in risk of adult diseases like prostate cancer.

Unlike our previous study, where we found no difference between cord blood leukocyte telomere length in black and white male neonates (1), in the current study we did find a racial difference as hypothesized (i.e., telomere length shorter in cord blood of black male neonates). Two previous studies investigated racial differences in newborn telomere length with conflicting results (35, 36). Okuda et al. (n=134) found no difference between black and white newborn cord blood leukocyte telomere length (35), whereas Drury et al. (n=66) found that black neonates had significantly longer telomeres than white neonates (36). There are a few possible reasons for the differences in findings among the four studies. First, the four studies adjusted for differing arrays of potential confounders (1, 36), if any (35). Second, some studies included both male and female neonates (35, 36), while our previous and current study only included males (1).

Drury et al. reported that telomere length was different by sex and race, with the longest telomeres in black females (36), whereas Okuda et al. included both sexes, but did not account for possible sex differences by race (35). Third, the four studies used different methods to measure telomere length, which may have differing accuracies in detecting small differences in telomere length: three used qPCR (1, 36) including the current study, and one used PCR-based terminal restriction fragment length (35). Fourth, all of these studies, including both of ours, had small samples sizes, thus we cannot rule out chance as an explanation for the differences in findings among the four studies.

In our current study, we found that, after age-adjustment, black mothers had shorter peripheral blood leukocyte telomere length than white mothers, which is in contrast to a previous study by Hunt et al., which found that black adult women had longer telomeres than white adult women (37). First, our study and that of Hunt et al. were conducted in populations with differing characteristics in the black and white women, such that the patterns of racial variation in telomere length could be different. Second, the two studies took into account different potential confounders. For example, Hunt et al. had no measure of education (37), whereas we adjusted for education, which attenuated the racial difference. Our findings in the mothers is also in contrast, at least on face value, to our prior finding that black men did not have shorter telomere length than white men in midlife either before or after taking into account factors that differ by race, and if anything telomere length was about 5% longer in black men (Chapter 3).

Our finding of a positive correlation between maternal and cord blood telomere length overall is consistent with previous studies in healthy women in the UK (38) and in women with HIV (39), although the correlation we observed was stronger than in these two studies. Unlike the other studies, we accounted for additional maternal factors when calculating this correlation, and showed that even after controlling for maternal and neonate factors, the positive correlation was not substantially changed overall, including within blacks and whites and within strata of maternal factors. We conclude that racial differences in cord blood telomere length (unadjusted) are not explained by racial differences in the strength of the correlation between maternal and cord blood telomere length.

An additional aim of this study was to evaluate whether factors associated with maternal and cord blood telomere length differed by race. With respect to maternal factors associated with maternal leukocyte telomere length, of the factors we evaluated only maternal age (inverse) and education (positive) were possibly associated with maternal telomere length overall and by race. With respect to maternal and neonate factors associated with neonate telomere length and by race, we found that maternal education (positive), pre-pregnancy BMI (inverse), and possibly pre-pregnancy physical activity (positive) were associated with cord blood telomere length overall, however, none of the mother's factors during pregnancy, e.g., maternal weight gain or physical activity was associated. In addition, birth length (positive) and possibly ponderal index (inverse) were associated with cord blood telomere length, whereas birth weight and placental weight were not associated with cord blood telomere length.

None of these associations differed by race, except possibly for the association for placental weight, for which there was a positive association for white neonates and no association for black neonates (P-interaction=0.05). Thus, we conclude that while some maternal and neonate factors are associated with cord blood telomere length, few of these associations differ by race.

Many aspects of this study merit discussion. With respect to strengths, this study used data and samples from a completed longitudinal study designed to evaluate racial differences in in utero biomarkers that may explain racial disparities in prostate cancer in adulthood. Because we conducted a longitudinal study in which we collected the mothers' blood each trimester, in the analysis of the correlation between maternal and cord blood telomere length we were able to use a blood sample from the first trimester when telomere length would be less influenced by the current pregnancy. Detailed data were available on the mothers before and during pregnancy and on their neonates at birth. In the current study, we implemented a thorough quality control protocol in the measurement of telomere length. The technicians running the telomere assay were blinded to race and to maternal or cord blood status. Maternal-neonate pairs were run on the same assay plate to avoid plate-to-plate variability between maternal and corresponding neonate samples. Because we noted plate-to-plate differences in overall mean telomere length, we adjusted for plate in the analyses of racial differences in telomere length.

With respect to possible limitations, we cannot rule out that our findings may have been affected by the fact that black adults are known to have a greater percentage of neutrophil granulocytes than white adults (40). Neutrophil turnover is greater than other types of leukocytes, and greater proliferation can result in shorter telomeres. At birth, the correlation between telomere length in granulocytes and leukocytes has been shown to be 0.97 (41), thus, racial differences in leukocyte subpopulations are unlikely to fully explain the racial differences in cord blood telomere length. We cannot rule out this explanation for the racial difference in leukocyte telomere length in the mothers, however. While we collected blood and detailed information from the mothers, we did not recruit the fathers. Some previous studies on adult children and their parents have reported that paternal and offspring telomere lengths are positively correlated, and that offspring telomere length is associated with paternal age (16, 17). Irrespective of paternal contribution, our findings support the hypothesis that maternal telomere length may influence offspring telomere length. Our samples size was small, which may have limited our ability to detect as statistically significant the size of the racial difference in telomere length that we observed in the mothers and their male neonates' cord blood. Finally, this study was conducted at a single institution and was limited to white and black maternal-neonate pairs only. We also examined maternal-cord blood leukocyte telomere length correlation stratified by race and stratified by maternal pre-pregnancy and pregnancy factors in only healthy women. We are uncertain about the generalizability of the findings to other white and black populations with characteristics that differ from our study population.

In conclusion, maternal factors age, education, and pre-pregnancy BMI were associated with male neonate cord blood telomere length, and maternal telomere length itself was associated with cord blood telomere length, an association that did not differ by race or after accounting for maternal or neonate factors. Our age-adjusted findings suggesting that black mothers and their male neonates both have shorter leukocyte telomeres than whites appear to be contrary to our previous findings in adult men (42) and in cord blood (1). However, after adjustment for maternal factors in the current study, the black-white difference in maternal leukocyte telomere length was attenuated and the black-white difference in cord blood telomere length was eliminated. Taken together, our results appear to support the hypothesis that telomere length differs by race at birth in part due to inherent differences between black and white mothers and in part due to maternal factors that differ by race. These findings may inform the racial disparity in risk of adult diseases like prostate cancer.

Chapter 4 References

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Chapter 4 Tables

Table 4.1 Maternal and male neonate factors by race, Expanded HUB Study, 2006-2007

	Race[b]		
	White	Black	P[a]
No.	35	24	
Maternal Factors			
Age (years, mean)	31	27	0.0003
Graduate degree (%)	77.1	16.7	<0.0001
Pre-pregnancy BMI (%)			
Normal (18.5-24.9 kg/m^2)	85.7	41.7	
Overweight (25-29.9 kg/m^2)	11.4	20.8	<0.0001[d]
Obese (≥ 30 kg/m^2)	2.9	37.5	
Exposure to cigarette smoke, active or secondhand (%)	40.0	45.8	0.7
Ever drinker (%)	91.4	83.3	0.4
Sedentary time (hrs/week, median)	13.5	12.2	0.9[e]
Physical activity (hrs/week, median)	7.1	9.7	0.4[e]
Number of previous births (%)			
0	60.0	37.5	
1	28.6	37.5	0.08[d]
2 or more	11.4	25.0	
Pregnancy Factors			
Sedentary time (hrs/week, median)	13.1	16.3	0.9[e]
Physical activity (hrs/week, median)			
1st trimester	4.6	7.2	0.4[e]
2nd trimester	5.0	6.3	0.5[e]
3rd trimester	3.9	5.1	0.7[e]
Alcohol use during pregnancy (%)			
Never drank	14.3	16.7	
Quit before pregnancy	42.9	70.8	0.04
Quit during pregnancy	42.9	12.5	
Overall weight gain (lbs, mean)	36.2	36.2	0.9
Weight gain (lbs, mean) by pre-pregnancy BMI			
Normal	35.4	34.0	
Overweight/obese	41.2	37.7	0.8
Weight gain within guidelines (%)	42.9	29.2	0.3
Normal	46.7	60.0	0.5
Overweight/Obese[c]	20.0	7.1	0.4

[a]T-test unless otherwise specified.
[b]Race was self-reported by the mother. Mother and father of child must have been of the same race to be eligible for the Expanded HUB Study.
[c]Of the 9 women who were overweight pre-pregnancy, only 1 white and 1 black woman gained weight within the guidelines. Of the 10 women who were obese pre-pregnancy, there were no women who gained weight within the guidelines.
[d]Cochran-Armitage test.
[e]Wilcoxon-Mann-Whitney test.

Table 4.1 (continued) Maternal and male neonate factors by race, Expanded HUB Study, 2006-2007

	Race[b]		
	White	Black	P[a]
Neonate Factors			
Birth weight (g, mean)	3541.2	3327.3	0.09
Birth length (in, mean)	20.3	19.4	0.006
Ponderal index (kg/m^3, mean)	2.6	2.8	0.1
Placental weight (g, mean)	723.2	672.3	0.2

[a]T-test unless otherwise specified.
[b]Race was self-reported by the mother. Mother and father of child must have been of the same race to be eligible for the Expanded HUB Study.
[c]Of the 9 women who were overweight pre-pregnancy, only 1 white and 1 black woman gained weight within the guidelines. Of the 10 women who were obese pre-pregnancy, there were no women who gained weight within the guidelines.
[d]Cochran-Armitage test.
[e]Wilcoxon-Mann-Whitney test.

Table 4.2B Maternal and male neonate factors[a] by tertile of umbilical cord blood telomere length and race, Expanded HUB Study, 2006-2007

	Umbilical cord blood leukocytes											
	Overall				White				Black			
	Telomere length tertiles			P-trend[b]	Telomere length tertiles			P-trend[b]	Telomere length tertiles			P-trend[b]
	Short		Long		Short		Long		Short		Long	
No.	19	19	17		10	12	11		9	7	6	
Maternal Factors												
Pre-pregnancy[c]												
Age (years)	29.6	29.8	28.5	0.6	31.2	31.5	31.0	0.9	27.9	26.9	23.8	0.2
Graduate degree (%)	33.3	52.6	72.2	0.03	50.0	90.0	80.0	0.2	20.0	11.1	33.3	0.9
BMI (kg/m^2)	26.6	24.9	22.1	0.01	23.2	22.5	21.0	0.1	30.4	28.9	23.9	0.07
Exposure to cigarette smoke (%)	50.0	31.6	44.4	0.9	50.0	20.0	53.3	0.7	50.0	44.4	0.0	0.2
Ever drank alcohol (%)	94.4	84.2	83.3	0.5	100.0	90.0	86.7	0.5	90.0	77.8	66.7	0.4
Sedentary time (hrs/week)	13.2	15.4	10.5	0.5	12.5	16.8	11.4	0.6	14.0	13.0	8.8	0.4
Physical activity (hrs/week)	7.05	7.24	11.1	0.08	5.0	5.96	10.0	0.03	9.3	9.4	13.0	0.7
Nulliparous (%)	55.6	47.4	38.9	0.4	37.5	50.0	33.3	0.8	70.0	44.4	66.7	0.8
Pregnancy[d]												
Overall weight gain (lbs)	31.7	37.0	38.8	0.1	34.5	33.4	39.6	0.2	28.7	43.1	37.2	0.2
Weight gain within guidelines (%)	27.8	42.1	50.0	0.2	25.0	60.0	46.7	0.5	30.0	22.2	66.7	0.5
Sedentary time (hrs/week)	12.2	15.5	15.0	0.3	12.1	14.1	13.8	0.3	12.3	18.3	17.4	0.7
Physical activity (hrs/week)	4.91	6.03	5.37	0.3	4.2	5.1	4.4	0.8	5.7	7.8	7.2	0.2

[a] Mean or prevalence (%)
[b] P-trend calculated using Cuzick's non-parametric trend test (means) or the Cochran-Armitage trend test (prevalence)
[c] Before the current pregnancy as reported on the questionnaire administered during the first trimester.
[d] Pregnancy factors occurred after the first trimester blood sample collection.

Table 4.2B (continued) Maternal and male neonate factors[a] by tertile of umbilical cord blood telomere length and race, Expanded HUB Study, 2006-2007

	Umbilical cord blood leukocytes											
	Overall				White				Black			
	Telomere length tertiles			P-trend[b]	Telomere length tertiles			P-trend[b]	Telomere length tertiles			P-trend[b]
	Short		Long		Short		Long		Short		Long	
No.	19	19	17		10	12	11		9	7	6	
Neonate Factors												
Birth weight (g)	3356	3618	3457	0.3	3373	3633	3583	0.2	3337	3593	3226	0.9
Birth length (in)	19.7	20.3	20.4	0.03	20.1	20.5	20.4	0.4	19.3	19.8	20.3	0.06
Ponderal index (kg/m^3)	2.69	2.67	2.50	0.07	2.55	2.61	2.58	0.9	2.84	2.79	2.35	0.03
Placental weight (g)	661.7	762.6	716.3	0.3	631.9	797.9	748.3	0.1	694.8	707.0	646.0	0.8

[a] Mean or prevalence (%)

[b] P-trend calculated using Cuzick's non-parametric trend test (means) or the Cochran-Armitage trend test (prevalence)

[c] Before the current pregnancy as reported on the questionnaire administered during the first trimester.

[d] Pregnancy factors occurred after the first trimester blood sample collection.

Table 4.2A Maternal pre-pregnancy factors[a] by tertile of maternal peripheral blood telomere length and race, Expanded HUB Study, 2006-2007

	Maternal peripheral blood leukocytes											
	Overall				White				Black			
	Telomere length tertiles			P-trend[b]	Telomere length tertiles			P-trend[b]	Telomere length tertiles			P-trend[b]
	Short		Long		Short		Long		Short		Long	
No.	20	20	19		11	12	12		9	8	7	
Maternal Factors												
Age (years)	30.4	29.7	28.3	0.3	30.7	33.2	30.3	0.7	30.0	24.5	24.9	0.04
Graduate degree (%)	47.3	60.0	50.0	0.9	70.0	92.3	66.7	0.8	22.2	0.00	25.0	0.9
BMI (kg/m^2)	26.0	24.9	24.0	0.2	22.5	22.5	22.3	0.7	30.2	28.5	26.9	0.2
Exposure to cigarette smoke (%)	57.9	25.0	45.0	0.5	60.0	15.4	50.0	0.8	55.6	42.9	37.5	0.5
Ever drank alcohol (%)	100.0	75.0	90.0	0.5	100.0	84.6	91.7	0.7	100.0	57.1	87.5	0.5
Sedentary time (hrs/week)	13.9	12.8	12.3	0.5	14.4	12.0	14.2	0.8	13.2	13.9	9.0	0.4
Physical activity (hrs/week)	5.20	9.93	9.47	0.1	4.45	9.46	7.25	0.2	6.1	10.6	13.3	0.4
Nulliparous (%)	55.0	50.0	42.1	0.4	36.4	41.7	41.7	0.8	77.8	62.5	42.9	0.2

[a] Mean or prevalence (%)
[b] P-trend calculated using Cuzick's non-parametric trend test (mean) or the Cochran-Armitage trend test (prevalence)

Table 4.3A Age-adjusted[a] change in maternal peripheral blood leukocyte telomere length per change in status of (binary) or per unit change in (continuous) maternal factors by race, Expanded HUB Study, 2006-2007

	Overall	P	White	P	Black	P	P-interaction[b]
Maternal Factors							
Age (years)	-0.05	0.04	-0.05	0.2	-0.09	0.02	0.5
Graduate degree (yes vs. no)	0.59	0.05	0.18	0.7	0.63	0.3	0.7
BMI (kg/m^2)	-0.03	0.1	0.01	0.9	-0.02	0.4	0.5
Exposure to cigarette smoke (yes vs.no)	-0.12	0.6	0.02	0.9	-0.15	0.8	0.7
Ever drank alcohol (yes vs. no)	-0.39	0.3	-0.17	0.8	-0.65	0.3	0.4
Sedentary time (hrs/week)	-0.01	0.4	-0.01	0.7	-0.03	0.2	0.6
Physical activity (hrs/week)	-0.002	0.9	-0.01	0.6	0.01	0.8	0.5
Nulliparous (yes vs. no)	-0.19	0.5	0.07	0.8	-0.05	0.9	0.6

[a] Change per year of age is not adjusted
[b] Interaction between race and each factor

Table 4.3B Age-adjusted[a] change in umbilical cord blood leukocyte telomere length per change in status of (binary) or per unit change in (continuous) male neonate factors by race, Expanded HUB Study, 2006-2007

	Overall	P	White	P	Black	P	P-interaction[b]
Maternal Factors							
Pre-pregnancy[c]							
Age (years)	0.01	0.8	-0.02	0.8	-0.02	0.6	0.9
Graduate degree (yes vs. no)	0.78	0.02	0.83	0.09	0.38	0.5	0.7
BMI (kg/m^2)	-0.05	0.05	-0.08	0.2	-0.02	0.5	0.4
Exposure to cigarette smoke (yes vs.no)	0.05	0.9	0.32	0.4	-0.30	0.5	0.3
Ever drank alcohol (yes vs. /no)	-0.33	0.4	-0.23	0.7	-0.48	0.4	0.7
Sedentary time (hrs/week)	-0.02	0.4	-0.01	0.7	-0.04	0.2	0.4
Physical activity (hrs/week)	0.01	0.7	0.02	0.5	0.01	0.7	0.8
Nulliparous (yes vs. no)	-0.12	0.7	-0.05	0.9	0.40	0.5	0.5
Pregnancy[d]							
Overall weight gain (lbs)	-0.003	0.8	-0.003	0.8	-0.003	0.8	0.9
Weight gain within guidelines (yes vs. no)	0.38	0.2	0.28	0.4	0.43	0.4	0.8
Sedentary time (hrs/week)	0.004	0.8	-0.001	0.9	0.005	0.8	0.8
Physical activity (hrs/week)	-0.01	0.7	-0.007	0.9	-0.004	0.9	0.9
Neonate Factors							
Birth weight (g)	0.0001	0.9	0.00003	0.9	-0.00002	0.9	0.9
Birth length (in)	0.25	0.1	0.01	0.9	0.27	0.2	0.4
Ponderal index (kg/m^3)	-0.45	0.3	0.13	0.8	-0.74	0.2	0.3
Placental weight (g)	0.001	0.1	0.002	0.03	-0.001	0.4	0.05

[a] Change per year of age is not adjusted
[b] Interaction between race and each factor
[c] Before the current pregnancy as reported on the questionnaire administered during the first trimester.
[d] Pregnancy factors occurred after the first trimester blood sample collection, and thus associations with maternal leukocyte telomere length were not determined.

Table 4.4 Racial differences in mean maternal peripheral blood and male neonate umbilical cord blood leukocyte telomere length, Expanded HUB Study, 2006-2007

	Telomere length									
	Maternal peripheral blood leukocytes					Umbilical cord blood leukocytes				
Adjustment[a]	White	95% CI	Black	95% CI	P	White	95% CI	Black	95% CI	P
No additional adjustment	2.49	(2.19, 2.79)	2.42	(2.05, 2.78)	0.8	2.99	(2.72, 3.27)	2.68	(2.35, 3.02)	0.2
Age	2.65	(2.35, 2.95)	2.18	(1.81, 2.56)	0.08	3.02	(2.73, 3.31)	2.64	(2.27, 3.01)	0.1
Age, education, pre-pregnancy BMI	2.52	(2.20, 2.84)	2.38	(1.97, 2.79)	0.6	2.88	(2.58, 3.19)	2.85	(2.46, 3.25)	0.9

[a]All models adjusted for telomere length assay plate

Table 4.5 Racial differences in unadjusted and adjusted Spearman correlations between maternal peripheral blood and male neonate umbilical cord blood leukocyte telomere length, Expanded HUB Study, 2006-2007

	Overall (N=55 pairs)		White (N=33 pairs)		Black (N=22 pairs)	
Adjustment	*r*	P	*r*	P	*r*	P
Unadjusted	0.63	<0.0001	0.63	<0.0001	0.69	0.0004
Race	0.64	<0.0001				
Age (years)	0.67	<0.0001	0.65	<0.0001	0.62	0.003
Age, Education (graduate degree)	0.65	<0.0001	0.65	<0.0001	0.63	0.003
Age, BMI (kg/m^2)[a]	0.65	<0.0001	0.69	<0.0001	0.60	0.006
Age, Nulliparity	0.67	<0.0001	0.65	<0.0001	0.63	0.003
Age, Physical activity (hrs/week)[a]	0.68	<0.0001	0.65	<0.0001	0.66	0.002
Age, Birth length (in)	0.66	<0.0001	0.64	0.0001	0.73	0.0004
Age, Ponderal index (kg/m^3)	0.67	<0.0001	0.64	0.0001	0.65	0.003

[a] Pre-pregnancy

Table 4.6 Racial differences in Spearman correlations between maternal peripheral blood and umbilical cord blood leukocyte telomere length overall and stratified by maternal and neonate factors, Expanded HUB Study, 2006-2007

	All			White			Black		
Maternal and neonate factors	N	*r*	P	N	*r*	P	N	*r*	P
Overall	55	0.63	<0.0001	33	0.63	0.0001	22	0.69	0.0004
						P-heterogeneity=0.5			
Age									
Younger than 30 years	25	0.57	0.003	9	0.63	0.07	16	0.56	0.02
30 years or older	30	0.64	0.0001	24	0.63	0.001	6	0.37	0.47
P-heterogeneity [a]			0.7						
Education									
Bachelor's degree or less	26	0.63	0.0005	8	0.69	0.06	18	0.57	0.01
Graduate degree	29	0.67	0.0001	25	0.61	0.001	4	1.0	<0.0001
P-heterogeneity [a]			0.8						
Pre-pregnancy BMI									
Normal	39	0.66	<0.0001	29	0.66	0.0001	10	0.79	0.006
Overweight/Obese	16	0.53	0.04	4	0.60	0.4	12	0.49	0.1
P-heterogeneity [a]			0.5						
Gain outside of guidelines	33	0.62	0.0001	18	0.67	0.002	15	0.55	0.03
Gain within guidelines	22	0.74	0.0001	15	0.63	0.01	7	0.86	0.01
P-heterogeneity [a]			0.4						
Parity									
Nulliparous	29	0.49	0.007	20	0.58	0.008	9	0.32	0.4
Parous	26	0.78	<0.0001	13	0.78	0.002	13	0.75	0.003
P-heterogeneity [a]			0.08						
Birth Length									
Less than 20 in	33	0.59	0.0003	17	0.67	0.003	16	0.49	0.05
Equal or greater than 20 in	22	0.74	0.0001	16	0.55	0.03	6	1.0	<0.0001
P-heterogeneity [a]			0.4						

[a] P-heterogeneity additionally stratified by race were not calculated due to small sample sizes

Appendix B: Primary data collection and quality control protocol

For the primary data collection portion of this dissertation, relative telomere length was measured in maternal peripheral blood leukocytes from their first visit at the beginning of pregnancy and corresponding umbilical cord blood leukocytes from their neonates. The samples were from participants in the Expanded Hormones in Umblical Cord Blood (EHUB) Study.

Records from each participant were cross-referenced with the index of stored samples and any male neonate with stored cord blood buffy coat and a stored buffy coat sample from their mother at the beginning of the study was included. Samples were removed from freezer storage, placed in dry ice, and transported to the laboratory of Dr. Meeker and Dr. Heaphy. Samples were placed in the transport box and random and thus should not have been tested in any particular pattern by date.

Cord blood leukocyte DNA was isolated and re-purified to remove potential residual PCR inhibitors using the DNeasy Blood and Tissue kit (Qiagen) and quantitative PCR was used to estimate the ratio of telomeric DNA to that of a single copy β-globin gene. 5 ng of genomic DNA was used in a 25 μL volume for either the telomere or β-globin reactions; each sample was run in triplicate. Each maternal/child pair was tested on the same plate to remove interplate variability. Each 96-well plate contained a no template negative control and two separate 5-point standard curves using leukocyte DNA; these standard curves allowed the PCR efficiency to be determined for each experimental run.

Each plate also included three samples isolated from a series of cell lines with known telomere lengths, ranging from 3-15 kb, as determined independently by telomere terminal restriction fragment analysis. Inclusion of these samples provided an additional quality control check. Maternal-child pairs were run on the same plate to account for plate-to-plate variation and the lab was blinded to sample type, maternal or umbilical cord blood. Samples were re-run if the coefficient of variation (CV) of either the telomere or the β-globin reaction was equal or greater than 5% or either the telomere or the β-globin values fell outside the range of the standard curve. For those samples that were re-run, the mean telomere Ct and β-globin Ct values were compared to the original means to assess validity of the measurements. The means were similar and to keep maternal-child pairs on the same plate, the data from the original run were used. Across all samples, the mean CV was 1.44% and 0.99% for the telomere and β-globin reactions, respectively.

Chapter 5: Conclusion

Purpose of this Dissertation

This dissertation combined three areas of research: fetal origins of adult disease, prostate cancer, and telomere biology. Racial variation in prostate cancer incidence and mortality in the United States is marked, with black men having a 1.56 times higher incidence and a 2.45 times higher mortality rate than white men (1). Despite extensive study, the only known prostate cancer risk factors are age, family history, and race (2), none of which is modifiable. Race is not modifiable but it is possible that factors correlated with race may be modifiable, and thus targets to decrease the racial disparity in this cancer. However, no modifiable risk factors in midlife that explain the disparity have been found. It had been suggested that the possibility of modifiable factors earlier in the life course may be able to explain some of the racial disparity in prostate cancer (3). This is the hypothesis we addressed in this dissertation.

The "Fetal Origins of Adult Disease" is a theory, first proposed by Dr. Barker (4), that exposures in utero may affect a fetus's structure and function, thus influencing health later in life. Our group previously began to address this hypothesis for prostate cancer and observed some racial differences in in utero factors, such as steroid hormones and growth factors (5-7). Also, previous studies have suggested associations between birth weight and prostate cancer (8) especially advanced disease (9-11). In this dissertation, we built on this work to continue to investigate the potential fetal origins of prostate cancer.

Studies of the influence of the in utero environment on later life risk of prostate cancer overall and by race are not feasible to conduct: since the average man is diagnosed with prostate cancer around age 65, one would have to enroll a very large cohort at birth and follow them until the usual age at diagnosis of 60-70 years or older. Thus, it is necessary to have markers of these potential in utero exposures that may be associated with prostate cancer overall and by race. Telomere length could be one such marker. When telomeres shorten past their critical length, chromosomal instability may occur and contribute to carcinogenesis (12, 13). With this biology motivating the work, our group previously found that shorter telomeres in stromal cells in benign prostate tissue is associated with prostate cancer (14), and that greater variation in telomere length among cancer cells coupled with shorter telomere length in stromal cells is associated with prostate cancer death in men with prostate cancer (15). Thus, we proposed that shorter telomeres, along with being a marker of cell proliferation, oxidative stress, and biological aging, may serve as biomarker of cancer risk and prognosis. With respect to the racial disparity in the burden of prostate cancer, this dissertation addressed the hypothesis that racial differences in telomere length and in the associations between exposures in utero and telomere length may help explain some of the racial disparity in prostate cancer, and potentially other adult diseases that show a racial disparity.

Summary of the Key Findings in this Dissertation

This dissertation had 3 specific aims and numerous hypotheses. The results of studies addressing the specific aims are reported in Chapters 2, 3, and 4. In this section, the key findings are summarized and discussed.

Aim 1

In Aim 1, we addressed two questions: 1) whether white blood cell telomere length differs between black and white males at birth (Chapters 2, 4) and midlife (Chapter 3), and 2) whether the correlation between mother and male child telomere length differs by race (Chapter 4). We had several hypotheses. (A) We hypothesized that babies born to black mothers would have shorter telomeres than those born to white mothers due to inherited racial differences in telomere length and/or racial differences in the prevalence or extent of in utero exposures that shorten telomeres. (B) Further, we hypothesized that black middle-aged men would have shorter telomeres than white middle-aged men following a lifetime of racial differences in exposure that shorten telomeres. (C) We also hypothesized that the telomere length difference in midlife would be greater than the difference at birth. (D) Finally, we hypothesized that there would be a positive correlation between newborn's and his mother's telomere length, and that the magnitude of the correlation would be different by race. We addressed hypothesis (A) in two studies. The first study, a cross-sectional study of 38 black and 38 white male neonates (HUB), showed no racial difference in cord blood leukocyte telomere length while the second, a longitudinal study of 22 black and 33 white mothers and their male neonates (EHUB), showed cross-sectionally that black neonates had shorter cord blood leukocyte telomeres than white neonates. While not statistically significant, in EHUB, the magnitude of the racial difference was about 10% and about 14% after adjustment for potential confounders.

While the HUB and EHUB studies included study populations with differing characteristics (e.g., maternal age and birth size), the method of measuring telomere length was the same and was measured in the same laboratory. The geometric mean cord blood telomere length for black (2.72) and white (2.73) neonates in HUB fell between the mean telomere length for black (2.68) and white (2.99) neonates in EHUB suggesting additional validity and comparability of the telomere measurements in HUB and EHUB. There are a few possible reasons for these differences in findings between HUB and EHUB. One is chance due to small sample sizes. No racial difference was observed in HUB and the observed racial difference in EHUB was not statistically significant. At the time we proposed the work, for our sample size we determined a minimum detectable difference in cord blood relative telomere length between black and white neonates of -0.71 in HUB and -0.87 in EHUB, for a two sided test with alpha=0.05 and a power of 80%. We observed black-white differences of +0.01 and -0.31, in HUB and EHUB respectively, which could mean that there is no racial difference in telomere length or the size of the racial difference in telomere length is modest and we did not have sufficient power in either study to detect a more modest racial difference.

A second possible reason is that these populations were recruited from different source populations. In HUB, any eligible delivery at Johns Hopkins Hospital was included versus EHUB, where maternal participants were recruited from the faculty practice at Johns Hopkins Hospital. This practice may consist of more white women of higher socioeconomic status involved in the university and more black women of lower socioeconomic status from the area around the hospital. In the first study, minimal key

information, including age and parity, was collected by delivery room nurses at the Johns Hopkins Hospital once eligibility was confirmed. Both black and white women in EHUB were older and had slightly higher age-adjusted parity than in HUB. It is possible that the women in the two studies also differed on other lifestyle factors like BMI, education, or other unmeasured factors that may be associated with both race and telomere length. Neonates in HUB were also slightly smaller than those in the EHUB, with black babies being about 120 g smaller and white babies being about 70 g smaller, on average.

With regard to hypotheses (B), that black middle-aged men would have shorter peripheral blood leukocyte telomeres than white middle-aged men, and (C) that the difference in mid-life would be greater than at birth, contrary to these hypotheses, we found that geometric mean telomere length was about 3% longer in black men than white men before adjustment, and about 7% longer after adjustment for lifestyle factors. These racial differences in telomere length were not statistically significant. At the time we proposed the work, for our sample size we determined a minimum detectable difference in relative telomere length between black and white men of -0.36 for a two-sided test with alpha=0.05 and a power of 80%. We observed black-white difference of +0.07. Thus, it is possible that there is no racial difference in telomere length in midlife since these differences are not statistically significant but given the magnitude and small sample size, it is also possible that we did not have sufficient power to detect a more modest difference (including one in the direction opposite to our hypothesis). However, our finding of 3-7% longer telomeres in black than white men, while not statistically

significant, is consistent (16-18) with other studies (~5% longer telomere in black than white men).

If we assume that our findings – that telomere length is longer in black men in midlife, but shorter in black neonates at birth (in EHUB) – are true, taken together our findings might imply slower telomere shortening across life in blacks compared to whites. Our speculation is in contrast to the findings of Rewak et al., who reported that black neonates had longer cord blood leukocyte telomeres, but similar telomere length in adulthood between blacks and whites, suggesting a greater difference between birth and adulthood in blacks than whites (19). Our speculation is also in contrast to the theory that catch-up growth, which has been seen to be greater in black infants compared to white and Asian infants (20) might lead to shorter telomeres in black adults since rapid growth has been associated with more DNA damage and oxidative stress (21).

It is possible that longer telomeres at birth lead to faster shortening, but we were not able to assess that in this dissertation since our populations of male neonates and adults are not the same people and from different birth cohorts. Our male neonates are from a more recent source population, being born between 2004-2006 versus our men in midlife who were enrolled roughly 20 years before and were already ages 40-75. That means these men were born anywhere from 6-8 decades before our neonates when there may have been very different pregnancy standards and obstetric recommendations. More mothers may have smoked, entered pregnancy leaner, gained less weight, and/or drank alcohol. These secular patterns may differ by race (e.g., changes in maternal nutritional

sufficiency by race over time). Thus racial differences in telomere length between HUB/EHUB and HPFS may not be directly comparable. More longitudinal studies of the same individuals across decades are warranted (which may not be feasible to perform), or studies with the ability to take cohort effects into consideration.

We tested in EHUB hypothesis D of Aim 1, that there would be a positive correlation between newborn and mother telomere length, given that telomere length is partially inherited (22, 23), and that this correlation would be different by race. Our findings supported the first part of our hypothesis: both black (r=0.69) and white (r=0.63) maternal-child pairs had strong positive telomere length correlations. However, in contrast to our hypothesis, we did not find a racial difference in this correlation. With our data, we could not determine the extent to which the strong mother-child correlation in telomere length is explained by inheritance versus shared environment.

Aim 2

In Aim 2, we assessed cross-sectional associations of child factors with venous umbilical cord white blood cell relative telomere length overall and by race. We had three hypotheses. (A) We hypothesized that in HUB (Chapter 2) and EHUB (Chapter 4) higher birth weight and extreme placental size (smaller and larger) would be associated with shorter telomere length. (B) In addition, in HUB (Chapter 2), we hypothesized that higher umbilical cord blood concentrations of testosterone, estradiol, insulin-like growth factor-1, insulin-like growth factor-2, and leptin, and lower concentrations of sex hormone binding globulin, insulin-like growth factor binding protein-3, and vitamin D would be

associated with shorter telomere length. (C) Finally, we hypothesized that the direction and magnitude of these associations would not differ by race (Chapters 2, 4), but would, in part, account for any racial differences in telomere length observed in Aim 1a because of racial differences in the prevalence of these child factors. With respect to hypothesis (A), we did not observe an association between either birth or placental weight and telomere length in HUB or EHUB. Our findings are consistent with another study on birth size and cord blood telomere length (24).

With respect to hypothesis (B), in HUB we did not find any associations between umbilical cord blood concentrations of hormones or growth factors with telomere length. With respect to hypothesis (C), we did not observe racial differences in the association between birth weight and placental weight with telomere length in HUB but we did see a slight difference by race in EHUB. We saw a small positive, statistically significant association between placental size and telomere length in white neonates but a small inverse association with telomere length in black neonates (p-interaction=0.05). We also saw a similar pattern for ponderal index, an additional measurement for neonate size. Our findings are somewhat consistent with the hypothesis that smaller placental size would lead to shorter telomeres although we did not hypothesize that there would be a racial difference in this association. However, the magnitude of the association placental size and telomere length was very small for both races and thus seems to agree with our conclusions regarding hypothesis (A), that there is no association between birth size and telomere length.

We expected that in utero factors that influence greater proliferation and thus, greater fetal growth and birth weight would be associated with shorter telomeres. In both HUB and EHUB, we found that black neonates were smaller than white neonates. The fact that black neonates were smaller would seem to suggest that black neonates would have longer telomeres than white neonates. Because black and white children do not differ in size on average, black babies are more likely to experience catch up growth (20). Possibly catch up growth (i.e., greater cell proliferation) in blacks could produce shorter telomeres in blacks compared to whites later in life. While in EHUB the racial difference in telomere length that we observed is compatible with hypothesis (A) in Aim 1a, it is not compatible with our observation in HUB and EHUB (and US birth statistics (25)) that black male neonates tend to be smaller than white male neonates. It is possible that we were not able to detect associations between neonate factors and telomere length or racial differences in these associations because the range of birth weight may have been too narrow overall and within each racial group. In HUB, an eligibility criterion was "normal" birth weight range (2500-4000 g). In EHUB, the range of birth weight was broader (2366-4714 g) but only 6 neonates (3 black and 3 white) qualified as "high birth weight" (>4000 g) and one as "low birth weight" (<2500 g). In a supplementary analysis in EHUB, high birth weight (>4000 g) versus normal birth weight was non-significantly inversely associated (β=-0.6, p=0.1) with telomere length, suggesting that our hypothesis could be correct for high birth weight.

The lack of association between child factors and cord blood telomere length may be due to the measurement of the child factors at a time that is not etiologically relevant for the

production of telomere shortening: we measured both child factors and telomere length at the same point in time. We do not have measurements of child factors throughout gestation. Additionally, it is possible that that the greater influence on a neonate's telomere length is maternal factors, as explored in Aim 3, and that child-related factors may not sufficiently reflect maternal factors to detect associations. Finally, it is possible that telomere length is not a marker of the in utero environment.

Aim 3

In Aim 3, we assessed the association of maternal factors, both pre-pregnancy and pregnancy, with male neonate cord blood telomere length in EHUB (Chapter 4). We had 3 hypotheses. (A) We hypothesized that pre-pregnancy obesity and excess weight gain during pregnancy, as well as other maternal characteristics, including higher parity (a risk factor for maternal obesity) would be associated with shorter umbilical cord white blood cell telomere length. (B) Further, we hypothesized that the direction and magnitude of the associations between maternal factors and cord blood telomere length would not differ by race. (C) Finally, we hypothesized that racial differences in the prevalence of the maternal factors would, in part, account for any racial differences in telomere length observed in Aim 1a.

With respect to hypothesis (A), as hypothesized pre-pregnancy BMI was inversely associated, while maternal education was positively associated with umbilical cord blood telomere length. Contrary to our hypotheses, none of the studied pregnancy factors was associated with umbilical cord telomere length, including gestational weight gain. With

respect to hypothesis (B), as hypothesized these two maternal factors were also associated with cord blood telomere length in both blacks and whites. With respect to hypothesis (C), as hypothesized, after adjusting for pre-pregnancy BMI (prevalence of normal BMI: black 41.7%, white, 85.7%) and education (prevalence of graduate degree: black 16.7%, white 77.1%), the racial difference in umbilical cord blood telomere length (mean, age-adjusted: black 2.64, white 2.64, P=0.1; age, BMI, and education adjusted: black 2.85, white 2.88) was eliminated, suggesting that maternal pre-pregnancy BMI and education, a potential proxy for higher socioeconomic status, explain much of the racial difference in cord blood telomere length that we observed in EHUB.

In additional to addressing the stated aims of this dissertation, we addressed several additional questions that inform the links among maternal and child factors, adult factors, telomere length at birth and mid-life, and race. (1) Are demographic and modifiable factors associated with peripheral blood leukocyte telomere length over and by race at midlife in the HPFS (Chapter 3)? (2) Does adjustment for factors from (1) influence the finding of a possibly longer telomere length in black compared to white men at midlife in the HPFS (Chapter 3)? (3) Do black mothers have shorter peripheral blood leukocytes than white mothers in EHUB (Chapter 4)? (4) Are maternal factors associated with maternal peripheral blood leukocyte telomere length overall and by race in EHUB (Chapter 4)?

With respect to (1), we found inverse associations between waist circumference, and suggestive inverse associations of smoking, inactivity, and testosterone with telomere

length overall. We saw similar patterns by race except for testosterone, it's metabolite dihydrotestosterone, and for 1,25(OH)2D, where we saw inverse associations in white men and positive associations in black men. With respect to (2), adjustment for modifiable lifestyle factors did not change the difference in telomere length between black and white men, as expected given that these factors did not differ in prevalence or extent by race in the HPFS.

With respect to (3), we found that, on average, black women had shorter, although not statistically significantly, peripheral blood leukocytes telomeres than white women in EHUB. Thus the racial difference in cord blood leukocyte telomere length we saw in the male neonates mirrored the racial differences in their mothers' peripheral blood leukocyte telomere length (after taking into account mother's age). Our findings of a racial difference in telomere length in the EHUB mothers, who were in their 20s and 30s at the time of blood draw, differs from finding possibly longer telomeres in black than white HPFS men, who were in their 50s to 80s at the time of blood draw. It is possible that the men are more similar in exposures that may cause accelerated telomere shortening than the women or that there were different secular trends that differed by race in these exposures, causing a greater difference in the younger than older population. It has also been seen that longer telomere lengths leads to faster telomere attrition such that members of older populations may have more similar telomere length than members of younger populations (17).

With respect to (4), we saw that maternal age was inversely associated with maternal peripheral blood leukocyte telomere length, which has been shown previously (26), but other maternal factors such as smoking (27, 28), obesity, and physical inactivity (29), which were expected to be inversely association with maternal telomere length, were not associated. Exposure to cigarette smoke (active or passive) seemed to be slightly inversely associated with telomere length among black women (albeit not statistically significant) whereas in white women exposure to cigarette smoke was not associated with telomere length. Also, having a graduate degree was positively associated with telomere length in both black and white women, but the association was stronger among black women; the association was not significant in either black or white women. Adjustment for pre-pregnancy BMI and education did not affect the racial difference in maternal telomere length to the extent that it affected the difference in neonate telomere length but it did attenuate the difference.

Aspects of the Research that Warrant Discussion

Several aspects of the research warrant discussion, including their influence on the inferences that can be drawn. The three studies used in this dissertation, HUB, EHUB, and HPFS were all well-defined and conducted studies. Each study sample (e.g. HUB, EHUB, and race-specific blood sample for HPFS) was specifically designed to examine differences in biomarkers by race. In HUB and EHUB, collection of cord blood (HUB) and enrollment (EHUB) were designed to examine biomarkers in cord blood black versus white neonates whose parents were of the same race. In HPFS, a subset of cohort members selected based on self-reported ancestry, black, white, and Asian, were invited

to provide a second blood sample for the purpose of investigating racial difference in biomarkers associated with prostate cancer risk in midlife (we did not include Asian men for the purposes of our study). While our HUB and EHUB sample sizes are small, similar studies of neonate differences by race have similar sample sizes. Our two cohort studies, EHUB and HPFS, included detailed information on lifestyle factors, and specifically in EHUB, both pre- and during pregnancy lifestyle factors. This wealth of information allowed us to investigate whether these factors may influence and possible account for any racial differences in telomere length.

While we had ample information on and blood from the mothers, aside from race, we had no paternal information. Thus, we could not address the influence of paternal exposures on telomere length or racial differences in telomere length, or the correlation between paternal and cord blood telomere length overall or by race. Previous studies have found that paternal age and telomere length are highly associated with telomere length in their children (30, 31). However, lack of paternal information does not affect the inferences we made regarding maternal influence on cord blood telomere length.

While we had extensive information on modifiable factors, HUB and EHUB did not collect detailed information on maternal stress, which has been studied in relation to cord blood telomere length elsewhere previously (32). In EHUB, the women were asked three questions related to maternal stress: “How do you feel now about being pregnant?,” “How does your husband or partner feel now about you being pregnant?,” and “How often did you feel depressed during the last week?” Few of the women responded

anything other than happiness regarding the pregnancy or almost never depressed and the few others said they were "unsure" about the pregnancy or depressed "some of the time" which were the next options on the list. Because of this lack of variety in response, we were unable to utilize these questions as measures of maternal stress.

HUB and EHUB did not collect detailed information on socioeconomic status (SES). In EHUB, one correlate of SES that was collected and that we investigated in relation to telomere length is maternal education, which was highly positively associated with both race and cord blood telomere length. A prior study observed an interaction between race and SES measures (e.g., poverty) in the association with telomere length, suggesting heterogeneity among races in the effect of socioeconomic factors on telomere length, possibly greater than between races (33). In contrast, in EHUB we did not find a racial difference in the association between education and telomere length. We cannot rule out that the lack of a racial difference in this association is due to insufficient power resulting from imbalanced distribution of education among the white and black women in the study, or that education does not adequately capture SES. In the HPFS, all of the men have graduate degrees, and thus, are likely to be of similar SES irrespective of race. While we could not evaluate the association between SES and telomere length and whether SES or race has the greater influence on telomere length, the SES homogeneity in the HPFS allowed us to investigate racial differences in telomere length unconfounded by SES.

Several aspects of the measurement of telomere length warrant discussion. The outcome for all our analyses was relative telomere length. To measure relative telomere length, our group used quantitative PCR (qPCR) to estimate the ratio of telomeric DNA to that of a single copy gene (β-globin) (34). This ratio is often referred to as the T/S ratio and is a unitless measurement. QPCR is used by our group due to our laboratory's expertise in the method and also due to the lower amount of DNA required compared to other methods and higher throughput, allowing for measuring in triplicate, one of our quality control measures. Telomere length can also be measured using Southern blotting, a method requiring larger amounts of DNA and more rarely used recently, which when samples with a known number of telomeric repeats are included, yields an estimate of telomere length in kilobases. Many studies referenced in this dissertation measured telomere length use Southern blotting, making direct comparison of the results among studies more difficult. However, the measurements of telomere length produced by qPCR and Southern blot are highly correlated (34), and so inferences about differences in telomere length between racial groups, for example, should be similar.

Among studies using qPCR to measure relative telomere length, the range of T/S ratios within a study has varied considerably across studies: in adults ages 19-37 years, one study found a range of 2-3 (16), another in adults ages 45-85 years found a range of 0.6-1.1 (35), and another in nationally representative sample of adults ages 20-85 years found an overall mean of about 1.0 (18). Each study had slightly more women than men who have been who seen to have longer telomeres than men. A study of umbilical cord blood found mean relative telomere length to be between 1.5-2 across sex and race (36). In our

three studies, relative telomere length had a range of 0.6-4.9 (geometric mean 1.49) in adult men (HPFS), 0.9-4.9 (arithmetic mean 2.71) in adult women (EHUB), 1.3-6.9 (geometric mean 2.90) in our first study of male neonates (HUB), and 0.8-5.5 (arithmetic mean 2.91) in our second study of male neonates (EHUB). Of the studies that provided relative telomere length ranges, our ranges are a bit wider and our means are higher than the study of older adults (35), and the study with ages ranging across all of adulthood (18). Our adult women had a similar mean T/S ratio to adults of a similar age and our cord blood means are similar to the other neonate study (36).

The shape of the distribution of relative telomere length differed among our studies despite having used the same measurement method in the same laboratory (Figure 5.1). In HPFS and HUB, the distributions were right-skewed, and thus in the analysis, we natural logarithm transformed relative telomere length and calculated geometric means. In EHUB, maternal and neonate telomere length distributions appeared to be normal, and thus we calculated arithmetic means. While none of our results for racial difference in relative telomere length was statistically significantly different, telomere length appeared to be slightly longer in black men in HPFS and neonates in HUB than whites, whereas in EHUB, telomere length appeared to be slightly shorter in black women and neonates than in whites. It is possible, given our small sample sizes, that these racial differences are an artifact of a few participants with long telomeres in one racial group. We performed sensitivity analyses dropping these potentially influential points and the conclusions did not change. Nevertheless, we cannot rule out that our findings are due to chance alone,

given that our studies were powered to detect moderate or larger racial differences in telomere length.

We noted qPCR plate-to-plate variation in the measurement of telomere length in EHUB, and thus, adjusted for plate in all analyses except those for mother-neonate pairs, as they were run on the same plate. Although the samples were plated randomly with respect to race, adjusting for plate slightly attenuated the racial difference in telomere length in EHUB. We did not adjust for plate in HUB and HPFS because plate information was not available. However, this further adjustment may have further reduced the difference in telomere length in the other studies. It is also possible that the differences were attenuated due to adjustment with small sample sizes which would also have been an issue in the other two studies as well. Overall, as long as plating was random with respect to race, as was the case with EHUB, there should not have been differences in interpretation and we can speculate that we still would have seen no difference. Another measurement difference among our studies is the year of telomere length measurement: HPFS and HUB both in 2009 and EHUB in 2014. Over the 6 years, the validity and/or precision of the method could have changed due small refinements in the assay protocol, changes in personnel and/or increase in the experience of personnel who performed the assay, and small technical issues with laboratory equipment, but again, the ranges and precision are similar so our T/S ratio distributions seem to indicate similar testing and quality across studies.

The quality of the DNA, and thus qPCR efficiency, may have varied across our three studies. The initial HPFS DNA extraction, which was performed elsewhere, did not yield DNA that was sufficiently pure for qPCR. Our group re-extracted the DNA. Some of the EHUB buffy coat aliquots contained a high percentage of red cells and required an additional round of purification. These possible differences in DNA quality could have led to non-differential error in the measurement of telomere length, but we do not expect that the extent of the error would have differed by race (samples were randomly ordered). In our study, we used leukocyte telomere length as a surrogate for tissue telomere length, and in the context of our particular research question, prostate tissue. While telomere length appears to be similar among leukocytes within an individual (37), smaller positive correlations between leukocyte and some tissue telomere lengths (e.g., brain, skin, fat, etc.) have been found in adults (38). In neonates, higher positive correlations have been found between foreskin tissue, cord blood, and leukocyte telomere length (39). No studies have been published on the correlation between prostate tissue and leukocyte telomere length although our group did find there to be no correlation between benign prostate tissue or prostate cancer cell and leukocyte telomere length (40). Thus, while not based on the prostate, it is possible that leukocyte telomere length may better reflect tissue telomere length earlier than later in life.

Finally, while we did not study people with cancer, our hypotheses were based on the associations between shorter telomeres and cancer. However, there may be differences in leukocyte telomeres leading up to the time of cancer diagnosis. A recent study found that while age-related telomere shortening was faster in people who eventually developed

cancer, this shortening decreased closer to the time of diagnosis, especially among those with prostate cancer, showing those who developed cancer to have longer telomeres than those who remained cancer free, possibly due to reactivation of telomerase (41). This could explain discrepancies in studies looking at the association between telomere length and cancer that find longer telomeres to be associated with cancer like the recent study by Julin et al. and members of our group (42). It is possible that longer telomeres in black men in HPFS compared to white do actually reflect a higher risk of cancer if they were older and closer to time of diagnosis which we cannot determine based on our study. Our findings do not imply that telomere length is not a good measure of DNA damage or oxidative stress or that is not on the pathway to carcinogenesis, our findings just do not provide additional insight into racial differences in prostate cancer risk. Our findings from EHUB do suggest that there is a maternal influence on neonate telomere length and this could, in turn, provide insight into future health risk and racial differences based on maternal modifiable factors. This possibility warrants future exploration.

Major Conclusions

While modifiable factors may be associated with telomere length in midlife, and maternal modifiable factors may be associated with telomere length in their male neonates, in this dissertation, we conclude that racial differences in telomere length do not explain the racial disparity in prostate cancer (or other diseases). We saw that adjustment for the modifiable factors that differed by race, pre-pregnancy BMI and education, a correlate of SES, eliminated the difference in telomere length between black and white neonates and to a lesser extent, the difference in telomere length between the black and white mothers

in EHUB. We speculate that telomere length may differ by race at birth and at midlife, but this racial difference is explained by racial differences in exposure to these modifiable factors. Unlike in EHUB, in HUB, we did not see a racial difference in telomere length. It is possible that the black and white women in HUB were more similar on modifiable factors that influence telomere length (but we did not have information on the factors that we found eliminated the racial difference in telomere length in EHUB to confirm), that there truly is no racial difference in telomere length in the HUB study population, or that we simply could not detect a racial difference in HUB due to sample size constraints (although the sample size is not dissimilar to EHUB). In HPFS, in contrast, there were no racial differences in adiposity or other modifiable factors that influence telomere length, and by design, no difference in SES and so possibly, with all of these factors being more similar, we saw a smaller difference than in the adult women. We also saw a difference in the opposite direction which may be due to other unmeasured confounders that when adjusted for, may have eliminated the small difference we saw between the black and white men.

In EHUB, we also saw that maternal and neonate telomere lengths are equally highly correlated in both blacks and whites, supporting the heritability of telomere length. However, we also found evidence that maternal modifiable factors influence cord blood telomere length, and that racial difference in the prevalence or extent of those factors may lead to racial difference in telomere length at birth. While we cannot make any definitive conclusions regarding explanations for the racial disparity in prostate cancer based on telomere length at birth, it seems plausible that if all mothers, irrespective of race, had

equal health promoting characteristics, then maybe black and white babies would start life with similarly long telomeres and thus, some racial disparities in health, possibly including prostate cancer, might be reduced.

Public Health Significance

Prostate cancer is the number one cancer among men in the United States and the racial disparity is pronounced (1). It is therefore important to identify modifiable factors that may underlie these disparities, so that targeted prevention strategies can be developed. In this dissertation we combined three research areas – fetal origins of adult disease, prostate cancer, and telomere biology – to develop one comprehensive hypothesis regarding the effects of fetal programming on future risk of prostate cancer that may help develop prevention strategies.

One key finding from this dissertation is potentially actionable. In one of our studies, we saw that maternal pre-pregnancy BMI and education were associated with both race and umbilical cord blood telomere length. In the analysis adjusted for maternal age, we saw that black neonates had shorter telomeres than white neonates but after additional adjustment for maternal BMI and education, the racial difference in telomere length disappeared. If neonate telomere length is the starting point for lifetime health, targeting these modifiable factors, maternal BMI and education, or what is possibly a proxy for other SES factors, could allow neonates to start life at the same point in terms of health as measured via telomere length and perhaps reduce the racial disparities seen not only in prostate cancer, but other adult diseases with marked racial disparities as well.

Future Research Directions

Finding a marker of fetal origins of adult disease is a necessity if it is to become a practical area of research. While not all of our studies showed differences in telomere length by race, our work adds to the field of telomere biology, specifically its usefulness as a measure of maternal influence on fetal health. We saw expected patterns, based on previous work, in modifiable factors in midlife, so the patterns we saw with regards to maternal and neonate factors and umbilical cord blood leukocyte telomere length seem informative. To explore whether or not these patterns and associations translate into causation of telomere shortening, and specifically whether they capture the cumulative in utero experience require further studies with larger sample sizes. The minimum detectable racial differences in telomere length that we estimated during the planning of our three studies were greater than our observed differences and thus we may have been underpowered to detect a true difference by race or the true effect of different midlife, maternal, and neonate factors. Future studies should also include multiple levels of exposures such as more detailed SES data. Race is both a genetic concept as well as a construct, so parsing out those two aspects is essential. It could also be useful to include genetic categories of "race" beyond self-reported race. Another addition to this research, to fully incorporate the life course, would be utilize existing birth cohorts or create new, more detailed birth cohorts, for longer follow-up and to assess longitudinal changes in telomere length instead of looking at separate cross-sectional studies. Future studies could also include pre-pregnancy and/or pregnancy interventions to assess if telomere length at birth is affected. While it is clear that pre-pregnancy and pregnancy health

interventions would be good for health overall, looking at changes in telomere length along with overall health could advance this area substantially.

Chapter 5 References

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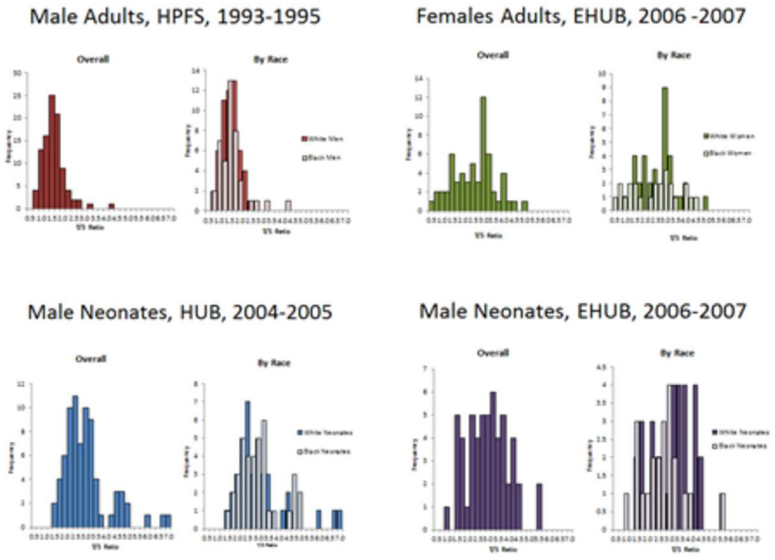
Chapter 5 Figures

Figure 5.1 Relative telomere length (T/S Ratio) distributions



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PRESENTATIONS

Weber, KA, Heaphy, CM, Giovannucci, E, Platz, EA, Meeker, AK. Midlife Racial Differences in Leukocyte Telomere Length and in Associations Between Modifiable Factors and Telomere Length. Presented at the Delta Omega Poster Competition, Baltimore, MD, 2015 and AACR Annual Meeting, Philadelphia, PA, 2015.

Weber, KA, Heaphy, CM, Rohrmann, S, Bienstock, JL, Monsegue, DD, Agurs-Collins, T, Platz, EA, Meeker, AK. Influence of In Utero Maternal and Child Factors on Cord Blood Leukocyte Telomere Length and Possible Differences by Race: Clues to The Racial Disparity in Prostate Cancer. Presented at the conference for Frontiers in Cancer Prevention Research, National Harbor, MD, 2013.