ATTACHMENT AND BIOBEHAVIORAL CATCH-UP PROTECTS TELOMERE LENGTH IN CHILDREN ADOPTED INTERNATIONALLY: RESULTS OF A RANDOMIZED CLINICAL TRIAL

by

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ABSTRACT

In a randomized clinical trial, we assessed whether a brief parenting intervention reduced telomere loss among children adopted internationally. Children (around 2 years old) adopted into families in the United States were randomly assigned to one of two intervention conditions. The parents of children assigned to the experimental intervention received Attachment and Biobehavioral Catch-up, a 10-session intervention targeting parental nurturance and sensitivity; parents of children in the control intervention condition received an intervention of the same duration, length, and frequency that targeted cognitive and physical development. A third non-randomized group was included with no intervention component. When assessed at about 5 years of age, children whose parents received the experimental intervention had longer telomeres (T/S ratio) than children in the other two groups. These results highlight the power of a brief intervention in remediating biological effects of early deprivation and indicate that experimental manipulation of telomere length is possible.
Chapter 1

INTRODUCTION

Early adversity in childhood is associated with a myriad of negative physical and mental health outcomes (Cicchetti & Lynch, 1993; Melchior, Moffitt, Milne, Poulton, & Caspi, 2007; Repetti, Taylor, & Seeman, 2002; Shonkoff et al. 2012). Biological mechanisms serve as a pathway through which early adverse experiences lead to negative outcomes, such as increased risk for tobacco dependence, drug or alcohol dependence, cardiovascular disease, immune function, PTSD, depression, anxiety, dissociation, and suicidal and self-injurious behavior (Cicchetti & Lynch, 1993; Damjanovic et al., 2007; Giudice, Ellis, & Shirtcliff, 2011; McEwen & Stellar, 1993; Repetti et al., 2002; Simon et al., 2006; Taylor et al., 1997). One plausible mechanism for this effect is telomere shortening, or the loss of nucleotide repeats that protect DNA against genomic damage (Tomiyama et al., 2012). Childhood adverse experiences, such as orphanage care, maltreatment, and witnessing violence, have also been shown to be associated with shorter telomeres (Drury et al., 2011; Drury et al., 2014; Price, Kao, Burgers, Carpenter, & Tyrka, 2013; Shalev et al., 2012; Tyrka et al., 2010). Most previous studies have involved correlational designs and are unable to address questions of causality. The present study used a randomized clinical trial to investigate whether intervening to modify parental sensitivity could protect telomeres among children adopted following early adversity.

Within orphanage care, children experience varying levels and types of deprivation, including deprivation of physical and basic needs (i.e. malnutrition and lack
of medical care), inadequate environmental stimulation, and insufficient social stimulation (Gunnar, Bruce, & Grotevant, 2000). Among children adopted internationally, these experiences are related to delays in physical growth, cognitive functioning, and social development (Gunnar et al., 2000; Nelson et al., 2007; O’Connor, Rutter, Beckett, Keaveney & Kreppner, 2000). Drury et al. (2012) investigated the effects of severe psychosocial deprivation on telomere length in Romanian children who had lived in institutional care. Children who spent a larger percentage of their early life in institutional care were found to have shorter telomeres in middle childhood than children who spent less time in institutional care (Drury et al., 2011). Upon adoption, children enter comparatively enriching environments with committed primary caregivers (Zeanah et al., 2003). The development of behavioral and biological regulatory capabilities of adopted children is dependent on the availability of nurturing and sensitive caregiving. Telomere length serves as an example of biological regulation, and has been associated with sensitive caregiving in populations who have experienced other types of childhood adversity (Asok et al., 2013). In the current study, we propose that an intervention targeting parental behaviors associated with biological buffering, such as nurturance and sensitivity, will be associated with telomere length in this population.

1.1 Telomere Length

Telomeres are repetitive TTAGGG nucleotide sequences that are located at the ends of chromosomal DNA. During DNA replication, an enzyme, DNA polymerase, copies the strand of DNA from the 5’ to 3’ direction, resulting in an ‘end replication
problem’ wherein small bits of DNA (50-100 base pairs) are lost each time the strand is copied. Thus, telomeres do not hold genetic information, yet they serve an important role by protecting against deterioration of meaningful, gene-encoding DNA (Blackburn, 2000). In certain cell types (i.e., germ cells and stem cells), telomeres are replenished by telomerase. However, telomerase is not present in many types of somatic cells, and the deterioration of the telomere eventually triggers cell senescence. Thus, telomere length is often used as a measure of cellular aging (Blackburn, 2001). In addition to this normative process, environmental factors may also lead to accelerated telomere attrition, (i.e. loss of telomere length over time). Oxidative stress (Zglinicki, 2002), cigarette smoking (Valdes et al., 2005), and radiation (Derradji et al., 2008) all influence the cellular environment and are associated with reduced telomere length. In later life, shorter telomeres are associated with a variety of physical diseases and mental health disorders, including cardiovascular disease, stroke, cancer, bipolar disorder, major depressive disorder, and schizophrenia (Price et al., 2013). As such, telomere length may play an important role in the biological embedding of environmental stress.

1.2 Telomere Length and Early Adversity

Numerous studies have revealed associations between different forms of childhood adversity and reduced telomere length. Retrospective reports of childhood maltreatment and adverse life events have been associated with shorter telomere length in adulthood in healthy adults, adults experiencing the stress of caregiving, and adults with an anxiety disorder (Tyrka et al., 2011; Kananen et al., 2010; Kiecolt-Glaser et al., 2011).
In one study, the difference in telomere length between adults who had and had not experienced childhood adversity was theorized to result into a 7-15 year difference in lifespan (Kiecolt-Glaser et al., 2011).

Several studies have found that the association between early adversity and telomere length begins in childhood. As previously reviewed, telomere length is associated with duration of orphanage care (Drury et al., 2011). In addition, Shalev and colleagues (2012) investigated the association between rate of telomere loss over time and exposure to violence in childhood (i.e., physical maltreatment, bullying, and witnessing domestic violence). Children who experienced multiple forms of violence exhibited an accelerated rate of telomere loss over children who had experienced one or no forms of violence during childhood (Shalev et al., 2012). Likewise, children who experienced high levels of family instability (i.e. witnessing violence, incarceration or suicide of a family member) had shorter telomeres than children who did not experience family instability (Drury et al. 2014). Asok et al. (2013) found that children identified by Child Protective Services as being at risk for neglect had shorter telomeres than a comparative low-risk sample.

Gender differences have emerged in studies of early adversity and telomere length. Drury et al. (2012) found that the relationship between telomere length and timing of orphanage care differed between genders. For females, percentage of time in institutional care at a baseline assessment (between 6 and 30 months) was predictive of telomere length in middle childhood percentage of time in institutional care at 54 months of age. For males, the converse was true; percentage of time in institutional care at a
baseline assessment was not predictive of telomere length, but percentage of time at 54 months of age was predictive of telomere length in middle childhood. In a study of high-risk children, Drury et al. (2014) found that females who witnessed violence had shorted telomeres than those who did not witness violence. However, there was no association between witnessing violence, or other forms of family instability, and telomere length in males. Gender differences in the association between telomere length and early adversity are also seen in animal models. Asok et al. (2014) examined telomere length in various brain regions of adult rats that experienced various care conditions (normative, foster-care, and maltreatment) during infancy. In adulthood, care condition was not associated with telomere length in adult males. However, telomere length was more flexible in adult females; females that experienced nurturing care had longer telomeres in the medial prefrontal cortex (mPFC) than females experiencing normal care or maltreatment. Female rats that experienced maltreatment care had longer telomeres in the amygdala than littermates that experienced normal care (Asok et al., 2014). Considered together, these studies suggest that telomere length may be differentially related with early adversity in males and females.

1.3 Potential Protective Mechanisms of Telomere Length

Various studies have reported factors that are associated with longer telomere length. Responsive parenting is not associated with telomere length in a sample of low-risk children (Asok et al., 2013). However, in a population of CPS-referred children, responsive parenting was directly associated with telomere length, such that children who
experienced responsive parenting had longer telomeres than children who did not receive responsive parenting, suggesting that responsive parenting may buffer high-risk children from the biological embedding of stress (Asok et al., 2013). In adulthood, longer telomeres have been associated with healthier diet, higher rates of physical activity, and lower body mass index (Lin, Epel. & Blackburn, 2012; Mirabello et al., 2009; Paul, 2011).

1.4 Intervention Effects on Telomere Length

Due to the important role of telomere length in future disease states, interventions that prevent telomere loss are exciting possibilities, but to date, most interventions have not been effective. One such intervention targeted positive physical health behaviors associated with longer telomere length. Adult participants were randomly assigned to receive a 6-month physical activity trial. Whereas telomere length did not differ between the intervention and treatment as usual (TAU) control group, change in telomere length was negatively associated with reduced sitting time (Sjögren et al. 2014). Given previous literature supporting an association between cortisol levels and telomere length (Choi, Fauce, & Effros, 2008; Epel et al. 2006; Kroenke et al. 2011; Tomiyama et al. 2012), Carlson et al. (2014) proposed that interventions associated with cortisol regulation in a sample of breast cancer survivors (mindfulness-based cancer recovery and supportive-expressive group therapy) would also be associated with telomere length. However, participants who received 10 weeks of mindfulness-based cancer recovery or a minimal-intervention control condition (supportive-expressive group therapy) did not experience
significantly different telomere loss from the no-intervention control group (Carlson et al., 2014). Taken together, interventions conducted with adults have been ineffective in altering telomere length.

Whereas interventions conducted with adults have not altered telomeres, intervening at an earlier age may be more efficacious because early childhood is a period of rapid change in telomere length (Zeichner et al., 1999). Parental factors, such as responsive parenting, are positively associated with telomere length among children who experience early adversity (Asok et al., 2013; Drury et al., 2014). Responsive parenting is associated with a variety of biological indicators of chronic stress in their children, including diurnal and stress-reactive cortisol, blood pressure, body mass index (BMI), and catecholamines (Albers, Riksen-Walraven, Sweep & de Weerth, 2008; Evans, Kim, Ting, Tesher, & Shannis, 2007; Gunnar, Broderson, Nachmias, Buss, & Rigatuso, 1996). Thus, interventions geared towards enhancing parenting, such as Attachment and Biobehavioral Catch-up, may be associated with telomere protection.

1.5 Attachment and Biobehavioral Catch-up

The Attachment and Biobehavioral Catch-up (ABC) intervention was designed to target parents’ nurturance (i.e., responding to their child’s distress) and responsiveness (i.e., following their child’s lead) through 10 sessions implemented in families’ homes. Through randomized clinical trials, the ABC intervention has been found to enhance parenting as well as to improve children’s ability to regulate behavior and physiology (Bernard, Dozier, Bick, & Carlson, 2012; Bernard, Dozier, Bick & Gordon, 2014; Bick &
Dozier, 2013; Dozier, Peloso, Lewis, Laurenceau & Levine, 2008; Lewis, Dozier, Ackerman & Sepulveda-Kozakowski, 2007; Lind, Bernard, Ross & Dozier, 2014). Of note, in a randomized clinical trial, the ABC intervention was shown to normalize another biological indicator of stress, diurnal patterns of cortisol production in neglected children (Bernard et al., 2014). These effects were maintained three years after children completed the intervention (Bernard, Hostinar, & Dozier, in press). Given the well-documented changes seen in children’s various regulatory capabilities following ABC, we propose that the intervention should also alter telomere length.
Chapter 2

METHOD

2.1 Participants

A total of 64 children were enrolled as participants. Of the 64, 49 children were recruited for a randomized clinical trial assessing the efficacy of an attachment-based parenting intervention for internationally adopted children and 15 were recruited separately for the current study and did not receive an intervention. The 49 children involved in the longitudinal study were randomly assigned to the experimental intervention condition in which parents received the Attachment and Biobehavioral Catch-up intervention (ABC; \( n = 28 \)) or the treatment control condition in which parents received the Developmental Education for Families intervention (DEF; \( n = 21 \)). The quality (i.e., A260/280 ratio) and quantity (i.e., ng/uL) of DNA extracted from 7 of the children was insufficient for telomere measurement by qPCR and these samples were excluded. Removing these participants resulted in a final sample of 57 children, with 24 children in the ABC group, 18 children in the DEF group, and 15 children in the non-randomized, no intervention group.

2.2 Procedure

For the randomized clinical trial, internationally adopted children were recruited through local adoption support agencies when the adoption process was complete. As soon as possible after children were adopted, families consented to participate in the longitudinal study. Each family was randomly assigned to receive the ABC or DEF
intervention and received weekly intervention sessions in their homes over a 10-week period. On average, families completed the intervention when the child was 27.95 months old ($SD = 9.81$). After completing the intervention, parents participated in semi-annual research visits. At the visit closest to the child’s 5th birthday, parents consented and children assented to telomere collection.

A separate, non-randomized sample was recruited through local adoption support agencies and online groups for internationally adopting parents. Research staff communicated with parents by phone or email to describe the protocol. Parents completed questionnaires about demographic information (i.e., parent and children’s age, race/ethnicity, etc.) and children’s pre-adoptive history.

For telomere sampling, a trained research assistant collected buccal cell samples by rubbing an Isohelix SK-1 buccal swab (Boca Scientific, Boca Raton, FL) on each cheek of a child’s mouth for 20 seconds per side. Buccal swabs were temporarily stored in a small cooler with ice packs during transport to the laboratory. For families that lived more than 100 miles from the laboratory, parents completed buccal cell sampling following the same procedures. Parents stored buccal swabs with DriCapsules (Boca Scientific, Boca Raton, FL), a compound that stabilizes DNA, and shipped samples overnight to the laboratory. Samples were immediately stored at -20°C upon arrival to the laboratory and then transferred to -80°C for long-term storage. Parents received compensation for their participation.
2.3 Interventions

Attachment and Biobehavioral Catch-up (ABC) consists of 10 hour-long sessions delivered once per week in families’ homes. The intervention was designed to enhance children’s attachment security and regulatory capabilities by improving parents’ nurturance (i.e. responsiveness to distressed child) and sensitivity (i.e. tendency to follow the child’s lead). During ABC sessions, the parent coach used several strategies to enhance these target behaviors. Specifically, parent coaches presented a rationale for parental nurturance and sensitivity based on research, used structured activities to provide opportunities for parents to practice the target behaviors, and used video feedback to highlight parent behaviors that were consistent or inconsistent with the ABC targets. Most importantly, ABC parent coaches commented on parent-child interactions during sessions. This “in the moment” feedback served to highlight target behaviors (i.e., nurturance and following the lead). Through previous randomized clinical trials, the ABC intervention has been shown effective in enhancing parent sensitivity, and in increasing the likelihood that children have secure attachments to parents, normative levels of cortisol production, and adequate executive functioning (Bernard et al., 2012; Bernard et al., 2014; Bick & Dozier, 2013; Dozier et al., 2008; Lewis et al., 2007; Lind et al., 2014).

Developmental Education for Families (DEF), the control condition, was matched to the ABC intervention in duration, frequency, and structure. Rather than focusing on enhancing parenting, the DEF intervention targeted motor and language development.
2.4 Relative Telomere Length

Relative telomere length was measured following procedures used by Asok et al. (Asok et al., 2013). The researcher conducting telomere assays was blind to sample conditions. DNA was extracted from buccal swabs via the Gentra Puregene Buccal Cell Kit (Qiagen, Valencia, CA). For telomere length analysis, the participant’s DNA was first quantified and assessed for purity via nanodrop spectrophotometry. Next, aliquots containing 10ng/µL of participant’s DNA were prepared. Finally, relative telomere length was measured via quantitative PCR (qPCR) (Cawthon, 2012; O’Callaghan & Fenech, 2011) on a Bio-rad CFX96 real-time PCR system (Bio-rad, Hercules, CA). The telomere (T) and single copy gene (S), acidic ribosomal phosphoprotein P0 (36B4), PCR reactions were conducted on separate 96-well plates, but in the same well positions. Reaction wells contained a 1x concentration of Power Sybr Green Master Mix (Life Technologies, Grand Island, NY), 20ng of participant’s DNA, and primers at a final concentration of 100nM for both telomeres (TelF 5’-CGGTTTGGTTTGGTTTGTTTGGTTTGGTTTGGTTTGGTTTGGTT-3’, TelR 5’-GGCTTGCGCTTACCCCTACCCCTACCCCTACCCCTACCCCTACCCCTACCT-3’) and 36B4 (36B4F 5’-CAGCAAGTGGGAAGGTGTAATCC-3’, 36B4R 5’-CCCATTCTATCATCAACGGG-TACAA-3’). Each plate contained a DNA standard isolated from the same cell type (buccal cells) spanning a 2-fold range between 2.5ng – 80ng. For each child, the qPCR amplification of the telomere (T) was compared to the amplification of a single copy gene (S) in order to compute a relative measure of telomere length, or T/S ratio. Relative telomere length was calculated by the formula: T/S = (2\(^{\Delta Ct \_tel}\))/(2\(^{\Delta Ct \_36B4}\)). Higher T/S
ratios indicate longer telomeres. Any triplicate that deviated by more than 1 cycle threshold (Ct) was excluded from the calculation of the sample average for both telomere and 36B4 assays. In these cases (< 2.74% of all triplicates) the remaining two replicates were used for the sample average. The inter-assay coefficient of variation was 0.92% for telomeres and 0.60% for 36B4. The intra-assay coefficient of variation for the individual triplicate samples analyzed was 0.92% for telomeres and 0.64% for 36B4.
Chapter 3

RESULTS

3.1 Preliminary Analyses

Preliminary analyses were conducted to examine differences in demographic variables between children who received each intervention (ABC, DEF) and the non-randomized group (NR) (Table 1). The proportion of boys and girls in each group did not differ, \( \chi^2 (2) = 1.06, p = .59 \), nor did the groups differ significantly in the number of months children had lived in orphanage care prior to adoption, \( F (2, 54) = 2.65, p = .08 \). Children were adopted from various countries in Asia (n = 35), Eastern Europe (n = 11), Africa (n = 8) and Central America (n = 3).

The proportion of children from each region did not differ between groups, \( \chi^2 (6) = 1.75, p = .94 \). However, the groups differed in child age at time of buccal cell collection, \( F (2, 54) = 5.17, p < .001 \). On average, NR children were older at time of telomere collection than children in the ABC, \( p = .01 \) and DEF groups, \( p = .01 \). Differences between ABC and DEF groups did not approach significance, \( p = .82 \). In light of this difference, we controlled for child age at time of telomere collection in primary analyses.

Associations between variables of interest and potential confounds were also examined. Telomere length was not correlated with months in orphanage care, \( r (57) = -.14, p = .29 \) or child age at sample collection, \( r (57) = -.10, p = .45 \). In addition, telomere length did not differ between region of adoption, \( F (3, 53) = 1.66, p = .19 \). Months in orphanage care prior to adoption was significantly associated with the age of telomere
collection, such that children who were older at the time of sample collection spent longer amounts of time in orphanage care than children who were younger, $r (57) = .59, p < .001$. Region of adoption was also associated with months in orphanage care, $F (3, 53) = 6.32, p = .001$, with children adopted from Eastern European countries spending longer amounts of time in orphanage care than children adopted from other regions. Region of adoption was not associated with age of telomere collection, $F (3, 53) = .23, p = .87$.

Males and females did not differ in telomere length, $F (1, 56) = 1.63, p = .21$, months in orphanage care, $F (1, 56) = .01, p = .91$, age at telomere collection, $F (1, 56) = .33, p = .57$, or region of adoption, $\chi^2 (6) = 5.41, p = .14$.

### 3.2 Primary Analyses

Of primary interest, analyses of co-variance (ANCOVA) were conducted to assess whether children in the ABC group had longer telomeres than children in the DEF and non-randomized group. Gender was included as an independent variable because it has proven to be important in previous investigations of telomere length in both human and non-human studies (Asok et al., 2014; Drury et al., 2011; Drury et al., 2014; Zhu et al., 2011). Data were analyzed as a 3 X 2 analysis of co-variance, with Group (ABC/DEF/Non-randomized) and Gender as independent variables, T/S ratio as the dependent variable, and age at telomere collection as a covariate. As hypothesized, a main effect for Group emerged, $F (2, 50) = 4.57, p = .02$. Fisher’s Least Significant Differences Test indicated that the ABC group had longer telomeres than both the DEF group, $p = .03$, and the non-randomized group, $p = .01$. The difference of primary interest
between the ABC and DEF groups represents a medium effect size, \( d = 0.58 \). The DEF and non-randomized groups did not differ from one another, \( p = .34 \) (see Figure 1 for group means). There was no main effect of Gender, \( F(2, 50) = 1.37, p = .25 \) or interaction effect of Gender by Group, \( F(2, 50) = 1.09, p = .25 \).

Subsequent analyses included the amount of time in orphanage care as an additional covariate, because previous research has shown that longer time spent in orphanage care is associated with shorter telomere length (Drury et al., 2011). A 3 (Group: ABC/DEF/Non-randomized) X 2 (Gender) analysis of covariance was conducted, with age at telomere collection and months spent in orphanage care included as covariates, and T/S ratio as the dependent variable. Again, a main effect for Group was present, \( F (2, 49) = 4.56, p = .02 \). There was no main effect of Gender, \( F (2, 50) = 1.38, p = .25 \) or interaction of Gender by Group, \( F(2, 50) = .88, p = .25 \).
Table 1: Demographic statistics ** $p < .01$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Randomized- ABC</th>
<th>Randomized- DEF</th>
<th>Non-Randomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>%</td>
<td>$n$</td>
<td>%</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>58.3</td>
<td>8</td>
<td>44.4</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>41.7</td>
<td>10</td>
<td>55.6</td>
</tr>
<tr>
<td>Region of Adoption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>15</td>
<td>62.5%</td>
<td>11</td>
<td>61.1%</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>4</td>
<td>16.7%</td>
<td>4</td>
<td>22.2%</td>
</tr>
<tr>
<td>Africa</td>
<td>3</td>
<td>12.5%</td>
<td>3</td>
<td>16.7%</td>
</tr>
<tr>
<td>Central America</td>
<td>2</td>
<td>8.3%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Months in orphanage care</td>
<td>Mean (SD)</td>
<td>Min - Max</td>
<td>Mean (SD)</td>
<td>Min - Max</td>
</tr>
<tr>
<td></td>
<td>13.21 (10.07)</td>
<td>0 - 35</td>
<td>9.39 (8.32)</td>
<td>0 - 24</td>
</tr>
<tr>
<td>Age (months)</td>
<td>Mean (SD)</td>
<td>Min - Max</td>
<td>Mean (SD)</td>
<td>Min - Max</td>
</tr>
<tr>
<td></td>
<td>64.67 (5.84)</td>
<td>59.96 – 80.99</td>
<td>64.16 (4.20)</td>
<td>60.29 – 72.74</td>
</tr>
</tbody>
</table>
Figure 1: Relative telomere length in children who were randomly assigned to intervention condition (i.e. ABC or DEF) and a non-randomized control sample. Error bars indicate standard errors of the mean. *p < .05, **p <.01
Chapter 4

DISCUSSION

The current study provides experimental evidence that an intervention to support parental nurturance and sensitivity was effective in protecting telomere length among children adopted internationally. Children who were randomized to receive the Attachment and Biobehavioral Catch-up intervention around age 2 had longer telomeres around age 5 than children who were randomized to receive a control intervention. These children also had longer telomeres than a non-randomized sample that did not receive an intervention. Gender was included in analyses, given that gender differences have been observed in previous samples, including children adopted internationally (Asok et al., 2014; Drury et al., 2011; Drury et al., 2014; Zhu et al., 2011). No gender interaction was observed, suggesting that the intervention was not differentially effective for boys and girls.

Duration of time in institutional care has also been associated with telomere length in previous research on children adopted internationally (Drury et al., 2011). Although the variable, months in orphanage care, was not associated with any key variables in the current study, potential relationships between variables may be masked by the overall intervention effect. Thus, we controlled for months in orphanage care in order to account for potential residual effects and to align with previous findings. Including this variable in the model did not alter the findings.
Under normative conditions, telomeres shorten during early childhood (Frenck, Blackburn, & Shannon, 1998; Zeichner et al., 1999), a process accelerated by early adversity (Drury et al., 2011; Drury et al., 2014; Shalev et al., 2012; Tyrka et al., 2010). Previous studies have shown that responsive parenting may protect against this accelerated shortening (Asok et al., 2013). The current study provides experimental evidence that an intervention geared towards increasing parental skills, such as nurturance and sensitivity, protect against accelerated shortening.

4.1 Strengths and Limitations

This study benefited from random assignment, which allows us to draw causal conclusions from the analyses. Although telomere data were not collected pre-intervention, investigation of demographic data suggests that random assignment was successful. In addition, the intervention was conducted with a heterogeneous sample, including children adopted from various regions with a variety of pre-adoptive experiences (i.e. length of time in orphanage care, or in the care of foster or birth parents). Regardless of the variation of these experiences, the intervention was successful at protecting telomere length.

There are several limitations of the current study. Because telomere length was not assessed prior to the intervention, the directionality and mechanisms through which telomere length was affected are unknown. Future research is needed to determine whether the current data represent a deceleration in telomere loss over time or an increase in telomerase activity, an enzyme responsible for rebuilding telomeres. The current study
also did not include a normative sample of similarly aged children. Thus, it is unknown if the telomere length of children who received Attachment and Biobehavioral Catch-up is normalized or simply improved.

### 4.2 Directions for Future Study

Given that this is the first study exhibiting successful experimental manipulation of telomere length, additional research is needed to identify the mechanisms through which this change occurred. Future research should include looking at specific biological mechanisms that may alter telomere length. Assays may be performed to determine what cellular mechanisms are at play. Telomerase is an enzyme responsible for rebuilding enzymes by replacing TTAGGG repeats to the 3’ end of the DNA strand after replication. The presence of telomerase in germ cells, lymphocytes, and cancer cells, allows these cell types to proliferate with no limit (Price et al., 2013). Though levels of telomerase in somatic cells are low, they have been associated with the increased shortening of telomere length (Blackburn, 2001). Likewise, dysregulated cortisol activity, such as increased levels of cortisol in response to a stressor and flatter diurnal cortisol slopes, has also been associated with shorter telomeres (Choi et al., 2008; Epel et al., 2006; Kroenke et al., 2011; Tomiyama et al., 2012). Investigating these various mechanisms would allow researchers to distinguish whether telomere length is affected by deficiencies in telomerase levels, cortisol dysregulation or a different aspect of the cellular environment.

The current study shows that children whose parents received the ABC intervention had longer telomeres than children whose parents received a control
intervention or no intervention. However, we did not investigate specific aspects of parenting behavior to parse apart what aspect of the intervention, if any, was altering telomere length. Future research should investigate levels of parental responsiveness and nurturance to determine which parental behaviors are important factors in regulation of telomere length.

The majority of studies on telomere length and early adversity are cross-sectional and provide insight into association between current, or retrospectively reported, environmental factors and telomere length. It is important to extend these findings and assess study participants prospectively. By assessing individuals at later time points, we may determine if telomere length and adversity in early childhood are associated with variation in telomere loss over time or mental and physical health outcomes at later time points. This longitudinal approach will allow telomere research to further elucidate the role of telomeres as a mechanism through which childhood adversity relates to future diseases.

4.3 Conclusion

Results from a randomized clinical trial indicate that children whose parents received a brief parenting intervention had longer telomeres than children whose parents received a control intervention. The present study significantly advances previous research on adversity and telomere length, as it suggests that experimental manipulation of telomere length in childhood is possible.
REFERENCES


doi:10.1016/j.jpeds.2010.08.007
Appendix

PERMISSION LETTER
Appendix

PERMISSION LETTER
DATE: October 10, 2012

TO: Mary Dozier, Ph.D.
FROM: University of Delaware IRB

STUDY TITLE: [385337-1] Cognitive Abilities, Health and Genetic Variation Among Internationally Adopted Children

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: October 10, 2012
EXPIRATION DATE: October 9, 2013
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # 3, 4, 7

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.
If you have any questions, please contact Jody-Lynn Berg at (302) 831-1119 or jiberg@udel.edu. Please include your study title and reference number in all correspondence with this office.
DATE: March 8, 2013

TO: Mary Dozier, Ph.D.
FROM: University of Delaware IRB

STUDY TITLE: [385337-2] Cognitive Abilities, Health and Genetic Variation Among Internationally Adopted Children

SUBMISSION TYPE: Amendment/Modification

ACTION: APPROVED
APPROVAL DATE: March 8, 2013
EXPIRATION DATE: October 9, 2013
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # 3, 4, 7

Thank you for your submission of Amendment/Modification materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

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If you have any questions, please contact Jody-Lynn Berg at (302) 831-1119 or jilberg@udel.edu. Please include your study title and reference number in all correspondence with this office.
DATE: September 27, 2013

TO: Mary Dozier, Ph.D.
FROM: University of Delaware IRB

STUDY TITLE: [385337-3] Cognitive Abilities, Health and Genetic Variation Among Internationally Adopted Children

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: September 27, 2013
EXPIRATION DATE: October 9, 2014
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # 3,4,7

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on the applicable federal regulation.

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Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.
If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.
DATE: October 9, 2014

TO: Mary Dozier, Ph.D.
FROM: University of Delaware IRB

STUDY TITLE: [385337-4] Cognitive Abilities, Health and Genetic Variation Among Internationally Adopted Children

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: October 9, 2014
EXPIRATION DATE: October 9, 2015
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # (3,4,7)

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on the applicable federal regulation.

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Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.
DATE: March 4, 2013

TO: Mary Dozier, PhD
FROM: University of Delaware IRB

STUDY TITLE: [437430-1] Health and Parenting Among a National Sample of Internationally Adopted Children

SUBMISSION TYPE: New Project

ACTION: APPROVED
APPROVAL DATE: March 4, 2013
EXPIRATION DATE: March 3, 2014
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # 3, 4, 7

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Expedited Review based on the applicable federal regulation.

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If you have any questions, please contact Jody-Lynn Berg at (302) 831-1119 or jilberg@udel.edu. Please include your study title and reference number in all correspondence with this office.
DATE: February 14, 2014

TO: Mary Dozier, PhD
FROM: University of Delaware IRB

STUDY TITLE: [437430-2] Health and Parenting Among a National Sample of Internationally Adopted Children

SUBMISSION TYPE: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: February 14, 2014
EXPIRATION DATE: March 3, 2015
REVIEW TYPE: Expedited Review
REVIEW CATEGORY: Expedited review category # 3,4,7

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

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If you have any questions, please contact Nicole Farnese-McFarlane at (302) 831-1119 or nicolefm@udel.edu. Please include your study title and reference number in all correspondence with this office.
DATE: February 10, 2015

TO: Mary Dozier, PhD
FROM: University of Delaware IRB

STUDY TITLE: [437430-3] Health and Parenting Among a National Sample of Internationally Adopted Children

IRB REFERENCE #: Continuing Review/Progress Report

ACTION: APPROVED
APPROVAL DATE: February 10, 2015
EXPIRATION DATE: March 3, 2016
REVIEW TYPE: Expedited Review

REVIEW CATEGORY: Expedited review category # (3,4,7)

Thank you for your submission of Continuing Review/Progress Report materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

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