


Article

Telomere Length as a Biomarker for Race-Related Health Disparities

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Abstract: Disparities between the races have been well documented in health and disease in the USA. Recent studies show that telomere length, a marker of aging, is associated with obesity and obesity-related diseases, such as heart disease and diabetes. The current study aimed to evaluate the connection between telomere length ratio, blood pressure, and childhood obesity. The telomere length ratio was measured in 127 children from both European American (EA) and African American (AA) children, aged 6–10 years old. AA children had a significantly high relative telomere to the single copy gene (T/S) ratio compared to EA children. There was no significant difference in the T/S ratio between normal weight (NW) and overweight/obese (OW/OB) groups of either race. Blood pressure was significantly elevated in AA children with respect to EA children. Hierarchical regression analysis adjusted for race, gender, and age expressed a significant relationship between the T/S ratio and diastolic pressure. Low T/S ratio participants showed a significant increase in systolic pressure, while a high T/S ratio group showed an increase in diastolic pressure and heart rate of AA children. In conclusion, our findings show that AA children have high T/S ratio compared to EA children. The high T/S ratio is negatively associated with diastolic pressure.

Keywords: telomere length; race; blood pressure; health disparities



Citation: Selvaraju, V.; Phillips, M.; Fouty, A.; Babu, J.R.; Geetha, T. Telomere Length as a Biomarker for Race-Related Health Disparities. *Genes* **2021**, *12*, 78. <https://doi.org/10.3390/genes12010078>

Received: 22 December 2020

Accepted: 7 January 2021

Published: 9 January 2021

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1. Introduction

Telomeres are present at the end of the chromosomes as shielding caps and protect the degradation targeted by the chromosome's cellular DNA damage. Telomeres consist of a repeated number of tandem sequences, TTAGGG, incorporated with a protein complex [1]. Telomere shows a substantial variation among individuals from birth and decreases during aging.

Obesity is a chronic disease in children and adults, developed in recent decades, and increases comorbidities in adulthood [2]. Worldwide obesity prevalence increased rapidly due to its high impact among children and adults [3]. Hence, it is imperative to treat young children to avoid obesity-related difficulties during their adulthood. The literature reveals that the correlation between obesity and telomere length have shown ambiguous results in adults. Some reports indicate a direct or inverse relationship between obesity and telomere length [4–7], but it differs in children. There is no significant difference in the telomere length reported between normal weight obese Caucasian children, but the obese adults had a shorter telomere length than the normal weight adults [8]. Very few studies discussed the correlation between telomere length and obesity due to lifestyle changes, suggesting that telomere length is either protected or extended while maintaining or reducing weight [4,5,9]. Even though overall telomere length is shorter in the obese state [10], studies conducted in older adults did not show any relationship between telomere length and obesity [5,7]. All individuals vary in telomere length with its reflection at birth [11], and the difference is observed throughout life [12,13]. Oxidative stress is a process that increases the shortening of telomeres during each cell division in somatic cells [14,15]. Inflammation is an additional

factor responsible for telomere reduction in leukocytes by increasing hematopoietic stem cells [16]. Obesity is the foremost independent risk factor for increasing aging and it is associated with metabolic diseases, such as hypertension, diabetes, cardiovascular disease, and cancer [17–21]. Even though the causal directions of these relationships persist to be defined [22,23], shorter telomeres are a well-established biomarker for various age-related conditions [24].

Recent studies have shown that telomere length, a marker of cellular aging, is used in studies of race-based health disparities and it is sensitive to effects of social stress [25–28]. Notably, African Americans have high levels of poor health, and it is expected for African Americans to have shorter telomeres than European Americans (EAs). Interestingly, recent findings show that African Americans (AAs) have longer telomere length in contrast to the expectations [29–33].

In industrialization, an age-related rise in pulse pressure is used as a phenotype of biological aging of the vasculature. In almost 95% of the hypertensive cases, hypertension is unknown [34]. Telomere attrition has been linked through hypertension and, during aging, it has been found in cells such as vascular endothelial cells, smooth muscle cells, and cardiomyocytes [35]. Therefore, in the current study, we examined the relationship between telomere length and blood pressure among EA and AA children. The relative telomere length to a single copy gene (T/S ratio) was calculated by comparing the telomeric DNA (T) level with the single copy (S) hemoglobin subunit γ one gene in salivary genomic DNA by quantitative real-time PCR. First, we tested whether the mean T/S ratio differed across the overall participants' races or weight category and low and high T/S ratio groups. Second, we tested the relationship between blood pressure and heart rate with low and high T/S ratios.

2. Materials and Methods

2.1. Study Subjects

The Auburn University Institutional Review Board for the Protection of Human Subjects in Research approved this study. The written consent was obtained from the participants and their parents before sample collection. By distributing the study flyer in the Lee and Macon counties in Alabama, participants were recruited from schools, after-school programs, through friends, and via participant referrals. Participants were recruited between ages 6–10 years, and a phone survey was conducted before recruiting to exclude the participants with health conditions such as diabetes and cardiovascular disease.

2.2. Anthropometric Measurements and Saliva Collection

The participants' body weight and height were measured, as described in our previous study [36]. The body mass index (BMI) percentile range was calculated according to the Centers for Diseases Control and Prevention (CDC), and the participants were grouped into normal weight and overweight/obese [37]. Saliva collection and isolation of salivary DNA was performed, as previously discussed [36]. The DNA isolated was used to measure the telomere length.

2.3. Blood Pressure Measurement

Measurement of blood pressure (BP) is known as an integral part of clinical examinations. The "gold standard" method was used to record the BP in participants as auscultatory, with an aneroid non-mercury manometer connected to a suitable cuff for the children. The systolic and diastolic pressure, along with heart rate, was recorded.

2.4. Telomere Length Measurement

The telomere length was measured as previously described in Cawthon et al. (2002), using a QuantStudio 3 real-time polymerase chain reaction (PCR) system [38]. The qPCR reaction mixture was performed with a volume of 20 μ l using the following reagents. Assays were performed using 96-well plates (Thermo Fisher Scientific, Waltham, MA, USA)

and the qPCR mix contained SYBR green PCR master mix (which included ROX passive dye), telomere primers (Tel 1, 5'-GGT TTT TGA GGG TGA GGG TGA GGG TGA GGG TGA GGG T-3' and Tel 2, 5'-TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA-3') (Integrated DNA Technologies, Inc., Coralville, IA, USA), and RNase free water to adjust the reaction volume. With this qPCR mix, a template DNA was added to perform the PCR. Simultaneously, single-copy gene qPCR was performed by preparing the qPCR mix of SYBR green PCR master mix with ROX passive dye, single copy number gene primers (hbg 1, 5'-GCT TCT GAC ACA ACT GTG TTC ACT AGC -3' and hbg 2, 5'-CAC CAA CTT CAT CCA CGT TCA CC-3') (Integrated DNA Technologies, Inc., Coralville, IA, USA), and RNase free water, along with template DNA. The telomere PCR and single-copy gene PCR was performed in separate 96-well plates matching the same well position of telomere PCR in the first plate and single-copy gene PCR on the second plate. The plates were prepared with six-point reference HeLa DNA (New England BioLabs, Ipswich, MA, USA) from the highest concentration of 150 ng/mL three-fold dilution in duplicates to make a standard curve. There were two negative control wells and four positive control wells (equivalent concentration of sample DNA template) on every plate. Plates were sealed with transparent optical adhesive covers (Thermo Fisher Scientific, Waltham, MA, USA) and loaded in the QuantStudio 3 real-time PCR system. The reaction was carried out using the following cycling profile of initiation for 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 52 °C for 20 s and extension at 72 °C for 45 s with signal acquisition; and then a melt curve was performed.

For each run of qPCR, amplification curves and melt curves were inspected to evaluate the sample and qPCR run quality, along with the absence of amplification in negative controls. Before calculating the T/S values, a coefficient variation of an inter and intra assay was examined. The relative measure of telomere length and T/S ratio in each sample was calculated in a separate excel sheet using the following formula:

$$\Delta Ct = Ct_{Tel} - Ct_{hbg}$$

$$\Delta Ct \text{ of control} = Ct_{Tel} - Ct_{control}$$

$$\Delta\Delta Ct = \Delta Ct - \Delta Ct \text{ of control}$$

$$\text{Relative T/S} = 2^{-\Delta\Delta Ct}$$

The low and high T/S ratio groups were separated based on the median T/S ratio. The low T/S ratio group denotes that the T/S ratio values were less than the median. On the other hand, the participant's T/S ratio was higher than the median, named a high T/S ratio group.

2.5. Statistical Analysis

All the data in the graphical representation was expressed as mean \pm SEM, $p < 0.05$ considered as significant. The descriptive and hierarchical regression analyses were performed using the Statistical Package for Social Sciences (SPSS, version 25, IBM, Armonk, NY, USA). We used hierarchical regression analysis, introducing covariates such as race, age, and gender to predict the dependent variables. A two tailed Students t-test was used to calculate the mean difference between the two groups. The graphs were prepared using Graphpad Prism version 8 (San Diego, CA, USA). The proportion graphs were prepared using the number of participants in each category of study. The statistically significant difference in proportion graphs was calculated using the online MedCalc software website (Ostend, Belgium).

3. Results

The anthropometric measurements, telomere length ratio, and blood pressure for low and high T/S ratio groups are listed in Table 1 from the 127 participants, aged 6–10 years. There was no significant difference observed between the low and high T/S ratio of age,

height, weight, and BMI. The T/S ratio did not show a significant difference between EA and AA children, normal weight (NW) and overweight/obese (OW/OB) groups. Systolic, diastolic pressure and pulse were among the low and high T/S ratio groups not showing significant changes. The mean T/S ratio of all participants was 1.06 ± 0.04 , with a median of 1.066. The mean T/S ratio of EA participants was 0.907 ± 0.04 (median 0.805) and AA participants was 1.23 ± 0.07 (median 1.216). The mean T/S ratios of NW and OW/OB participants were 1.07 ± 0.04 and 1.04 ± 0.07 , respectively.

Table 1. General characteristics of the study population.

	Low T/S Ratio (64)	High T/S Ratio (63)	<i>p</i> Value
Age (year)	8.40 ± 0.19	8.19 ± 0.18	$p < 0.427$
Height (cm)	133.01 ± 1.42	130.89 ± 1.45	$p < 0.297$
Weight (kg)	32.39 ± 1.34	32.07 ± 1.42	$p < 0.868$
BMI (kg/m ²)	17.98 ± 0.41	18.21 ± 0.45	$p < 0.696$
T/S Ratio			
All	0.739 ± 0.02	1.38 ± 0.05	$p < 0.0001$
EA	0.724 ± 0.02	1.26 ± 0.04	$p < 0.0001$
AA	0.776 ± 0.06	1.44 ± 0.08	$p < 0.0001$
NW	0.750 ± 0.03	1.35 ± 0.05	$p < 0.0001$
OW/OB	0.726 ± 0.04	1.43 ± 0.11	$p < 0.0001$
Blood Pressure			
Systolic Pressure (mmHg)	100.52 ± 1.31	101.61 ± 1.72	$p < 0.610$
Diastolic Pressure (mmHg)	66.05 ± 1.24	67.38 ± 1.40	$p < 0.477$
Heart rate (BPM)	84.52 ± 1.58	84.51 ± 1.57	$p < 0.997$

European American (EA); African American (AA); normal weight (NW); overweight/obese (OW/OB); telomere length/ single copy gene (T/S ratio); values are expressed as mean \pm SEM.

To determine the difference in the telomere length ratio between races, the calculated T/S ratio was analyzed by the *t*-test. AA participants showed a significantly ($p < 0.0001$) higher T/S ratio compared to EA participants (Figure 1a). The telomere length ratios among races for NW and OW/OB groups were also compared. The NW and OW/OB participants did not show any significant differences in either EA participants (NW- 0.922 ± 0.05 ; OW/OB- 0.883 ± 0.05) or AA participants (NW- 1.235 ± 0.07 ; OW/OB- 1.22 ± 0.14) (Figure 1b,c).

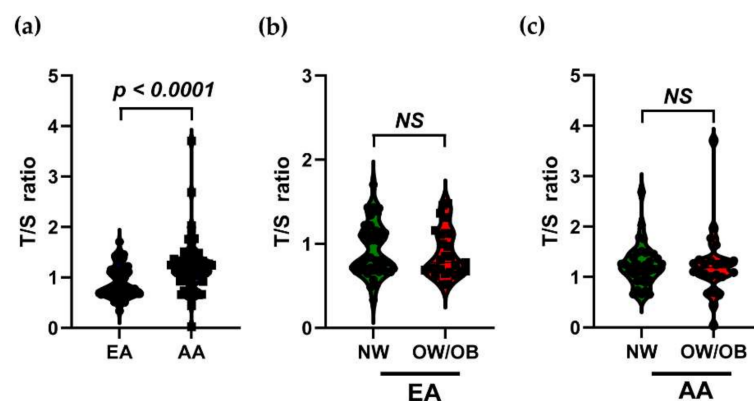


Figure 1. The violin plot with data points shows the distribution of the T/S ratio in EA and AA children. (a) The mean T/S ratio is compared between EA and EA children. The T/S ratio of NW and OW/OB children is compared in EA (b) and AA children (c). Values are expressed as mean \pm SEM. European American (EA); African American (AA); normal weight (NW); overweight/obese (OW/OB); telomere length/ single copy gene (T/S ratio); Not significant (NS).

Based on the median T/S ratio, the study group was divided into two: low and high T/S ratios. The proportion of the low T/S ratio participants was significantly higher in the

EA group (70.31%) than the AA group (29.69%). On the contrary, a greater proportion of high T/S ratio participants were found in the AA group (63.49%) in comparison to the EA group (36.51%), as shown in Figure 2a. The proportion of participants with low T/S ratios in both NW and OW/OB groups of the AA participants decreased significantly ($p < 0.01$) compared to EA participants of NW and OW/OB groups. However, the proportion of high T/S ratios in both NW and OW/OB groups of AA participants significantly ($p < 0.05$) increased in comparison to corresponding EA participants (Figure 2b). This result suggests that the T/S ratio in AA participants significantly increased compared to EA participants, but there was no difference between the NW and OW/OB groups with EA or AA children.

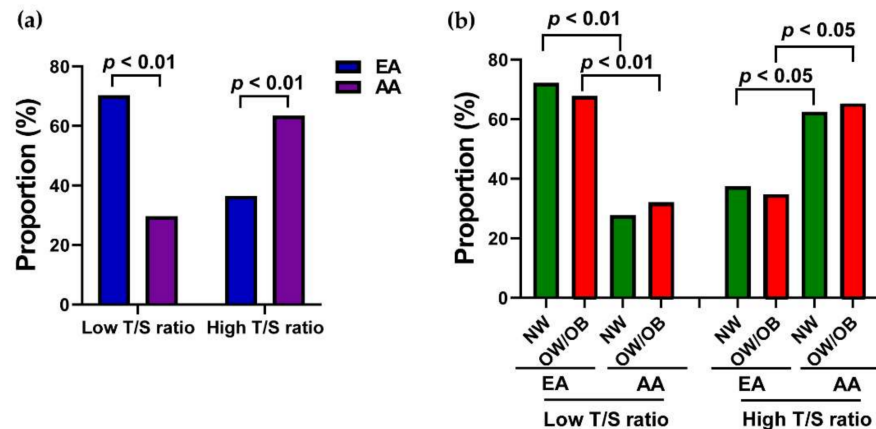


Figure 2. Proportion of participants with low and high T/S ratio groups. (a) The proportion difference participants with low and high T/S ratios in EA and AA children. (b) The proportion difference among NW and OW/OB children of EA and AA participants with low and high T/S ratios.

Next, the difference in the circulatory pressure between race, NW, and OW/OB participants was determined. The systolic blood pressure ($p < 0.004$) and diastolic pressure ($p < 0.002$) among AA participants significantly increased compared to the EA group (Figure 3a,b). However, there was no difference observed between AA and EA participants' pulse rates (Figure 3c). The OW/OB participants of both the EA and AA groups had significantly higher systolic blood pressure compared to NW participants. However, the diastolic pressure and pulse rate among NW and OW/OB groups of EA and AA participants did not show significant changes (Figure 4a,b). This suggests that the systolic and diastolic pressure increased in the AA participants compared to EA participants but only the systolic pressure in OW/OB was greater than the NW group of EA and AA participants.

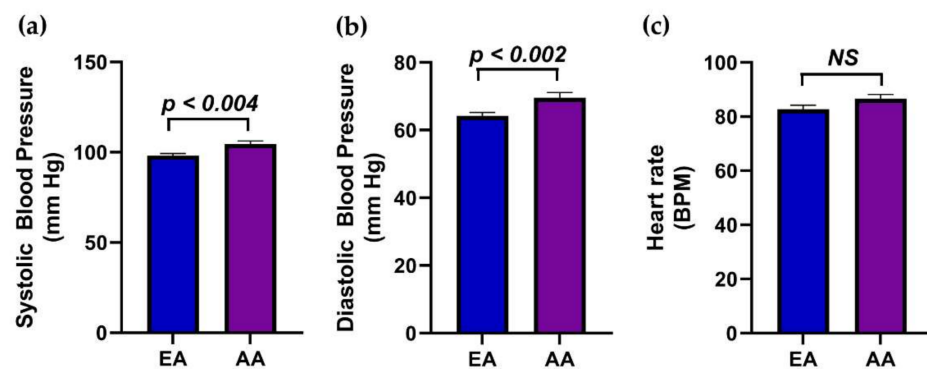


Figure 3. The blood pressure of EA and AA participants. The difference in the (a) systolic pressure, (b) diastolic pressure, and (c) heart rate between EA and AA children. Values are expressed as mean \pm SEM.

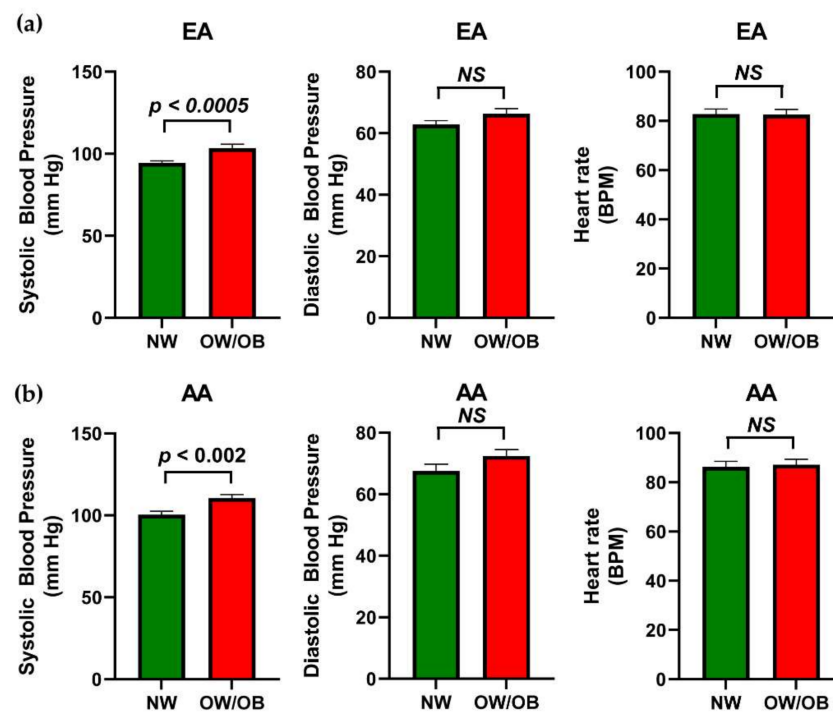


Figure 4. Comparison of blood pressure in NW and OW/OB children of EA and AA participants. The bar graphs shows the differences in the blood pressure in the NW and OW/OB groups of (a) EA participants and (b) AA participants. Values are expressed as mean \pm SEM.

The differences in the blood pressure and heart rate of the low and high T/S ratio participants were determined between EA and AA groups. The systolic pressure was significantly increased ($p < 0.004$) in AA participants compared to EA participants with low T/S ratios. However, there was no difference in diastolic pressure and heart rate with low T/S ratios (Figure 5a). AA participants with a high T/S ratio observed significantly increased diastolic pressure ($p < 0.01$) and heart rate ($p < 0.01$) compared to the EA group. Nevertheless, systolic blood pressure did not significantly change in high T/S ratio participants (Figure 5b). This explains that the AA children with low T/S ratios had higher systolic pressure and those with high T/S ratios had higher diastolic pressure and heart rate compared to EA children.

The relationship between the T/S ratio and circulatory pressure for unadjusted and adjusted hierarchical regression was analyzed. Race, gender, and age of the participants were entered to adjust for covariates. Both adjusted and unadjusted hierarchical regression was performed with all participants for low and high T/S ratio groups between T/S ratios, systolic, diastolic pressure, and heart rate. Even though systolic pressure showed positive and diastolic pressure, it showed a negative correlation with the T/S ratio. There was no significant difference observed in the β -coefficient of unadjusted regression analysis. After adjusting for race, gender, and age, diastolic pressure showed a significant negative correlation ($\beta = -0.327$; $p < 0.049$) with high T/S ratio participants (Table 2). This suggests that children with high T/S ratios are negatively correlated with diastolic pressure.

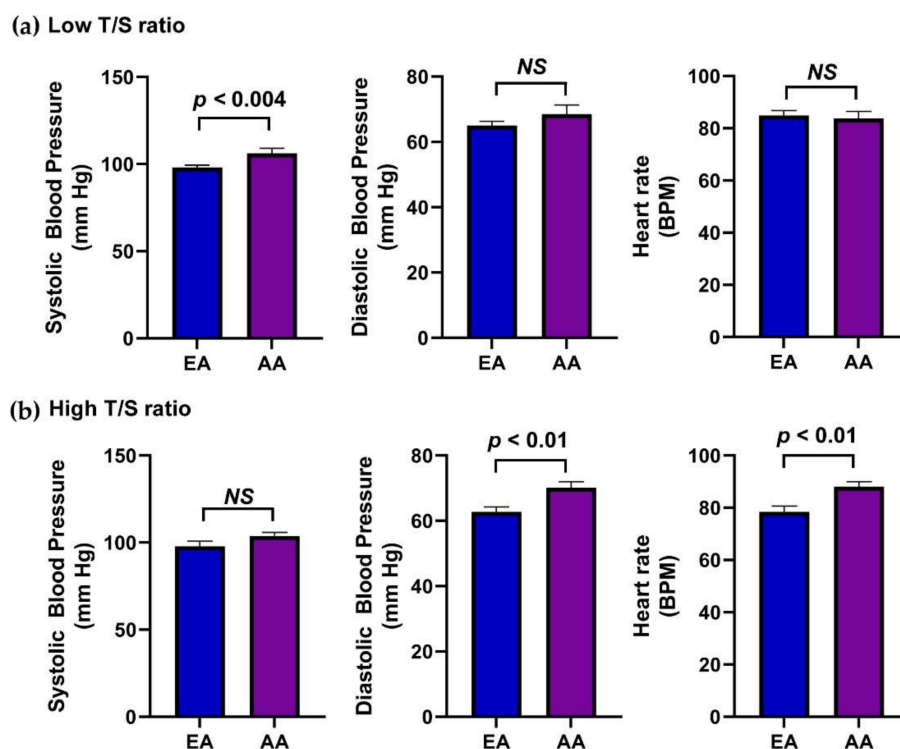


Figure 5. Comparison of blood pressure in EA and AA children with low and high T/S ratios. Bar graphs shows the differences in blood pressure in EA and AA participants with (a) the low T/S ratio and (b) the high T/S ratio. Values are expressed as mean \pm SEM.

Table 2. The relationship between T/S ratio and blood pressure by hierarchical regression analysis (Adjusted for race, gender, and age).

Parameters	Unadjusted						Adjusted					
	B	SE	B-Coefficient	95% Confidence Interval for b		p-Value	B	SE	B-Coefficient	95% Confidence Interval for b		p-Value
				Lower Bound	Upper Bound					Lower Bound	Upper Bound	
All participants												
Systolic Pressure	0.005	0.004	0.136	−0.003	0.014	0.242	0.003	0.004	0.079	−0.006	0.011	0.497
Diastolic Pressure	−0.005	0.005	−0.113	−0.015	0.005	0.333	−0.007	0.005	−0.162	−0.016	0.002	0.143
Pulse	0.000	0.003	0.012	−0.006	0.007	0.891	−0.002	0.003	−0.057	−0.008	0.004	0.508
Low T/S ratio participants												
Systolic Pressure	0.000	0.003	0.017	−0.006	0.006	0.923	−0.001	0.004	−0.051	−0.008	0.006	0.800
Diastolic Pressure	−0.003	0.003	−0.135	−0.009	0.004	0.444	−0.001	0.003	−0.077	−0.008	0.005	0.679
Pulse	−0.001	0.002	−0.093	−0.005	0.002	0.474	−0.001	0.002	−0.090	−0.005	0.003	0.495
High T/S ratio participants												
Systolic Pressure	0.007	0.005	0.251	−0.002	0.017	0.122	0.006	0.005	0.205	−0.004	0.016	0.228
Diastolic Pressure	−0.010	0.006	−0.259	−0.021	0.002	0.109	−0.012	0.006	−0.327	−0.024	0.000	0.049
Pulse	0.003	0.004	0.085	−0.006	0.011	0.508	−0.001	0.005	−0.034	−0.010	0.008	0.809

Unstandardized coefficient (B); standard error (SE). The bold number shows the significant.

4. Discussion

The current study includes 68 EA and 59 AA (total 127) children with an age range of 6–10 years. The study population was divided into low and high T/S ratios based on the median. We did not observe differences in the T/S ratio according to age, height, weight, or weight category. The results agree with previous studies in children and adult populations [39,40]. This may be due to the narrow range of ages and sample sizes used in the study. The mean T/S ratio in the AA children increased compared to the EA children. Similar results were found by Rewak et al., with significantly longer leucocyte telomere length (LTL) in Blacks than Whites at birth and adulthood [41]. Several studies showed longer LTL on average in AA participants [29–33]. In adulthood, shorter leukocyte telomeres are correlated with BMI in women [42] and waist-hip ratios in both genders [13]. However, previous studies on childhood obesity and telomere length showed an inconclusive result. The NW and OW/OB children in both EA and AA participants did not show any significant changes in the mean T/S ratio. These results are in correlation with Zannolli et al., where the leukocyte telomere length did not show any difference between obese and non-obese Italian children [8]. The participants were separated based on the T/S ratio's median into low and high T/S ratio groups. In the high T/S ratio group, the proportion of the AA children was more compared to EA children, and the proportion of participants of NW and OW/OB children in the AA group showed higher numbers. This result clearly correlates with the previous results published by Rewak et al. on the childhood telomere length [41].

Systolic and diastolic pressure was significantly increased in AA children compared to EA children. Globalization increases systolic blood pressure throughout life [43,44]. Diastolic blood pressure is also increased in early life, but it tends to decrease in aged persons [45]. Arterial aging has predominantly increased the stiffness of central elastic arteries, which is the factor that regulates pulse pressure. The factors that increase the biological aging of vessel formation, including essential hypertension [46], other diseases, such as diabetes [47], and higher amounts of salt intake [48], independently increase the arterial stiffness. Connecting all these processes proposes that aortic blood pressure might show a phenotype of aging and is one of the major risk factors for cardiovascular disease [49–51].

The systolic pressure of the OW/OB children of both EA and AA races was significantly increased than in NW children. The overall T/S ratio was high in AA participants, and the diastolic blood pressure and heart rate also increased in the same participant group. Our results are consistent with earlier cross-sectional findings of race/ethnicity differences in telomere length at different age groups. In particular, Rewak et al. showed a longer telomere length in the AA population [41]. The study conducted with NHANES data suggests that cardiovascular health is associated with smaller leucocyte telomere lengths and race/ethnicity [52]. Numerous studies have consistently reported that more prevalence of hypertension in AA participants than EA participants is the primary reason for increased cardiovascular disease occurrence in the AA community [53]. Based on the median of the T/S ratio, significantly increased diastolic pressure and heart rate was observed in the high T/S ratio of AA participants. The shorter telomere lengths are correlated with hypertension in adults [54]. We found that systolic pressure was only significantly increased in AA children with a low T/S ratio. Additionally, our results suggest that the characteristic is only a significant analyst of telomere length in adulthood and not in childhood. Aydos and Tukun (2007) describe no correlation between systolic pressure, diastolic pressure, BMI, and telomere length [55]. However, in this study, the hierarchical regression analysis, after adjusting with covariates such as race, gender, and age, indicates that children with high T/S ratios are negatively correlated with diastolic pressure.

Some limitations need to be acknowledged. We used a real-time PCR method to measure the telomere length ratio with higher assay variability in general than the traditional telomere restriction fragment method [56,57]. The plate-to-plate variability effect was minimized using the corresponding well layout of the telomere primer and single-copy

gene [9]. The results should also be considered preliminary due to the small sample size in the study.

5. Conclusions

In this study, we explored the connection between telomere length ratio, blood pressure and childhood obesity. Our results demonstrated that AA children have a greater mean T/S ratio and systolic and diastolic pressure compared to EA children. The OW/OB children of both EA and AA groups have higher systolic pressure compared to NW children. AA children with low T/S ratios have increased systolic pressure and children with high T/S ratios have increased diastolic pressure and heart rate than EA children. The children with high T/S ratio are negatively correlated with diastolic pressure. This study validates the use of non-invasive salivary measurement of telomere length and addresses the gap between the T/S ratio and blood pressure among children.

Author Contributions: Conceptualization, T.G. and V.S.; methodology, V.S., M.P., and A.F.; formal analysis, V.S., M.P., and A.F.; project administration, J.R.B. and T.G.; funding acquisition, A.F., J.R.B. and T.G.; supervision J.R.B. and T.G.; writing—original draft preparation V.S.; writing—review and editing, all. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Alabama Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, USA. Department of Agriculture to Jeganathan Ramesh Babu and Thangiah Geetha. The Undergraduate Research Fellowship and the Fred and Charlene Kam Endowed Fund for Research Excellence in Nutrition-Dietetics, Auburn University was funded to Anna Fouty.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Auburn University (Protocol # 17-364 MR 1709).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study is available on request from the corresponding author.

Acknowledgments: We would like to thank all the children for their participation.

Conflicts of Interest: The authors declare that they have no competing interests.

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