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Telomere length dynamics in early life: the blood-and-muscle model

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ABSTRACT: Telomere length (TL) trajectories in somatic tissues during human growth and development are poorly understood. We examined a blood-and-muscle model during early life, focusing on TL trajectories in leukocytes, representing the highly proliferative hematopoietic system, and skeletal muscle, a minimally proliferative tissue. Leukocyte TL (LTL) and skeletal muscle TL (MTL) were measured in 28 fetuses and 73 children. LTL and MTL were highly variable across individuals (SD: fetal LTL = 0.72 kb, MTL = 0.72 kb; children LTL = 0.81 kb, MTL = 0.82 kb) but were highly correlated within individuals (fetuses, $r = 0.76$, $P < 0.0001$; children, $r = 0.87$, $P < 0.0001$). LTL was shorter than MTL in fetuses (10.63 *vs.* 11.01 kb; $P = 0.0004$) and children (8.46 *vs.* 9.40 kb; <0.0001). The LTL-MTL gap was smaller in fetuses than children. TL in children was inversely correlated with body mass index (BMI) (LTL: -0.047 ± 0.016 kb/BMI, $P < 0.005$; MTL: -0.037 ± 0.017 kb/BMI, $P = 0.03$). We conclude that variations in TL across adults and differences in TL between somatic tissues are largely established in early life. Because TL plays a significant role in aging-related diseases, insight into the factors that fashion TL in somatic tissues during early development should contribute to an understanding of the relationship of TL with these disease and longevity in humans.—Sabharwal, S., Verhulst, S., Guirguis, G., Kark, J. D., Labat, C., Roche, N. E., Martimucci, K., Patel, K., Heller, D. S., Kimura, M., Chuang, D., Chuang, A., Benetos, A., Aviv, A. Telomere length dynamics in early life: the blood-and-muscle model. FASEB J. 32, 529–534 (2018). www.fasebj.org

KEY WORDS: fetus · children · leukocytes

Epidemiologic studies have mostly used blood to examine associations of telomere length (TL) with a host of human conditions. A consensus has emerged based on measurements of leukocyte TL (LTL): whereas individuals with short LTL are prone to atherosclerotic cardiovascular disease (CVD) (1, 2), individuals with long LTL are at a higher risk of major cancers (3–6). Such findings raise the question of whether the associations of LTL with these disease categories reflect interindividual LTL variation that is already evident early in life, variation in the rate of age-dependent LTL attrition afterward, or both.

Longitudinal studies of LTL dynamics (LTL at birth and its age-dependent shortening thereafter) that follow individuals across their life course from birth onward could answer this question. However, many decades would pass before the outcomes of such studies are known. Instead, we have proposed a model that uses information on LTL and skeletal muscle TL (MTL) to generate quasi-longitudinal information on TL dynamics in the individual (7). This blood-and-muscle model is based on the following premise: LTL and MTL are similar in early life. However, LTL, which reflects TL in the highly proliferative hematopoietic system, undergoes faster shortening than TL in the minimally proliferative skeletal muscle. Therefore, the difference between LTL and MTL reflects relative LTL shortening since early life. We originally developed this blood-and-muscle model in dogs (8) and subsequently examined it in adult humans (9). Here we report our findings on the blood-and-muscle model during the first 2 decades of life, including intrauterine life.

ABBREVIATIONS: BMI, body mass index; CVD, cardiovascular disease; LTL, leukocyte telomere length; MTL, muscle telomere length; TL, telomere length

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MATERIALS AND METHODS

Subjects

Muscle biopsy samples (from the quadriceps) and blood were obtained from nonviable, electively aborted fetuses, the sex of which was unknown (gestational age 15–22 wk; mean 18 wk). Muscle and blood samples were obtained from children (aged 0.3–19.7 yr; mean 11.7 yr) undergoing various orthopedic surgical procedures at the University Hospital of the Rutgers New Jersey Medical School. During surgery, blood was drawn from the venous line, while samples (~25 mg) were obtained *via* biopsy from a skeletal muscle in the surgical field. Specimens from fetuses and children were kept on ice until arrival in the laboratory (~30–60 min after collection), where they were frozen at −80°C. Children with major congenital diseases, chromosomal abnormalities, muscle disease, infection, autoimmune disease, and cancer were excluded. This research was approved by the institutional review board of the New Jersey Medical School; written informed consent was obtained from the legal guardian, and when appropriate, assent was also obtained from the subject.

TL measurements

DNA was extracted by the phenol/chloroform method, and aliquots were resolved in 1% (w/v) agarose gel to test for DNA integrity (10). No sample showed evidence of DNA degradation. TL measurements were performed by Southern blot analysis of the terminal restriction fragments as previously described (10). Samples were digested with restriction enzymes *Hin*fI (10 U)/*Rsa*I (10 U) (Roche, Basel, Switzerland); they (and DNA molecular weight ladders) were then resolved in 0.5% agarose gels. After 16 h, the DNA was depurinated, denatured, and neutralized. The DNA was transferred to positively charged nylon membranes and hybridized at 65°C with the digoxigenin-labeled telomeric probe, which was detected by digoxigenin luminescence (Roche) and exposed on X-ray film. The measurement repeatability, as determined by the intraclass correlation coefficient, was 0.99 (95% confidence interval, 0.817, 1.0) and 0.98 (95% confidence interval, 0.81, 1.0) for LTL and MTL, respectively. The repeatability of the means of 2 duplicates (used in the analysis), known as the extrapolated repeatability, was 0.995 and 0.991 for LTL and MTL respectively.

Statistical analysis

Descriptive values are expressed as means ± SD, with minimal and maximal values. Student's *t* test was used for continuous variables and the χ^2 test for discrete variables. Comparisons of TL with MTL within subjects were performed by a paired Student's *t* test; $P < 0.05$ was regarded as statistically significant. In children, the body mass index (BMI) association with TL was examined using a mixed model, with TL of muscle and leukocytes introduced as dependent variables, and donor identity as a random effect. Statistical analyses were carried out by NCSS 9 (NCSS Statistical Software, Kaysville, UT, USA) and JMP 9.0.1 (JMP Statistical Discovery, Cary, NC, USA) statistical software.

RESULTS

General characteristics of the 28 fetuses (mean ± SD gestational age 18 ± 2 wk) and 73 children (aged 11.7 ± 4.1 yr) with TL measurements are summarized in **Tables 1 and 2**.

TABLE 1. *General characteristics of fetuses*

Parameter	All
Number	28
Gestational age (wk)	18.4 ± 1.9 (15.0–22.0)
African American (*n*)	26
Hispanic (*n*)	1
Other (*n*)	1
LTL (kb)	10.63 ± 0.72 (8.70–11.88)
MTL (kb)	11.01 ± 0.72 (9.39–12.31)

Data are presented as means ± SD with min–max range in parentheses unless otherwise indicated. Sex of fetuses was not known.

LTL was shorter than MTL in fetuses (10.63 *vs.* 11.01 kb; $P = 0.0004$) and children (8.46 *vs.* 9.40 kb; <0.0001). There was no apparent effect of muscle location in children (lower extremities, upper extremities, and back) on MTL. **Figure 1** displays data and linear regressions of the relation between LTL and MTL in fetuses and children. At any age, individuals with short (or long) LTL also displayed comparatively short (or long) MTL (fetuses, $r = 0.76$, $P < 0.0001$; children, $r = 0.87$, $P < 0.0001$). The line of identity is the theoretical alignment when LTL and MTL are equivalent, presumably in early embryonic development. For comparison, we also include the regression line of LTL *vs.* MTL (95% confidence interval) in adults from our previously published work (9). The alignment of the LTL-MTL regression line in the fetuses was much closer to the line of identity than that of children, whose LTL-MTL regression line was closer to the line of identity than in adults. Thus, with age, the LTL-MTL alignment progressively shifted downward away from the line of identity because age-dependent LTL shortening was faster than MTL shortening.

Regardless of cell type, TL was longer in fetuses than children, whose TL was longer than the TL of adults (mean age 44 yr) (Tables 1 and 2, **Fig. 2*A, B***). The difference between LTL and MTL (*i.e.*, LTL-MTL) and the difference between LTL and MTL scaled to MTL [*i.e.*, (LTL − MTL)/MTL] were smaller in fetuses than children, whose LTL-MTL was smaller than that of adults (Fig. 2*C, D*). Of note, in the fetuses, the LTL-MTL gap was significantly different from 0 (mean ± SD 0.38 ± 0.50 kb; $P = 0.0005$), suggesting that the LTL-MTL gap started developing during early fetal life.

This study was designed to test whether the basic principles of the blood-and-muscle telomere model in adults also hold during the first 2 decades of life, including intrauterine growth. It did not have the power (11) to examine the impact of variables such as sex and ethnicity on TL parameters in children (12, 13). That said, in light of the strong ethnic imbalance evident between fetuses and children, a sensitivity analysis restricted to African Americans (26 fetuses and 32 children) was undertaken and confirmed the main study findings ($P < 0.0001$ in all analyses in Fig. 2). In addition, a mixed model that included TL of both leukocytes and muscle as dependent variables and cell type (leukocytes *vs.* muscle), age, sex, ethnicity (African Americans *vs.* other ethnicities), and BMI as the independent variables showed that TL decreased with

TABLE 2. *General characteristics of children*

Parameter	All	Boys	Girls
Number	73	38	35
Age (yr)	11.7 ± 4.1 (0.3–19.7)	11.1 ± 4.7 (0.3–17.3)	12.3 ± 3.4 (3.3–19.7)
African American (*n*)	32	12	20*
Hispanic (*n*)	27	16	11
Other (*n*)	14	10	4
BMI (kg/m²)	21.7 ± 5.9 (12.5–44.8)	21.0 ± 6.3 (12.5–44.8)	22.4 ± 5.6 (12.9–35.1)
LTL (kb)	8.46 ± 0.81 (6.48–10.31)	8.40 ± 0.82 (6.61–10.20)	8.53 ± 0.80 (6.48–10.31)
MTL (kb)	9.40 ± 0.82 (7.45–11.37)	9.29 ± 0.84 (7.45–11.00)	9.52 ± 0.79 (7.64–11.37)

Data are presented as means ± SD with min–max range in parentheses unless otherwise indicated. *$P < 0.05$.

increasing BMI (**Fig. 3**; Supplemental Table S1*A*). In this model, neither the association with age or BMI depended on tissue type (interactions added to model in Supplemental Table S1*A*; age × cell type: $F_{1,70} = 0.005$, $P = 0.9$; BMI × tissue: $F_{1,70} = 1.60$, $P = 0.2$). The latter is confirmed when fitting the model in Supplemental Table S1*A* for the tissue types separately (LTL: −0.047 ± 0.016 kb/BMI, $P < 0.005$; MTL: −0.037 ± 0.017 kb/BMI, $P = 0.03$). Omitting 2 individuals with a BMI of >31 kg/m² did not change this result (LTL: −0.044 ± 0.019 kb/BMI, $P < 0.025$; MTL: −0.037 ± 0.019 kb/BMI, $P = 0.05$). Thus, a high BMI was associated with short TL independent of tissue type.

DISCUSSION

The findings of the present study extend the blood-and-muscle model, originally developed in adults, to fetuses and children. It underscores a fundamental phenomenon: although LTL and MTL are highly correlated, TL in leukocytes, representing the highly proliferative hematopoietic system, is shorter than TL in the minimally proliferative skeletal muscle. The shorter LTL than MTL is already evident during intrauterine life—as early as the 18th gestational week. That said, the gap between LTL and MTL progressively widens because of the faster pace of shortening with age of LTL than MTL. Given that TL variation across individuals is much wider than intra-individual variation between LTL and MTL, notwithstanding the age-dependent widening of the LTL-MTL gap, an individual with a short or long LTL displays correspondingly short or long MTL. We have shown this to be the case for somatic tissues with different proliferative activities in adults (9), and now we show this to be the case in fetuses and children. Such information is necessary for resolving unanswered questions about the meaning of the associations of LTL with age-related diseases (7).

Because of its availability, blood, and hence LTL, has been typically used in epidemiologic and population genetic studies examining the role of TL in aging and its related diseases. It was originally thought that LTL is a passive biomarker (*i.e.*, a telomeric clock, of human aging and that the short LTL in subjects with atherosclerotic CVD reflects a faster pace of this clock) (14). However, the telomeric clock metaphor has overlooked the wide interindividual variation in TL across fetuses, as shown in this work and in newborns (15–17), indicating a different baseline 0 clock time for each individual. Thus, the telomeric clock concept might be valid only if, for a given age, LTL is scaled to LTL at birth, which is not feasible in most epidemiologic or clinical settings.

The view of LTL as a biomarker that charts the pace of aging has given way to recent thinking that TL, as expressed in LTL, might play a causal role in a host of aging-related diseases, including CVD and major cancers (7, 18). The considerable LTL variation across newborns and children suggests that having short or long LTL, which is highly heritable (19, 20), precedes by decades the onset of LTL-associated diseases. In addition, LTL-associated alleles have been used to construct genetic risk scores for diseases known to be associated with short or long LTL (21–23). These findings diminish the possibility of reverse causality (*i.e.*, that such diseases shorten or lengthen LTL).

On the basis of longitudinal studies, as adults get older, they typically maintain their LTL ranking (*e.g.*, long, average, or short) compared to peers (24). However, whether this LTL tracking finding also extends to the first 2 decades

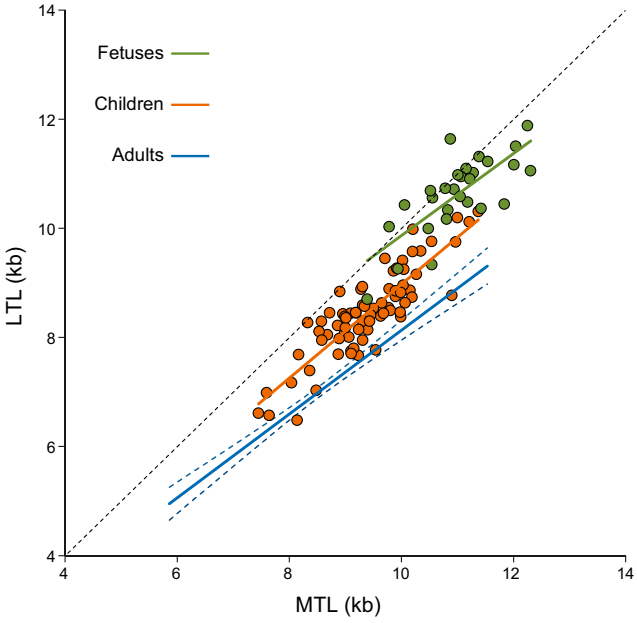

Figure 1. LTL *vs.* MTL. Black dotted line, line of identity; blue line and dotted blue lines, regression line and 95% confidence interval of LTL and MTL data in adults (9).

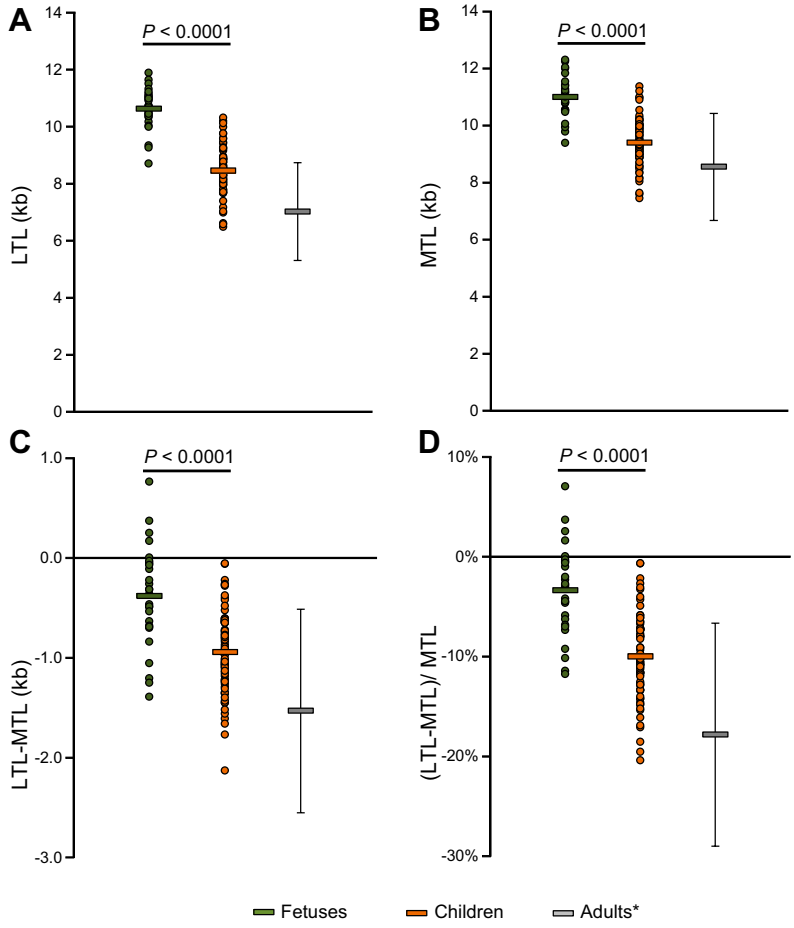

Figure 2. TL parameters in fetuses and children compared to adults. Adult TL parameters (9) are displayed as means ± 2 SD for comparison with those of fetuses and children. Fetuses were on average in 18th wk of gestation; children and adults were on average 12 and 44 yr old. Horizontal bars indicate mean values.

of life has been unknown. The blood-and-muscle TL model, originally tested in adults (9) and now extended to the first 2 decades of life, suggests that this is the case. Moreover, the present findings further support the notion that as expressed in leukocytes, variation in TL across adults of the same age largely reflects variation in TL that is already displayed across fetuses and children (15–17).

Such a conclusion does not preclude the possibility that some individuals might have a faster or slower age-dependent TL attrition than their peers at any time during their life course. However, it underscores that the high LTL variability across individuals is already expressed *in utero* and in children. Therefore, the mechanisms behind the association between LTL and disease might be better understood when we learn what factors determine TL in newborns and developing children.

The difference between LTL and MTL is already observed in fetuses, perhaps because telomerase is robustly active only during early embryonic life (25, 26). At a mean age of 11.7 yr, the LTL-MTL gap is already established as a major portion of the difference between LTL and MTL in adults at the mean age of 44 yr (Figs. 1 and 2) (9). Thus, variation in TL attrition in leukocytes and muscle across individuals during the first decade of life explains a large proportion of the LTL-MTL gap in adults.

Obese children display short LTL (27), and LTL is inversely related to BMI in adults (28, 29). The inverse relation between BMI and TLs in both leukocytes and muscle in the present study suggests a role of increased body mass during growth in fashioning TL, expressed not only in leukocytes but other somatic tissues in adults. Moreover, individuals who enter adult life with short TL are likely to

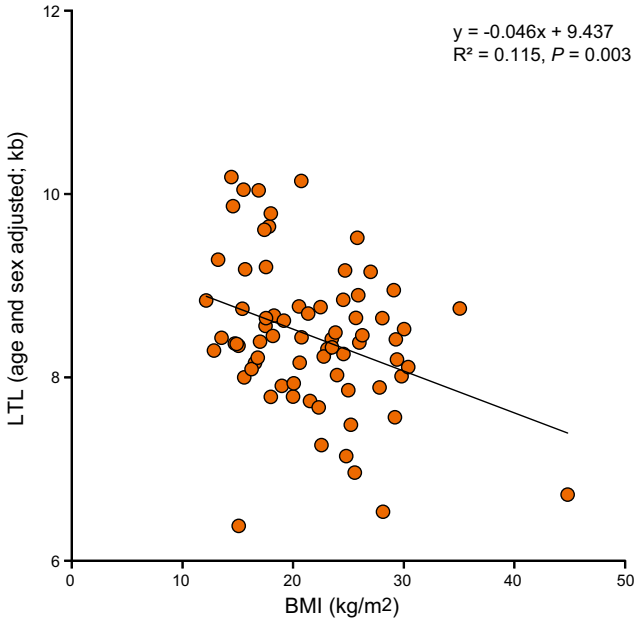

Figure 3. LTL *vs.* BMI in children. LTL was adjusted for age and sex. See Supplemental Table S1 for statistical details.

have short TL for their remaining life course, as shown here and previously (2, 9). In this light, it is noteworthy that based on 2.3 million individuals, a recent study reported that BMI in late adolescence predicts propensity to CVD later in adulthood (30). Given the association between short LTL and CVD (1, 2), might the influence of BMI during adolescence on the propensity to CVD later in life be mediated in part through TL?

Finally, we would like to underscore the strength and limitation of this study. We used the highly reproducible Southern blot method (10, 31) of TL measurement to showcase the capability of the blood-and-muscle model to capture TL trajectories during early life. However, our sample size was modest and not necessarily representative of the general population. Future large-scale studies that use this model are likely to increase our understanding of a host of preconception, intrauterine, and early extrauterine factors that exert lasting influences on TL trajectories during the entire life course. This knowledge is valuable for insight into mechanisms that explain the connections between TL and diseases of aging, including atherosclerotic CVD and major cancers. FJ

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AUTHOR CONTRIBUTIONS

S. Sabharwal helped design the study, collected surgical specimens, and participated in writing the article; S. Verhulst performed statistical analysis and participated in writing the article; G. Guirguis helped in designing the study of fetuses, collected samples, and helped write the article; J. D. Kark participated in study design and helped write the article; C. Labat performed statistical analysis and contributed to the writing of the article; N. E. Roche participated in study design and collection of fetal samples; K. Martimucci participated in collecting fetal tissues; K. Patel and D. S. Heller participated in collecting fetal tissues and study design; M. Kimura directed TL measurements; D. Chuang and A. Chuang participated in sample collections from children; A. Benetos participated in study design, statistical analysis, and writing the article; and A. Aviv oversaw the project and participated in writing the article.

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