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Telomere Length and Age-dependent Telomere Attrition The Blood-and-Muscle Model

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Telomere Length and Age-dependent Telomere Attrition -The Blood-and-Muscle Model-

Short title: Telomere Dynamics & The Blood and Muscle Model

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ABSTRACT

Short telomere length (TL) is associated with atherosclerotic cardiovascular disease (ACVD) and other age-related-diseases. It is unclear whether these associations originate from having inherently short TL or a faster TL attrition before or during disease development. We proposed the Blood-and-Muscle Model in order to assess TL dynamics throughout life course. Our objective was to measure TL in leukocytes (LTL) and in skeletal muscle (MTL) which served as a proxy of TL at birth. The MTL-LTL delta represented life-long telomere attrition. Blood draws and skeletal muscle biopsies were performed on 35 Lebanese individuals undergoing surgery. Following DNA extraction, LTL and MTL were measured by Southern blot. In every individual aged between 30 to 85 years, MTL was longer than LTL. With age, MTL and LTL decreased, but the MTL-LTL delta increased by 14 bp/year. We validated the Blood-and-Muscle Model that allowed us to identify TL, TL at birth and lifelong TL attrition in a cross-sectional study. This model can be used in larger cross-sectional studies to evaluate the association of telomere dynamics with age-related diseases onset and progression.

Keywords: Aging, Leukocytes, Muscle, Telomere, Southern Blot.

INTRODUCTION

In replicating somatic cells, telomeres, the nucleoproteic structures at the extremities of chromosomes, go through progressive shortening that eventually induces replicative senescence (Blackburn 2005; Van Deursen 2014). During early intrauterine life, the enzyme telomerase adds telomere repeats to the DNA termini (Blackburn 2005), thereby compensating telomere length (TL) shortening. However, telomerase is largely repressed in somatic tissues during extrauterine life (Morrison et al. 1996; Yui et al. 1998) leading to telomere attrition with age.

Many clinical studies have demonstrated the association between short leukocyte telomere length (LTL) and atherosclerotic cardiovascular diseases (ASCVD) (McGrath 2007; Willeit 2010; Weischer 2012; Haycock 2014; D'Mello 2015), arterial aging (aortic stiffness (Benetos et al. 2001), coronary artery calcifications (Hunt et al. 2015; Ormseth et al. 2016), and severity of atherosclerotic plaques in the coronary arteries (Scheller Madrid et al. 2016)), and diminished survival (Deelen et al. 2014). Inflammation and oxidative stress are central factors in aging-related vascular injury, contributing to atherosclerosis and arterial stiffness (Ross 1999). Since both inflammation and oxidative stress are known to increase telomere attrition *in vitro* (von Zglinicki 2002) and in animal models (Cattan et al. 2008), shortened LTL has been considered as a marker of the chronic burden of inflammation and oxidative stress leading to vascular injury (Sahin et al. 2011). On the other hand, many findings support the idea that short LTL precedes by several decades the clinical manifestations of ASCVD and related metabolic risks (Codd et al. 2013; Chen et al. 2014; Zhao et al. 2014; Scheller Madrid et al. 2016; Verhulst et al. 2016). Consequently, LTL, may be an active determinant in the development of the disease later in life, and hence plays a role in causal pathways rather than being exclusively a risk marker for ASCVD (Toupance et al. 2017; Shabharwal et al. 2018; Benetos et al. 2018). Such outcomes raise the question of whether the associations of LTL

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with the diseases cited above reflect: 1) an inter-individual LTL variation already apparent since development; 2) a variation in the rate of age-dependent LTL attrition afterward, or 3) both (Shabharwal et al. 2018). As a matter of fact, LTL dynamics are mainly determined by: a) an Inter-individual variation of 4 to 6 kb in leukocyte TL commonly displayed in newborns (Rufer et al. 1999; Okuda et al. 2002; Akkad et al. 2006) and throughout the human lifetime (Rufer et al. 1999; Barbieri et al. 2009; Benetos et al. 2011); and b) an intra-individual variation, age-dependent LTL attrition that is highly variable among individuals (Chen et al. 2011; Benetos et al. 2011).

Longitudinal studies of LTL dynamics in which individuals are monitored from birth onward, could help to determine the timing in the widening of the LTL gap between subjects who develop ASCVD versus those who do not. However, this might be time- consuming and extremely costly (Benetos et al. 2018). On the other hand, cross-sectional studies would be more suitable, yet they would require large sample of individuals with a large age range and they would determine age-dependent LTL attrition only by group, but not individually (Benetos et al. 2011). Instead, we have proposed the blood-and –muscle model by considering skeletal muscle TL (MTL) and Leukocyte TL (LTL) to generate quasi-longitudinal data on TL dynamics in the individual (Aviv et al. 2015). The mean skeletal muscle shows almost no change between the ages of 20 to 80 years (Ponsot et al. 2008), suggesting little replicative activity, whereas leucocytes undergo tremendous turnover (Summers et al. 2010). In this pilot study, we tested a model of TL dynamics to evaluate the possible role of inherently short TL versus a faster age-dependent LTL attrition in an ethnic group other than individuals of European ancestry, such as in Lebanese adults. The validation of this model could allow us to study the relation between telomere dynamics and development of age-related disease such as ACVD as well as metabolic and neurodegenerative diseases in bigger cohorts.

MATERIALS AND METHODS

The Blood-and-Muscle Model

We applied the blood-and-muscle model to gain further understanding of TL dynamics in a Lebanese population. The designed model uses 1) TL in skeletal muscle, a minimally replicating somatic tissue, as an internal reference or a proxy of TL early in life, 2) TL in Leukocyte, a highly proliferative hematopoietic system, and 3) the difference between leukocyte and skeletal muscle TLs (ΔLMTL) which provides a broad account of leukocyte TL dynamics or the extent of LTL shortening over the life course of the individual or an estimate of LTL attrition up to the sampling age (**Figure 1**) (Benetos et al. 2011, 2018; Shabharwal et al. 2018).

We initially developed this blood-and muscle model in dogs (Benetos et al. 2011), subsequently examined it in adult humans (Daniali et al. 2013), then during the first 2 decades of life, including intrauterine life (Shabharwal et al. 2018), as well as in ASCVD patients of European descentance (Benetos et al. 2018).

Study design and population

This research is part of the ITS (International Telomere Study) that examined the role of telomere dynamics in arterial aging and the development of atherosclerosis in 3 centers: France, Greece, and Lebanon in 158 subjects classified as ACVD patients/ Morbid obese /Renal failure patients/ Control subjects. Here, we only presented data from Lebanese patients (N=35 subjects). We focused our work on measuring TL in skeletal muscle and leucocytes of patients undergoing surgery. For this purpose, we enrolled men and women (≥ 20 year-old) admitted for surgery including inguinal herniorrhaphy, cardiovascular (CV) surgery or pacemaker/ defibrillator implantation. We excluded

patients with an active malignancy, or history of chemotherapy/radiotherapy, or went through a cancer surgery.

Data collection

The study was approved by the research ethics committee of the hospitals, and all participants signed an informed consent form. At that time, the doctor in charge collected socio-demographics data (age, sex, body weight, height, educational level), clinical data (clinical history, medications, blood pressure, and heart rate), the standard blood chemistry data (lipids, glycemia, blood count, renal function), cigarette (current, ex-smoker, non-smoker), and alcohol consumptions. Just prior to surgery, 5 to 10ml of blood were extracted from each patient on EDTA tubes. During the surgery, 100 to 200 mg of skeletal muscle biopsies in the surgical field were performed. Biopsies were weighed out before storing them with the blood tubes in -80°C freezer.

DNA extraction and telomere length measurements

DNA was extracted by the phenol/chloroform method from peripheral blood leukocytes and skeletal muscle (≈ 100 mg) as previously described (Kimura et al. 2010b). Briefly, total leukocytes were obtained from whole blood after red cell osmotic lysis and muscle tissue was pulverized in liquid nitrogen. Pelleted leukocytes and ground tissue were lysed overnight in presence of proteinase K. Protein, RNA and other contaminants were removed by phenol-chloroform organic extraction and DNA recovered by absolute ethanol precipitation. After 70% ethanol washing, DNA was solubilized in Tris-EDTA buffer and DNA quantity determined by UV spectroscopy. Aliquots were resolved in 1% (w/v) agarose gel to test for DNA integrity. No sample showed evidence of

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DNA degradation. DNA extractions were performed in Beirut, Lebanon at the Lebanese University. All DNA samples were then sent to University of Nancy, France for TL measurements. LTL and MTL measurements were performed by Southern blots of the terminal restriction fragments, as previously described (Kimura et al. 2010b). Briefly, DNA samples were digested (37°C) overnight with restriction enzymes *HinfI* and *RsaI* (Roche Diagnostics GmbH, Germany). Digested DNA samples and DNA ladders were resolved on 0.5% (wt/vol) agarose gels. Then, 23 hours later, the DNA was depurinated, denatured, neutralized, and transferred onto a positively charged nylon membrane (Roche) using a vacuum blotter (Biorad, Hercules, CA). Membranes were hybridized at 42°C with the digoxigenin-labeled telomeric probe after which the probe was detected by the digoxigenin luminescent detection procedure (Roche) and exposed on charge-coupled device camera (Las 4000, Fuji). Measurements were performed in duplicate on separate gels with analyses based on the average of these 2 measurements. The baseline and follow-up samples from each individual were run in adjacent lanes. The interassay coefficient of variation for the duplicate measurements (on different gels) was 1.2%.

Statistical Analysis

Continuous variables are presented as mean $\pm$ SD and discrete variables as percentages. Telomere length values are presented and compared with or without adjustment to age and sex. Bivariate relationships between continuous variables were determined using Pearson's correlation coefficients. For categorical variables, analyses were carried out using general linear models (ANOVA). A p value < 0.05 was considered significant. Statistical analyses were carried out using the NCSS 9 statistical software package (NCSS, Kaysville, UT).

RESULTS

A- International Telomere Study- Lebanese population

Characteristics of participants of this study are summarized in **Table 1**. The total of recruited subjects were 35, aged in average 60.4 year-old (± 12.4), in majority men (94%) with an average BMI of $26.4(\pm 3.9)$. Almost half of them were active smokers or ex-smokers (46%). They presented some cardiovascular risk factors, such as hypertension (40%), dyslipidemia (23%), diabetes (14%), and atheroma (11%).

LTL and MTL were strongly correlated ($R^2=0.50$; $p < 0.0001$), but MTL was longer (8.60 ± 0.52 kb) than LTL (6.84 ± 0.67 kb) in all subjects ($n=35$) (**Table 1 & Figure 2**). The mean difference MTL-LTL that represents lifelong LTL attrition was 1.76 ± 0.48 kb (**Table 1**).

B- Effect of Age on TL:

Both LTL and MTL shortened with age, but only LTL showed a statistically significant attrition with time ($p=0.02$) (**Figure 3A**). MTL tended to show a much slower shortening with age ($p=0.35$) (**Figure 3B**); Indeed, the slope of the effect of age was much steeper for LTL (**Figure 3A**; LTL = - 21bp/year) than MTL (**Figure 3B**; MTL = - 7bp/year). In addition the delta (MTL - LTL) increased significantly with age ($p=0.03$) with a rate of 14bp/year (**Figure 3C**).

C- Smoking and BMI Associations With LTL and MTL

Both age- and sex-adjusted LTL and MTL showed no statistical significant difference between never smokers versus smokers (LTL, never-smokers = 6.83 ± 0.14 kb and smokers = 6.85 ± 0.16 kb, $P=0.91$; MTL, never-smokers = 8.58 ± 0.12 kb and smokers = 8.61 ± 0.13 kb, $P=0.88$) (Note that the smokers group is composed of current and ex- smokers). No significant association was either

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observed between age- and sex-adjusted BMI and LTL or MTL (BMI, kg/m², regression slope coefficient 0.029 ± 0.030 kb, $P=0.34$, for LTL, and 0.001 ± 0.025 kb, $P=0.96$ for MTL).

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DISCUSSION

The objective of this study was to determine whether the blood-and-muscle TL model might reveal insight into telomere biology in an ethnic group other than individuals of European ancestry, such as the Lebanese population. The findings of the present pilot study, part of the International Telomere Study (ITS), mostly confirm the blood-and-muscle model developed in adults by our team in the French and Greek populations (Benetos et al. 2018), and North-American (black and white) populations (Daniali et al. 2013), as well as in mammals (Benetos et al. 2011). It highlights an age-dependent TL attrition in leukocytes and muscle across more than 5 decades (individuals aged from 30 to 85 years) of adult life, despite different replicative status (**Figure 3**). It shows essentially the strong correlation between MTL and LTL, yet with LTL being shorter than MTL, and this gap widening gradually due to the faster rate of LTL attrition compared to MTL attrition (**Figure 2**). Indeed, we demonstrated that MTL tended to display a much slower shortening with age than LTL (**Figure 3**). In addition, the delta MTL-LTL of participants aged 60.4 ± 12.4 years in this study was 1.76kb which was very similar to the one found in a study we performed in a larger French population of 259 individuals, the TELARTA study, with participants of 63 years of age and a delta of 1.87kb (Benetos et al. 2018). Results obtained in a younger population (mean age of participants of 44 years) showed a smaller delta of 1.55kb (Daniali et al. 2013) confirming that the MTL-LTL delta is widening with age.

Our results underscore that TL varies much more inter-individually than intra-individually between tissues (even if there is an age-dependent widening of the delta MTL-LTL): an individual with a short or long LTL shows correspondently a short or long MTL. There is, therefore, an intra-individual correlation in TL across the somatic tissues in humans (Youngren et al. 1998; Okuda et al. 2002; Kimura et al. 2010a), and other mammals (Benetos et al. 2011; Smith et al. 2011).

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However a study of our group also using the blood-and-muscle model has shown that, despite this synchrony, difference in LTL between groups can arise from different lifelong attrition (MTL-LTL) in individuals with similar MTL (Benetos et al. 2018). This study in adults did not find any difference in the pace of MTL-LTL gap widening between groups indicating that the difference stems from TL dynamics before adulthood. Another blood-and-muscle model study in fetuses and children (Shabharwal et al. 2018) support that hypothesis showing TL to be mainly determined very early in life with a 1kb widening of delta MTL –LTL already registered before adulthood (Shabharwal et al. 2018). More specifically, variation in TL attrition in leukocytes and muscle across individuals during the first decade of life explained a large proportion of the MTL-LTL gap in adults. Consequently, it is of interest to understand the factors that can affect human TL dynamics during this critical period (**Figure 4**).

Finally, we would like to underline the *strengths and limitations of this study*: 1) First, our study design was cross-sectional and we used tissues obtained during elective surgeries from individuals of different ages to assess TL dynamics; however, most of the surgeries were an inguinal herniorrhaphy which can cause a selection bias; 2) Second, our sample size was very small (only n=35), however we tried to counterbalance this limitation by the high reproducibility of the Southern blot measurements of TL. Moreover, we replicated these findings in the other populations (French and Greek) of the ITS (unpublished data).

In summary, the ITS is the third study following the Daniali et al study and the TELARTA study using the blood-and-muscle TL model in adults. Skeletal muscle can now be considered a reference for deriving proxy of TL data at birth. Hence, there is no particular need to perform lifelong longitudinal studies any longer since the validation of the Blood-and-Muscle Model allowed us to have access to TL, TL at birth and lifelong TL attrition through a cross sectional study (**Figure 5**).

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Therefore, future studies applying this model will allow us to study the relation between telomere dynamics and development of age-related disease such as ACVD, metabolic and neurodegenerative diseases. In addition, longitudinal studies during the first decade of life will determine how early extra-uterine factors might influence TL dynamics with consequences during the entire life course.

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DISCLOSURE

No conflict of interest.

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REFERENCES

- Akkad A., Hastings, R., Konje, J.C., Bell, S.C., Thurston, H., and Williams, B. 2006. Telomere length in small-for-gestational-age babies. *BJOG*. **113**:318–323. [PubMed: 16487204]
- Aubert, G., Baerlocher, G.M., Vulto, I., Poon, S.S., and Lansdorp, P.M. 2012. Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. *PLoS Genet*. **8**: e1002696. (doi:10.1371/journal.pgen.1002696)
- Aubert, G. and Lansdorp, P.M. 2008. Telomeres and aging. *Physiol. Rev.* **88**:557–579. (doi:10.1152/physrev.00026.2007)
- Aviv, A. 2018. The mitochondrial genome, paternal age and telomere length in humans. *Phil. Trans. R. Soc.* **B 373**: 20170210. <http://dx.doi.org/10.1098/rstb.2017.0210>
- Aviv, A., Kark, J. D., and Susser, E. 2015. Telomeres, atherosclerosis, and human longevity: a causal hypothesis. *Epidemiology* **26**: 295–299.
- Aviv, A. and Jerry W. Shay, J.W. 2018. Reflections on telomere dynamics and ageing-related diseases in humans. *Phil.Trans. R. Soc.* **B 373**: 20160436. <http://dx.doi.org/10.1098/rstb.2016.0436>
- Barbieri M, Paolisso G, Kimura M, Gardner JP, Boccardi V, Papa M, Hjelmberg JV, Christensen K, et al. 2009. Higher circulating levels of IGF-1 are associated with longer leukocyte telomere length in healthy subjects. *Mech Ageing Dev.* **130**:771–776. [PubMed: 19913048]
- Benetos, A., Kimura, M., Labat, C., Buchoff, G.M., Huber, H., Labat, L., Xiaobin Lu, and Aviv, A. 2011. A Model of Canine Leukocyte Telomere Dynamics Aging Cell. **10**(6): 991–995. doi:10.1111/j.1474-9726.2011.00744.x.
- Benetos, A., Toupance, S., Gautier, S., Labat, C., Masayuki, K., Rossi, P.M., Settembre, N., Hubert, J., et al. 2018. Short Leukocyte Telomere Length Precedes Clinical Expression of Atherosclerosis The Blood-and-Muscle Model. *Circ Res.* 122:616-623. DOI: 10.1161/CIRCRESAHA.117.311751.
- Blackburn EH. 2005. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett.* **579**:859–862. doi:10.1016/j.febslet.2004.11.036.
- Cattan, V., Mercier, N., Gardner, J. P., Regnault, V., Labat, C., Mäki-Jouppila, J., Nzietchueng, R., Benetos, A., et al. 2008. Chronic oxidative stress induces a tissue specific reduction in telomere length in CAST/Ei mice. *Free Radic Biol Med.* **44**(8):1592–8.
- Chen, W., Kimura, M., Kim, S., Cao, X., Srinivasan, S.R., Berenson, G.S., Kark, J.D., and Aviv, A. 2011. Longitudinal vs. Cross-Sectional Evaluations of Leukocyte Telomere Length Dynamics: Age-Dependent Telomere Shortening is the Rule. *J Gerontol Biol Sci Med Sci.* **66**:312–319.

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Chen, S., Yeh, F., Lin, J., Matsuguchi, T., Blackburn, E., Lee, E.T., Howard, B.V., and Zhao, J. 2014. Short leukocyte telomere length is associated with obesity in American Indians: the strong heart family study. *Aging (Albany NY)*. **6**:380–389. doi: 10.18632/aging.100664.

Codd, V., Nelson, C. P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J. L., Hottenga, J. J., and Fischer, K. 2013. Identification of seven loci affecting mean telomere length and their association with disease. *Nat. Genet.* **45**:422–427 e1–e2.

Dalgård, C., Benetos, A., Verhulst, S., Labat, C., Kark, J.D., Christensen, K., Kimura, M., Kyvik, K.O., et al. 2015. Leukocyte telomere length dynamics in women and men: menopause vs age effects *International Journal of Epidemiology*, **44**(5):1688–1695 doi: 10.1093/ije/dyv165

Daniali, L., Benetos, A., Susser, E., Kark, J.D., Labat, C., Kimura, M., Desai, K., et al., 2013. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun.* **4**:1597. doi: 10.1038/ncomms2602.

Deelen, J., Beekman, M., Codd, V., Trompet, S., Broer, L., Hägg, S., Fischer, K., Thijssen, P.E. 2014. Leukocyte telomere length associates with prospective mortality independent of immune-related parameters and known genetic markers. *Int J Epidemiol.* **43**(3):878–886. doi:10.1093/ije/dyt267.

De Meyer, T., Rietzschel, E.R., De Buyzere, M.L., De Bacquer, D., Van Criekinge, W., De Backer, G.G., Gillebert, T.C., Van Oostveldt, P., et al. 2007. Paternal age at birth is an important determinant of offspring telomere length. *Human molecular genetics*. **16**(24):3097–102. Epub 2007/09/21. doi: 10.1093/hmg/ddm271 PMID: 17881651.

D'Mello, M.J., Ross, S.A., Briel, M., Anand, S.S., Gerstein, H., and Paré, G. 2015. Association between shortened leukocyte telomere length and cardio-metabolic outcomes: a systematic review and meta-analysis. *Circ Cardiovasc Genet.* **8**:82–90. doi: 10.1161/CIRCGENETICS.113.000485.

Hansen, M.E., Hunt, S.C., Stone, R.C., Horvath, K., Herbig, U., Ranciaro, A., Hirbo, J., Beggs, W., et al. 2016. Shorter telomere length in Europeans than in Africans due to polygenetic adaptation. *Human Molecular Genetics*. **25**(11):2324–2330. DOI: 10.1093/hmg/ddw070

Haycock, P.C., Heydon, E.E., Kaptoge, S., Butterworth, A.S., Thompson, A., and Willeit, P. 2014. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ.* **349**:g4227.

Hunt SC, Chen W, Gardner JP, Kimura M, Srinivasan SR, Eckfeldt JH, Berenson, G.S., Aviv, A. 2008. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. *Aging cell.* **7**(4):451–8. Epub 2008/05/09. doi: 10.1111/j. 1474-9726.2008.00397.

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Hunt, S.C., Kimura, M., Hopkins, P.N., Carr, J.J., Heiss, G., Province, M.A., and Aviv, A. 2015. Leukocyte telomere length and coronary artery calcium. *Am J Cardiol.* **116**:214–218. doi: 10.1016/j.amjcard.2015.03.060.

Kimura, M., Gazitt, Y., Cao, X., Zhao, X., Lansdorp, P.M., and Aviv, A. 2010a. Synchrony of telomere length among hematopoietic Cells. *Exp Hematol.* **38**:854–859. [PubMed: 20600576]

Kimura, M., Stone, R.C., Hunt, S.C., Skurnick, J., Lu, X., Cao, X., Harley, C. B., and Aviv, A. 2010b. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. *Nat. Protoc.* **5**:1596–1607

McGrath, M., Wong, J.Y., Michaud, D., Hunter, D.J., and De Vivo, I. 2007. Telomere length, cigarette smoking, and bladder cancer risk in men and women. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* **16**:815–819.

Mangino, M., Christiansen, L., Stone, R., Hunt, S.C., Horvath, K., Eisenberg, D.T.A., Kimura, M., Petersen, I. 2015. DCAF4, a novel gene associated with leucocyte telomere length *J Med Genet.* **52**:157–162. doi:10.1136/jmedgenet-2014-102681

Morrison, S. J., Prowse, K. R., Ho., P. and Weissman, I. L. 1996. Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity* **5**:207–521.

Nawrot, T.S., Staessen, J.A., Gardner, J.P., and Aviv, A. 2004. Telomere length and possible link to X chromosome. *Lancet (London, England).* **363**(9408):507–10. Epub 2004/02/21. doi: 10.1016/s0140-6736(04) 15535-9 PMID: 14975611.

Okuda, K., Bardeguet, A., Gardner, J.P., Rodriguez, P., Ganesh, V., Kimura, M., Skurnick, J., Awad, G., et al. 2002. Telomere length in the newborn. *Pediatr Res.* **52**:377–381. [PubMed: 12193671]

Ormseth, M.J., Solus, J.F., Oeser, A.M., Bian, A., Gebretsadik, T., Shintani, A., Raggi, P., and Stein, C.M. 2016. Telomere length and coronary atherosclerosis in rheumatoid arthritis. *Rheumatol.* **43**:1469–1474. doi: 10.3899/jrheum.151115

Ponsot, E., Lexell, J., and Kadi, F. 2008. Skeletal muscle telomere length is not impaired in healthy physically active old women and men. *Muscle Nerve.* **37**:467–472. [PubMed: 18236464]

Ross R. 1999. Atherosclerosis is an inflammatory disease. *Am Heart J.* **138**(5 pt 2):S419–S420.

Rufer, N., Brümmendorf, T.H., Kolvraa, S., Bischoff, C., Christensen, K., Wadsworth, L., Schulzer, M., and Lansdorp, P.M. 1999. Telomere fluorescence measurements in granulocytes and T lymphocyte subsets point to a high turnover of hematopoietic stem cells and memory T cells in early childhood. *J Exp Med.* **190**:157–167. [PubMed: 10432279]

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Sabharwal, S., Verhulst, S., Guirguis, G., Kark, J.D., Labat, C., Roche, N.E., Martimucci, K., Patel, K., et al. 2018. Telomere length dynamics in early life: the blood-and-muscle model. *FASEB J.* **32**:529–534. doi: 0.1096/fj.201700630R.

Sahin E, Colla S, Liesa M, Moslehi, J., Müller, F.L., Guo, M., Cooper, M., Kotton, D. et al. 2011. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature.* **470**:359–365. [PubMed: 21307849]

Scheller Madrid, A., Rode, L., Nordestgaard, B.G., and Bojesen, S.E. 2016. Short telomere length and ischemic heart disease: observational and genetic studies in 290 022 individuals. *Clin Chem.* **62**:1140–1149. doi: 10.1373/clinchem.2016.258566.

Sidorov, I., Kimura, M., Yashin, A., and Aviv, A. 2009. Leukocyte telomere dynamics and human hematopoietic stem cell kinetics during somatic growth. *Exp. Hematol.* **37**, 514–524. doi:10.1016/j.exphem.2008.11.009

Smith, Jr D. L., Mattison, J.A., Desmond, R.A., Gardner, J.P., Kimura, M., Roth, G.S., Ingram, D.K., Allison, D.B., et al. 2011. Telomere dynamics in rhesus monkeys: no apparent effect of caloric restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **66**:1163–1168.

Stone, R. C., Horvath, K., Kark, J. D., Susser, E., Tishkoff, S. A., and Aviv, A. 2016. Telomere length and the cancer–atherosclerosis trade-off. *PLoS Genet.* **12**: e1006144.

Summers, C., Rankin, S.M., Condliffe, A.M., Singh, N., Peters, A.M., and Chilvers, E.R. 2010. Neutrophil kinetics in health and disease. *Trends Immunol.* **31**:318–324. [PubMed: 20620114]

Toupance, S., Labat, C., Temmar, M., Rossignol, P., Kimura, M., Aviv, A., and Benetos, A. 2017. Short telomeres, but not telomere attrition rates, are associated with carotid atherosclerosis. *Hypertension.* **70**:420–425. doi:10.1161/HYPERTENSIONAHA.117.09354.

Van Deursen, J.M. 2014. The role of senescent cells in ageing. *Nature.* **509**(7501):439–46. doi: 10.1038/nature13193 PMID: 24848057; PubMed Central PMCID: PMC4214092.

Verhulst, S., Dalgård, C., Labat, C., Kark, J.D., Kimura, M., Christensen, K., Toupance, S., Aviv, A. et al. 2016. A short leukocyte telomere length is associated with development of insulin resistance. *Diabetologia.* **59**:1258–1265. doi: 10.1007/s00125-016-3915-6.

von Zglinicki T. 2002. Oxidative stress shortens telomeres. *Trends Biochem Sci.* **27**(7): 339 – 44.

Weischer, M., Bojesen, S.E., Cawthon, R.M., Freiberg, J.J., Tybjaerg-Hansen, A., and Nordestgaard, B.G. 2012. Short telomere length, myocardial infarction, ischemic heart disease, and early death. *Arteriosclerosis, thrombosis, and vascular biology.* **32**:822–829.

Willeit, P., Willeit, J., Brandstatter, A., Ehrlenbach, S., Mayr, A., Gasperi, A., Weger, S., Oberhollenzer, F., et al. 2010. Cellular aging reflected by leukocyte telomere length predicts

advanced atherosclerosis and cardiovascular disease risk. *Arteriosclerosis, thrombosis, and vascular biology*. **30**:1649–1656.

Yui, J., Chiu, C. P., and Lansdorp, P.M. 1998. Telomerase activity in candidate stem cells from fetal liver and adult bone marrow. *Blood*. **91**: 3255–3262.

Youngren, K., Jeanclos, E., Aviv, H., Kimura, M., Stock, J., Hanna, M., Skurnick, J., Bardeguet, A., et al. 1998. Synchrony in telomere length of the human fetus. *Hum Genet*. **102**:640–643. [PubMed:9703424]

Zhao, J., Zhu, Y., Lin, J., Matsuguchi, T., Blackburn, E., Zhang, Y., Cole, S.A., Best, L.G., et al. 2014. Short leukocyte telomere length predicts risk of diabetes in American Indians: the strong heart family study. *Diabetes*. **63**:354–362. doi: 10.2337/db13-0744.

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Legend Tables**Table 1: Characteristics of the participants of the ITS study (Lebanese population)**

BMI indicates body mass index; LTL, leukocyte telomere length; MTL, Muscle Telomere length.

Data are expressed as mean $\pm$ SD or %.

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Legend Figures

Figure 1: The Blood-and-Muscle Model

In order to examine different elements of TL dynamics, the designed blood-and –Muscle model uses: 1) TL in skeletal muscle, a low proliferative index replicating somatic tissue, 2) TL in leukocyte, a highly proliferative index replicating somatic tissue, and 3) the difference between leukocyte and skeletal muscle TLs, $\Delta(\text{MTL-LTL})$, which provides lifelong LTL attrition.

In humans, at birth and in utero TL is highly **synchronized** (equivalent) among cells from different organs and tissues within the individual (Okuda et al. 2002; Youngren et al. 1998; Kimura et al. 2010a; Benetos et al. 2011). During adulthood, LTL becomes **shorter** compared to MTL, thus widening the gap between LTL and MTL. However, TL in tissues stays synchronized (correlated), such that individuals with short (or long) MTL are likely to have short (or long) LTL.

LTL indicates leukocyte telomere length; MTL indicates muscle telomere length;

Figure 2: Leukocyte telomere length vs Muscle telomere length in the International Telomere Study (Lebanese data). Dotted black line represents line of identity (LTL= MTL). The relation between MTL and LTL (blue line) is very strong. The line of identity is higher than the blue line: in all subjects MTL is longer than LTL. LTL indicates leukocyte telomere length and MTL, muscle telomere length.

Figure 3. Telomere length models vs Age in the International Telomere Study (Lebanese data). **A.** Leukocyte telomere length (LTL) vs Age. LTL decreases with age; Slope of -0.021 kb corresponds to a telomeric attrition of 21 bp/year. **B.** Muscle telomere length (MTL) vs Age. MTL decreases with age; Slope of -0.007 kb = telomeric attrition of 7 bp/year: weaker than LTL: Skeletal muscle tissue with low proliferative index. **C.** Difference between leukocyte telomere length (LTL) and muscle telomere length (MTL); Delta (MTL – LTL) increases with age with a rate of 14 bp/year in adults.

Figure 4. Factors affecting human TL dynamics

(a) *Variation within somatic cells of the individual and variation across individuals:* TL varies in different somatic tissues depending of their different replicative history; Indeed, tissues of low proliferative index such as skeletal muscle and fat show longer telomeres than the ones of high proliferative index such as the skin and the haematopoietic system (Daniali et al. 2013; Shabharwal et al. 2018). This variation in TL of somatic cells is observed intra-individually and inter-individually. Certainly, intra-individual differences in TL across leucocyte lineages (Kimura et al. 2010a) or somatic cells (Daniali et al. 2013; Shabharwal et al. 2018) are much narrower than inter-individual TL variations (Aviv and Shay 2018).

(b) *Telomere length dynamics during growth:* The rate of LTL shortening is considerably faster during early stages of human growth and development than throughout adulthood (Aubert et al. 2012; Aubert and Lansdorp 2008), due to the expansion of the haematopoietic system through replications in tandem with the growing soma (Sidorov et al. 2009; Aviv and Shay 2018).

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(c) *The heritability effect on telomere length*: LTL is highly familial with about 65% heritability (Stone et al. 2016). DCAF4 is a novel gene that contributes to LTL variation in humans (Mangino et al. 2015; Aviv and Shay 2018).

(d) *The sex effect on telomere length* : Females have longer LTL than males (Nawrot et al. 2004; Stone et al. 2016) since an estrogen-response element is present in the promoter of the catalytic subunit of telomerase, thus the estrogen can potentially stimulate telomerase (Dalgard et al. 2015; Aviv and Shay 2018).

(e) *The paternal-age-at-conception (PAC) effect on telomere length*: Offspring of older fathers have longer LTL than those of younger fathers (De Meyer et al. 2007; Stone et al. 2016). Actually, variation in the production of ROS due to underlying mitochondrial DNA polymorphism and heteroplasmy might differentially influence TL in sperm by mediating age-dependent selection of germ stem cells with long telomeres which would be transmitted to offspring (Aviv, 2018; Aviv and Shay 2018).

(f) *The ancestry effect on telomere length* African Americans (Hunt et al. 2008) and sub-Saharan Africans (Hansen et al. 2016) have longer LTL than individuals of European ancestry (Stone et al. 2016) because of a high parasitic/infectious load in Africa, which requires a robust immune response throughout the life course and consequently a high proliferative potential due to long TL in cells of the haematopoietic hierarchy (Aviv and Shay 2018).

(g) *Environmental factors*: socioeconomic status, smoking, sedentary lifestyle, energy intake, and perhaps mental stress might be associated with a short LTL (Stone et al. 2016).

Figure 5: Validation of the blood-and-muscle model and benefit for future studies

In a cross-sectional study with only one measurement of LTL, it is impossible to determine if the short or long LTL observed in a subject is due to LTL at birth or LTL attrition. **B.** In a cross-sectional study using the blood-and-muscle model, it is possible with only one time point to evaluate LTL and lifelong LTL attrition at once thanks to the MTL measurement. This study confirmed that MTL experiences very little attrition over the course of 5 decades and can therefore serve as a proxy of LTL at birth. The $MTL - LTL$ delta represents lifelong LTL attrition. LTL indicates leukocyte telomere length; MTL indicates muscle telomere length.

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Table 1

Number	35
Age (years)	60.4±12.4
Gender (Women %)	6 %
Weight (kg)	80.7±13.3
Height (cm)	174.5±4.9
BMI (kg/m²)	26.4±3.9
Smoking (%)	46 %
Atheroma (%)	11 %
Hypertension (%)	40 %
Diabetes (%)	14 %
Dyslipidemia (%)	23 %
LTL (kb)	6.84±0.67
MTL (kb)	8.60±0.52
MTL-LTL (kb)	1.76±0.48