Telomere Length

26

Measurement and Application as a Biological Indicator – Links with Anthropometry in Lifestyle Intervention

A. Marti del Moral and G. Zalba Goñi

Contents

A. Marti del Moral (✉)
Center for Nutrition Research and Department of Nutrition, Food Science and Physiology, Faculty of Pharmacy and Nutrition, University of Navarra and IdiSNA, Pamplona, Spain

Navarra Institute for Health Research (IdiSNA), Pamplona, Spain

CIBER Physiopathology of Obesity and Nutrition (CIBERobn), Carlos III Health Institute, Madrid, Spain
e-mail: amarti@unav.es

G. Zalba Goñi
Department of Biochemistry and Genetics, School of Science, University of Navarra, Pamplona, Spain
e-mail: gzalba@unav.es

V. B. Patel, V. R. Preedy (eds.), *Biomarkers in Nutrition*, Biomarkers in Disease: Methods, Discoveries and Applications,
https://doi.org/10.1007/978-3-031-07389-2_31

Abstract

Abnormal telomere shortening underlies patients' risk of developing age-related degenerative diseases, including metabolic and cardiovascular diseases, and cancers. The measurement of telomere length (TL) and the detection of excessively short telomeres emerge as an important new tool in biomedical research and clinical practice. The first section reviews the main methods for measuring telomere length and compares the technologies, pointing out their advantages and limitations. The second section provides information on the role of TL as a biomarker of response in lifestyle intervention aimed to reduce cardiometabolic risk in adult and pediatric populations. There is evidence that substantial weight loss is able to lower chronic inflammation and adipose tissue oxidative stress which can lead to promote TL conservation and DNA repair, thus reducing telomere attrition. Inconsistent results concerning the benefit of lifestyle intervention on TL suggest the need for more studies – probably devoted to the measurement of ***excessively short telomeres*** – before its clinical application in routine use as biomarker.

Keywords

Short telomere · High throughput · Obesity · Anthropometric indexes · Dietary intervention · Lifestyle changes · Telomere length · Pediatric population · Mediterranean diet

Abbreviations

BMI	Body mass index
BMI-SDS	Body mass index standard deviation score
bp	Base pairs
MD	Mediterranean diet
MET	Metabolic equivalent
MMqPCR	Monochrome multiplex qPCR
PA	Physical activity
PCR	Polymerase chain reaction
PPARG2	Peroxisome Proliferator Activated Receptor Gamma 2
Q-FISH	Quantitative Fluorescence in situ Hybridization
qPCR	Quantitative PCR
STAR	Single telomere absolute-length rapid
STELA	Single telomere length analysis
TL	Telomere length
TRF	Terminal restriction fragment
T/S ratio	Telomere/Single copy gene ratio
WHO	World Health Organization

Introduction

Abnormal telomere shortening increases the susceptibility of patients to develop degenerative diseases associated with age, diabetes mellitus, cardiovascular diseases, and cancers. The first section reviews the main methods for measuring telomere length and compares the technologies, pointing out their advantages and limitations. The second section provides information on the role of TL as a biomarker of response in lifestyle intervention aimed to reduce cardiometabolic risk in adult and pediatric populations.

Section 1. Telomere Length: Measurements

Telomeres are regions of repeating noncoding nucleotide sequences (5′-TTAGGG-3′ in humans) at the end of each chromosome. Their main function is to protect the ends of the chromosomes from degradation and maintain the integrity of the chromosomes. The size of human telomeres ranges between 8 and 15 kb after birth, and decreases around 150 bp with each replication. The gradual shortening of telomeres causes them to reach a minimum critical length, which causes the cell to enter a state of senescence.

Abnormal telomere shortening increases the susceptibility of patients to develop degenerative diseases associated with age, diabetes mellitus, cardiovascular diseases, and cancers. In this context, the measurement of telomere length (TL) has experienced growing interest in the field of biomedicine, due to its potential use as a biomarker that can be used both in the diagnosis of diseases associated with age and different cancers, as well as in the response of patients to various therapies to treat these pathologies. Despite this, at present there are still significant technical challenges that limit its use as a daily tool in clinical practice. In order to solve this deficiency, the ideal method for the determination of TL should be simple, precise, and reproducible in any biomedical research laboratory, and fast and high throughput.

In this section, we will review the most used methods to measure TL, comparing them and discussing their advantages and weaknesses (Table 1). Specifically, we will focus on the following methods (Fig. 1):

- Terminal restriction fragments analysis, the gold standard for telomere determination, involves a Southern blot procedure of restriction fragments of genomic DNA.
- Quantitative polymerase chain reaction (PCR), a procedure in which the quantification of telomeres are referred to a single copy gene.
- Quantitative fluorescence in situ hybridization (FISH) approaches, in which the measurement involves the use of digital fluorescence microscopy.
- Single telomere length analysis (STELA), a method based on PCR, after the ligation of a linker to the ends of the chromosomes.
- Single telomere absolute-length rapid (STAR) assay, a novel digital real-time PCR approach.

Table 1 Comparative characteristics of TL measurement methods

Method	Applicability	Amount of starting material	Processing time	Labor intensive	Throughput	Dynamic range	Telomere length distribution	Absolute length
TRF	Research and commercial	> 1 μg	2 days	Yes	32 samples	0.8–20 kb	Yes (semi-quantitative)	Yes
qPCR	Research	10–20 ng	<2 h	No	384 samples	Measures relative length	No	No
Metaphase Q-FISH	Research	20 cells	2 days	Yes	10 samples per week	0.15–80 kb	Yes	Yes
High-throughput interphase Q-FISH	Research and commercial	10,000 cells	2 days	Yes	96 samples	0.2–80 kb	Yes	Yes
STELA, U-STELA	Research	50–200 pg	2 days	Yes	32 samples	0.4–20 kb	Yes, for short telomeres	Yes
STAR	Research	< 1 ng	<3 h	No	48 samples	0.2–320 kb	Yes	Yes

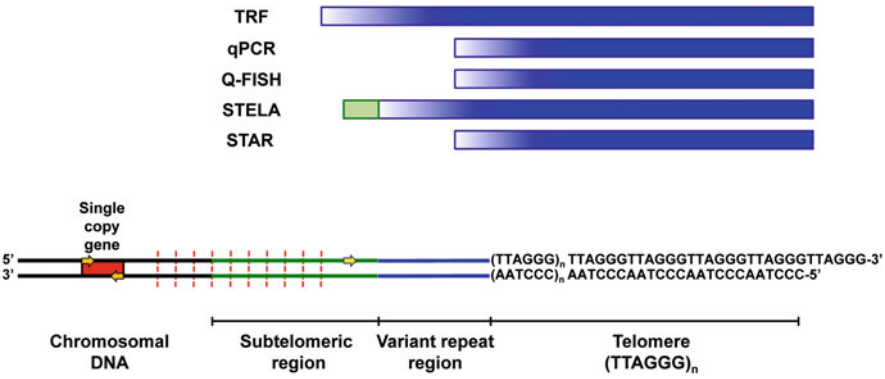

Fig. 1 Representation of chromosomal ends and the procedures for telomere length estimation. Telomeres are represented with the TTAGGG and AATCCC sequence repeat, the variant region (composed of degenerate TTAGGG repeats and telomere associated repeats) is represented by a thick blue line, the subtelomeric region is shown as a thick green line, and the rest of chromosomal DNA is reproduced as a thick black line where a single copy gene (red box) can be amplified by PCR using specific primer (orange arrows). Frequent cutting restriction endonucleases are also illustrated (red dotted lines). The telomere length estimation of the used techniques is represented as the blue colored boxes on the top: TRF includes part of the variant repeat region and subtelomeric region which are not digested by the frequent cutting restriction endonucleases. qPCR methods compare the amplification of telomeric DNA with the amplification of a single copy gene (red box) and can include part of the variant repeat region. Q-FISH probes recognize pure telomeric repeats but tandems of these can be also part of the variant repeat region. STELA uses a specific primer of the subtelomeric region (yellow arrow), but since this region is well characterized it can be subtracted to the telomere length quantification. STAR measures by PCR the absolute lengths and quantities of individual telomeres, after digestion of genomic DNA with restriction endonucleases

Terminal Restriction Fragments (TRF)

TRF analysis was the first technique used to measure TL and is known as the "gold standard" in telomere quantification (Montpetit et al. 2014). This approach is used to calibrate and validate other methods. The procedure begins when genomic DNA is digested with a combination of four base-pair restriction enzymes. These enzymes do not cut telomeres due to the repetitive nature of this sequence (TTAGGG)n, so the telomeric DNA remains intact; the rest of the chromosomal DNA is cut into small fragments (<800 bp). Electrophoresis of the digested genomic DNA and subsequent analysis by Southern blot allows the detection of uncut telomeric fragments, using a radioactive or nonradioactive (TTAGGG)n-labeled probe (Kimura et al. 2010). Each sample will present a smear that represents all the telomeric fragments, the result of the integration of the differences in the length of the 96 telomeres present in each cell, and the differences among the different cells that make up the sample. After quantifying the intensity of the labeled DNA smear and comparing with a DNA ladder with known fragment sizes, it is possible to infer the average.

TRF analysis is a method accurate and with relatively small coefficient of variation that does not require costly or specialized equipment, which provides an

absolute quantification in kilobases of average TL. Since it is not chromosome specific, the result obtained represents the mean TL of the whole simple. Moreover, it is a labor intensive and time-consuming procedure (5–7 days) that requires a great amount of starting DNA (1 μg), thus conditioning its application preferentially to TL determination in blood samples rather than in other tissues. In addition to the characteristic canonical sequence of telomeres, they also contain a subtelomeric region consisting of non-canonical, degenerate telomere repeats, whose length may vary depending on the endonuclease cocktail used. Thus, TRF analysis may overestimate the TL that explains the high variability of results found among different research laboratories. A last disadvantage is its low sensitivity to detect short telomeres (2 kb or less), which have few TTAGGG tandem sequences. Short telomeres are a characteristic of diseases related to senescence and aging, so the inability to detect them represents a significant lack of information (Vera and Blasco 2012).

Some of these limitations can be minimized by quantifying the telomeres of specific chromosomes after sorting by flow cytometry, by using pulsed-field electrophoresis that increases the resolution of the gel, or by reducing the necessary starting amount of genomic DNA (TRF slot) (Marti and Zalba 2017).

Quantitative PCR Methods

Quantitative PCR (qPCR) methods are easy assays that allow the quantification of TL in a small amount of DNA (ng or less). They are rapid, relatively low-cost methods that do not require the use of expensive or specialized equipment and are easily suitable for high-throughput performance, which has led to their use in genetic and epidemiological studies (Cawthon 2002). With this strategy, the canonical sequences of telomeres are specifically counted, without producing interferences derived from the subtelomeric regions.

Due to the repetition of the TTAGGG sequence along the telomeres, the first generation primers used to amplify them were complementary to each other, which favored the formation of primer dimers instead of amplifying the target DNA sequence. This complication was partially saved by the design of primers that bound to the C- and G- regions, but were mismatched at the other bases (Cawthon 2009). In this approach, the telomere (T) measurement is normalized against the measurement of a reference single copy gene (S), which provides a T/S ratio that acts as a relative index of the mean length of telomeres. The amplifications of T and S are carried out in separate tubes, which compromise the precision of the determination.

In an updated approach, both the T and S measurements could be performed within the same tube (Cawthon 2009). In this case, simultaneous amplification generates two amplicons that have relevant differences that allow them to be identified and quantified separately within the same tube. The telomeric amplicon has a relatively low melting temperature (≈81 °C) and is quantified in earlier cycles of the reaction, when the amplicon levels of the single copy gene are not yet detectable. On the other hand, the single copy amplicon has a melting temperature

(≈91 °C) higher than the telomeric one, and it is quantified in subsequent cycles and at a temperature higher than that used for the quantification of the telomeric amplicon. The increase in the quantification temperature allows to measure only the fluorescence generated by single copy amplicon, avoiding interference from the much more abundant telomeric amplicon signal. This method reduces operator-generated variability (pipetting errors), is more economical, and correlates better with other methodological approaches.

Although initially the PCR methods only provided a relative measure of telomeres (the T/S ratio), and not an absolute value (kilobases), this limitation can be solved if a standard curve of telomeric fragments of known length is used in the procedure (Montpetit et al. 2014). Similar to TRF analysis, this methodology only provides the mean TL of a sample; it cannot quantify the length of telomeres in a single cell or a specific chromosome, and it is unable to detect the presence of short telomeres.

A major shortcoming of qPCR approaches is the high variability between determinations made by different laboratories, which limits comparisons between different studies. This may be because the intra- and inter-assay coefficients of variation of qPCR are higher than those found with other procedures (Aviv et al. 2011; Lindrose et al. 2021). This may probably be associated with the absence of standardized protocols (use of different reagents and PCR machines or different single copy genes), so that their optimization could result in a substantial decrease in these variabilities, as has already been suggested by some researchers (Pejenaute and Zalba 2017).

The applicability of qPCR is limited to samples that are normal diploid, and its use is not suitable in cancer studies in which the reference single copy gene may have been duplicated or lost due to aneuploidy, alterations that occur in the majority cancer cells (Lai et al. 2018).

Quantitative FISH

The Quantitative Fluorescence in situ Hybridization (Q-FISH)) technology is based on the hybridization of a fluorescent probe complementary to the TTAGGG repeats sequence of telomeres (Lansdorp et al. 1996; Rufer et al. 1998; Dweck and Maitra 2021). Each probe binds to three TTAGGG telomeric repeats so there is a direct proportion between fluorescence intensity and TL, which can be detect and quantify by digital fluorescence microscopy (Metaphase and Interphase Q-FISH) or flow cytometry (Flow-FISH). A synthetic peptide nucleic acid molecule is mostly used as the fluorescent probe rather than the classical oligonucleotide probe. The probe only binds to the perfect TTAGGG telomeric repeats, so partial hybridizations with the non-canonical repeats of the subtelomeric region cannot occur. However, this probe can also recognize tandems of TTAGGG repeats in intrachromosomal regions, which may generate some false-positive results and produce the consequent overestimation of TL. Q-FISH assays are performed directly in cells which could be fresh, frozen, fixed, or embedded in paraffin, which allow to determine TL of a single

cell (Flow-FISH and Interphase Q-FISH), as well as the telomeric end of a single chromosome (Metaphase Q-FISH). Interestingly, Q-FISH has a low detection limit, which makes it suitable for quantifying critically short telomeres. In fact, this technique made it possible to demonstrate that cell viability and chromosomal stability critically depended on the presence of the shortest telomeres, and not on their average length.

In **metaphase Q-FISH**, the cells are stopped in metaphase and spread on a slide, after which the DNA is fixed and denatured to proceed to hybridize the telomeric DNA with the fluorescent probe. Finally, the images are visualized and acquired with a fluorescence microscope or with a digital imaging system that allows quantifying the fluorescent signals of the telomeres, which are finally transformed into kb values, thanks to the use of control samples of known length (Lansdorp et al. 1996; Pejenaute and Zalba 2017). Metaphase Q-FISH can determine the size of each telomere from a specific chromosome, with a very precise and sensitive estimation of TL (the detection limit is less than 0.15 kb). This technique is capable of detecting chromosomes with critically short telomeres or discovered chromosomes, an advantage of the technique given the relevance of short telomeres in diseases related to senescence and aging. Besides, this technique can combine TL quantification with karyotype analysis and the study of chromosomal abnormalities (chromosome fusion), and is capable of measuring TL even in a small number of rare cells. Unfortunately, metaphase Q-FISH is not applicable in non-dividing cells and is very difficult in samples with a low proliferation rate. This method is time-consuming, expensive, and labor-intensive, limiting its use in large epidemiological studies. It requires a suitable fluorescence microscope and digital camera equipment, which is expensive and requires a properly trained technician (Lai et al. 2018). Furthermore, there is a limitation in the fluorophores that can be used to label the telomeric probe.

Interphase Q-FISH is performed on interphase cells instead of metaphase chromosomes, which solves the deficiency of Q-FISH in metaphase with respect to its limitation of applicability only in proliferating cells, is less laborious, and requires less time (Montpetit et al. 2014; Lai et al. 2018). Nevertheless, it has the disadvantage that it is not able to measure the size of the telomeres of individual chromosomes or to detect the free ends of telomeres. In contrast, the interphase Q-FISH provides a good estimate of the frequency of short telomeres. An enhancement of interphase Q-FISH is high-throughput Q-FISH (HT Q-FISH), a validated, accurate, and sensitive technology, which evaluates interphase nuclei with high-throughput microscopy in a 384-well format. HT Q-FISH is a precise and sensitive technique capable of measuring TL in almost any type of cell. Its speed and automat (it can process 384 samples with 1000 nuclei per sample in 2 h) makes it suitable for large epidemiological studies, being capable of calculating the frequency of short telomeres and even studying individual telomere spots in a cell. However, it cannot detect telomere free ends and requires confocal microscopy equipment.

The **Flow-FISH methodology** combines the basic concepts of FISH applied to the measurement of TL with the use of cell sorting by flow cytometry, which allows FISH to measure TL in individual cells after they have been classified into different

subpopulations (Rufer et al. 1998; Baerlocher et al. 2006). This technique requires cells in suspension so it is applied mainly in the measurement of telomeres of hematopoietic cells. In a first step, nucleated cells are isolated from the sample and prepared for flow cytometry. The DNA is then denatured (applying heat and formamide under controlled conditions), which allows it to hybridize with a fluorescent probe, preferably a peptide nucleic acid probe, which specifically recognizes telomeric DNA. After the corresponding washes, the DNA is counterstained, using a nonspecific DNA fluorescent dye (4′,6-diamidino-2-phenylindole, DAPI), which allows the quantification of the specific fluorescent signal of the telomeres to be normalized. Finally, the acquisition and flow cytometer analysis takes place. For this, cells are sorted and passed one by one through the lasers and the fluorescent signals, both from the telomeres (specific) and from the rest of the DNA (nonspecific) are measured (Lauzon et al. 2000; Baerlocher et al. 2002). The use of control cells of known TL allows the fluorescent signal to be converted into absolute TL values. The reproducibility and precision of this procedure depends on all the experimental steps and conditions (temperature, reagent concentrations, incubation times ...) being perfectly optimized. This technique requires a low number of cells (less than 100,000) per single, and some of the steps in the procedure can be automated, thus requiring less time compared to Q-FISH in metaphase. These characteristics make Flow FISH an accurate method suitable for medium-scale studies. Flow FISH can be adapted for higher throughput, thus permitting some larger scale studies on human lymphocytes. On the other hand, flow FISH only measures mean TL values per cell and cannot detect individual telomeres or telomere free ends. Cells in the sample need to be fixed, so the chemicals used can affect cell surface epitopes or hybridization efficiency. Furthermore, a laborious technique requires a high level of skill to be performed correctly and a flow sorting equipment (Dweck and Maitra 2021).

In **telomapping**, Q-FISH is performed on biopsies of any type of tissue and thus provides added value in preclinical and clinical studies using formaldehyde-fixed paraffin-embedded archival samples.

STELA Methods

STELA, which was designed to study the short arms of sex chromosomes, measures the abundance of the shortest telomeres using a combination of ligation, PCR-based methods and Southern blot analysis (Montpetit et al. 2014; Baird et al. 2003; Dweck and Maitra 2021). STELA is a highly accurate technique that is able to measure TL ranging from 406 bp to 20 kb. STELA technique is capable to measure the length of telomeres in specific chromosomes and detecting critically short telomeres even with limited starting material (few picograms of DNA or as few as 50 cells), which makes this method a good choice for the study of rare cells and no dividing senescent cells. Although it requires no specialized equipment for telomere analysis, it is a labor intensive and technically challenging technique that requires good optimization of the procedure so it has a low-throughput nature and it is not applicable to large studies.

Not all chromosomal ends have unique sequences, which restricts, a priori, the number of chromosome ends that can be analyzed with this technique. The development of Universal STELA (U-STELA) procedure solved this problem (Bendix et al. 2010). This improved method uses small amounts of DNA and is capable of detecting telomeres at all ends of chromosomes, which makes it possible to monitor changes in the shortest telomere of cells, although it is not effective in detecting TL greater than 8 kb.

U-STELA allows the detection of critical short telomeres in all chromosomes using small amounts of DNA, solving the limitation of conventional STELA. However, it is less useful when measuring mean TL and like conventional STELA is not able to detect telomeres longer than 20 kb.

Single Telomere Absolute-Length Rapid (STAR)

The STAR assay is a novel high-throughput digital real-time PCR approach that measures the absolute lengths and quantities of individual telomeres, in less than 3 h. The STAR assay is an accurate, reproducible, powerful tool that enables the use of TL distribution as a biomarker in disease and population-wide studies (Luo et al. 2020).

Digital PCR platforms allow the random encapsulation of individual molecules, with high throughput and minimal reagent consumption (Fig. 2). After digesting the genomic DNA with restriction enzymes that do not cut within the telomeric sequence, the telomere-specific primers and the qPCR mixture are added, and the mixture is compartmentalized (nanoliters) on the digital PCR chip, with the dilution

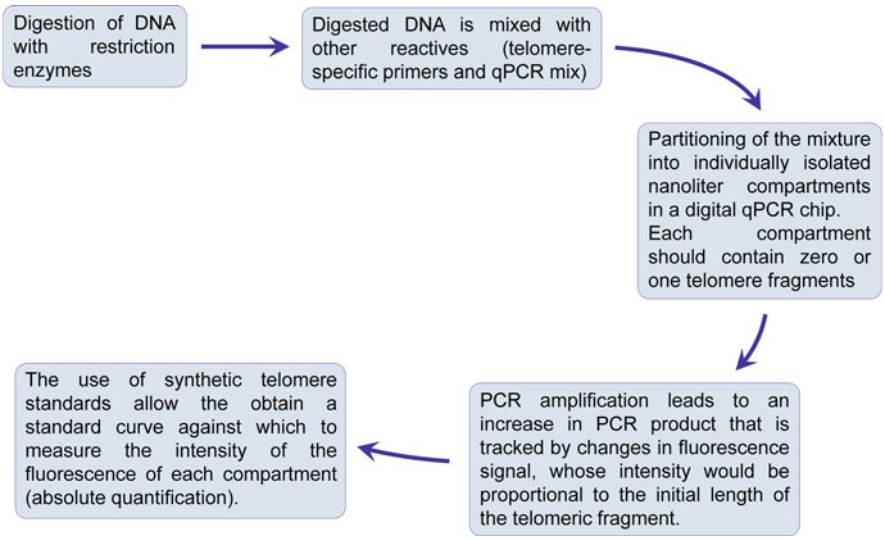

Fig. 2 Representative scheme of the STAR assay procedure

adequate to ensure that each compartment will contain only one telomeric fragment (Dweck and Maitra 2021). With the development of well-characterized standards, this approach allows to measure absolute TL. Thus, combining multiple short, synthetic telomeric sequences (90 bp sequences) that serve as long telomere standards, the STAR assay has a wide dynamic range (0.2–320 kb). Finally, this procedure unbiases comparison across laboratories.

Main Remarks

Numerous methods allow the measurement of TL, although there is no single technique capable of being precise, sensitive, fast, and simple at the same time. Depending on its strengths and limitations, choosing the most appropriate method is critical.

On the one hand, the type of measurement obtained is crucial, since some methods only quantify the mean length of telomeres in a sample (such as TRF and qPCR), of individual cells (FISH flow), or of telomeric stains (interphase Q-FISH). U-STELA and Q-FISH in metaphase can measure the length of individual telomeres of all chromosomes. Given the relevance that the possibility of detecting extremely short telomeres or free ends has acquired in recent years, this critical aspect must be considered when selecting the appropriate method (metaphase Q-FISH, STELA, U-STELA and STAR).

Regarding the type of sample used, all Q-FISH methods require live cells, while TRF, qPCR, STELA, and STAR use DNA directly. Metaphase Q-FISH requires proliferating cells and cannot be performed with senescent cells. On the other hand, and regarding the availability of starting material, TRF is the method that requires the largest amount of sample, while STELA, metaphase Q-FISH, interphase Q-FISH, and STAR require the least amount.

The quantification of telomeres in mice should not be performed with STELA and U-STELA since the upper limit of detection (20 kb) is below the size of murine telomeres. Lastly, TRF, metaphase Q-FISH, and STELA can process a small number of samples, while qPCR, interphase Q-FISH, flow FISH, and STAR are suitable for large-scale studies.

Section 2. Telomere Length: Links with Anthropometry in Lifestyle Intervention

The prevalence of obesity is raised in children and adult's populations worldwide and, due to its impact on the cost of the healthcare system (nearly 21% of all medical spending in the USA), is a number one public health problem. In 2016, globally approximately 39 million children under 5 years of age were obese and 340 million children and adolescents (5–19 years) were overweight or obese (WHO 2021). In Europe 59% of the subjects have either overweight or obesity. Recently, the Europe

Commission recently had classified obesity as a chronic relapsing disease, gateway to other noncommunicable diseases (Burki 2021).

According to the World Health Organization (WHO) obesity is described as an excess fat mass that increases the risk of morbidity, impairment of physical, psychological, or social well-being and/or mortality. It is a multifactorial disease whose etiology involves genetic, metabolic, hormonal, behavioral, environmental, psychological, economic, and social factors (Burki 2021; Marti and Aguilera 2018). It is characterized by an excessive accumulation of energy as fat in the body, leading to an increase in body weight in relation to the expected value according to sex, height, and age. Inflamed or dysfunctional adipose tissue releases inflammatory mediators that trigger low-grade systemic inflammation with an imbalance of oxidative stress that affects the different organs (liver, pancreas, kidney, vascular endothelium, muscle, among others) and increases cardiometabolic risk (Marti and Aguilera 2018). Hence, there is evidence from epidemiological studies that obesity and mortality rate are related, and that as the BMI increases, the risk of suffering from diabetes, cardiovascular disease, cancer, or others diseases also increases.

Inflammation and Oxidative Stress Linked to Obesity and TL

Obesity is associated with both a low-grade inflammation and oxidative stress processes increasing cardiometabolic risk (Marti and Aguilera 2018). Oxidative stress could be defined as the imbalance between production of reactive oxygen species and the antioxidant systems, leading to oxidative damage to cells. Reactive oxygen species are chemical species containing free radicals, namely, hydroxyl radical (OH–), superoxide anion (O_2–), and hydrogen peroxide (H_2O_2). Reactive oxygen species production in adipocytes is mainly caused by the catalytic activity of nicotinamide adenine dinucleotide phosphate oxidase (Marti and Aguilera 2018). Excess adipocytes in obesity lead to increased reactive oxygen species and, as a consequence, increased oxidative stress. Thus, several studies have correlated obesity with oxidative stress (Marti and Aguilera 2018).

Obesity-related inflammation and oxidative stress may modify telomere length (Marti and Zalba 2017). The production of reactive oxygen species could damage all components of the cell, including proteins, lipids, and DNA. The repetitive DNA sequences at telomeres adopt a specific structure called a t-loop structure that is composed of shelterin complexes that protect the DNA. Telomeres are highly sensitive to oxidative stress damage due to their high content of guanines. In fact, short and dysfunctional telomeres are the starting point for cellular senescence, cell death, and DNA instability, being 8-oxo-7,8-dihydroguanine the reactive oxygen species most abundant in senescent cells (Welendorf et al. 2019).

In the literature the relationship between BMI and TL was robustly analyzed in young, middle-aged and older subjects in a meta-analysis with data from 146,114 individuals (87 observational studies). Higher BMI was associated with shorter telomeres, especially in younger subjects (18–60 years old). Notably, telomere loss was −3.99 bp and −7.67 bp per unit increase in BMI in the total sample and in

young adults, respectively (Gielen et al. 2018). A substantial number of observational studies have described associations between modifiable lifestyle behaviors and TL (Arsenis et al. 2017; Marti et al. 2017; Galiè et al. 2020; Canudas et al. 2020; Ojeda-Rodríguez et al. 2021; Alonso-Pedrero et al. 2022), which has led to growing interest in determining whether intervention studies based on lifestyle changes would influence TL.

Lifestyle Factors for Obesity Development

Childhood and adolescence are stages of life in which children acquire and learn healthy or unhealthy lifestyle habits. In this sense, the environment in which these young people live will influence their behavior. As already mentioned, obesity has been described as the result of an imbalance between energy intake and energy expenditure. Today, children have been described as being in an "obesogenic" environment that is characterized by sedentary behaviors and unhealthy diets (Lanigan 2018).

In addition, the family environment can influence children's adiposity through different pathways: (1) the availability of different types of food in the home, (2) the dietary patterns followed by the family, and (3) eating behaviors, where parental attitudes toward children's eating behavior have been shown to have an effect on adiposity levels (Brown and Perrin 2018). Recently, it has been described that unhealthy dietary patterns established during childhood (< 2 years of age) continue into adolescence (Luque et al. 2018).

In relation to lifestyle, in modern society food and drink are more accessible than ever and few children need to engage in a high level of physical activity to get around or choose to do so in their leisure time. Inadequate diet due to excess energy intake, increased consumption of highly palatable foods and sugary drinks, skipping breakfast and snacking between meals are risk behaviors for obesity. Regarding the relationship between physical activity and obesity, high levels of moderate to vigorous PA have been shown to have a protective effect on obesity, while sedentary behavior is a risk factor. Furthermore, the promotion of physical activity in the family context is recommended as a preventive strategy for pediatric obesity (Foster et al. 2018). It should be noted that children's and young people's leisure time is increasingly physically inactive, with time spent watching television and playing video games (sedentary activity) playing a major role.

Obesity has increased over the last 50 years, reaching high levels in many countries. It is considered the number one health problem of the twenty-first century and multiple approaches have been proposed to reduce the number of obese individuals. As obesity in childhood continues into adulthood, treatment and prevention in the youth population is a key factor in reducing its incidence.

Intensive lifestyle programs have been shown to be the best approach to reduce excess weight. Obesity is characterized by excess energy intake and low energy expenditure. For this reason, strategies aimed at modifying these aspects should be considered. They consist of a combination of three areas: dietary treatment,

promotion of physical activity, and behavioral therapy. Therefore, a multidisciplinary team composed of physicians, dietitians, psychologists, nurses, and physical activity experts is needed to conduct lifestyle interventions. They are the gold standard for reducing cardiometabolic risk through lifestyle change and weight loss.

Dietary Treatment

Dietary recommendations for obesity include nutritional counseling, reducing refined carbohydrates, baking, meat and saturated fat intake, and increasing consumption of fruits, vegetables, whole grains, fish, and legumes. There are different dietary patterns that have been shown to be effective for weight loss and improvement of metabolic comorbidities. These patterns are known under the following names: Optimized Mixed Diet, the Mediterranean Diet (MD), the New Nordic Diet, and the dietary approach to stop hypertension, also known as the DASH dietary pattern.

The Mediterranean Diet is a healthy dietary pattern typical of countries bordering the Mediterranean Sea such as Greece, Italy, Spain, and others. High adherence to the DM has been found to be associated with lower increases in BMI (Tognon et al. 2014). It is characterized by a high consumption of vegetables (2 servings per day), fruits (3 servings per day), whole grains, legumes, nuts and olive oil, a moderate consumption of poultry, fish, and dairy products; and a lower consumption of processed meats. This pattern is accompanied by different lifestyle factors, such as the way food is cooked, the use of spices and herbs instead of salt, physical activity or the enjoyment of food with family or friends. Obese children following a lifestyle intervention with DM have been shown to decrease BMI-SDS and some components of metabolic syndrome such as triglycerides, HDL-cholesterol, and glucose levels. The presence of metabolic syndrome was reduced in 45% of participants (Velázquez-López et al. 2014). Furthermore, in the PREDIMED study, following a Mediterranean-type dietary pattern in adult subjects at high cardiovascular risk has been shown to be a prevention strategy in cardiovascular disease endpoints (Estruch et al. 2018; García-Calzón et al. 2017).

Promoting Physical Activity

As mentioned above, obesity is the result of an imbalance between energy intake and energy expenditure, with physical activity being a key point in the treatment of obesity. The main problems of obese young people are: (1) lack of physical activity (PA) and (2) high levels of sedentary activity.

The WHO advises that children and adolescents should spend more than 60 min per day in moderate to vigorous physical activity that leads to an increase in heart rate of at least 65–70%. This recommendation was based on evidence of favorable associations between PA and adiposity or cardiometabolic biomarkers. Several studies report that European children do not reach this recommendation, with the average time spent in moderate to vigorous PA being 36 min/day (Konstabel et al. 2014). Neither obese nor overweight European children and adolescents follow the PA recommendations (Hughes et al. 2006). A recent meta-analysis has shown that obese children are less active than those of normal weight (Poitras et al. 2016).

On the other hand, lifestyle interventions based on dietary changes and promotion of physical activity or behavioral therapy have shown to be more effective than dietary interventions alone (Henriksson et al. 2018).

Notably, sedentary lifestyles have been considered one of the main risk factors for obesity. In this line, several global organizations have expressed the recommendation of not spending more than 2 h a day on screen activities such as: watching TV, playing video games, and spending time with tablets or smartphones. Screen time is used to extrapolate sedentary behavior because it accounts for the majority of sedentary time in children and is associated with adverse health effects (Henriksson et al. 2018).

Furthermore, it is important that lifestyle interventions do not focus physical activity recommendations on a single behavior (PA, sedentary or sleep time), but take into account all 24 h of the day. A recent review found that children and adolescents with lower levels of adiposity were those with a combination of optimal physical activity and behavior (i.e., high PA, low sedentary time, and sufficient sleep) (Chaput et al. 2017).

Behavioral Therapy

One of the main goals of lifestyle intervention is to achieve an improvement in anthropometric and metabolic outcomes, and to maintain the habits that have led to this improvement (van Hoek et al. 2016). In this context, behavioral therapy during lifestyle intervention is a key element to achieve improvement in eating behaviors and PA. This therapy encompasses various actions including counseling on self-monitoring of PA and eating behaviors, stimulus control, action planning, goal setting, and other behavior modification strategies (van Hoek et al. 2016).

Given that children and adolescents live with their families and that parents exert an important influence on healthy behaviors, it is important that behavioral interventions include the family. Lifestyle interventions that take the family into account have been described as more successful than those that only focus on children (Wilfley et al. 2017). The increasing rates of childhood and adolescent obesity appear to be the result of an interaction between genetic predisposition with lifestyle factors such as excess caloric intake and reduced physical activity. With this "obesogenic" environment, it is difficult to maintain a micro-environment that protects children and adolescents from obesity. The physical activity levels of parents or guardians, their eating habits, television viewing, and other sedentary attitudes play a special role. Therefore, intervention program based on lifestyle changes are more effective when the family is involved.

Lifestyle Intervention Studies

Most studies that have evaluated TL and lifestyle factors are cross-sectional analyses, which have not been able to consider cause and effect (García-Calzón and Marti 2017). But, intervention studies are a very important tool in epidemiology. The fundamental difference with cohort studies is that in intervention studies, the exposure is decided and allocated by the researcher and its effect is evaluated. These

studies represent the last link in the progression of epidemiological reasoning, because if properly designed and executed, they provide the strong and direct epidemiological evidence to judge the existence of a causal relationship between an exposure and an effect.

A recent review indicated that interventions involving multiple lifestyle modification components (i.e., diet and exercise) aimed to weight loss may influence TL (Qiao et al. 2020). They showed that six of the seven selected studies reported a significant intervention impacts in delaying telomere shortening suggesting that weight loss could reversed accelerated telomere shortening. It is crucial to design lifestyle intervention (based on diet, PA, or both types of recommendations) to be effective for maintenance of telomere length and also to determine to what extent weight loss is central.

Here we summarized human studies assessing the effect of lifestyle intervention aimed to reduce cardiometabolic risk on TL in adult and pediatric populations in Table 2 (Ojeda-Rodríguez et al. 2018a, b, 2020). For all of them TL was measured in blood cells by qPCR. Studies measuring TL should pay meticulous attention to the protocol and method used to measure TL. In line with this, in our studies we considered several points to reduce potential measurement error: TL was measured by MMqPCR, in which the quantification of telomeres and the single copy gene was performed in a single reaction and DNA samples corresponding to different time points (e.g., 0, 2, and 12 months) were run by triplicate in the same plate. Briefly, there are three lifestyle intervention studies that we performed, one in adult subjects at high cardiovascular risk (the PREDIMED-Navarra) and two in pediatric populations with abdominal obesity (the EVASYON project, and IGENOI study):

- The PREDIMED is a large randomized clinical trial of dietary intervention in subjects at high cardiovascular risk with the main objective of finding out whether the Mediterranean diet supplemented with extra virgin olive oil or nuts prevents the onset of cardiovascular disease compared to a low-fat diet (Estruch et al. 2018). (More information is available ISRCTN 35739639 and at http://www.predimed.org/.)
- The EVASYON project consists of the development, implementation, and evaluation of the effectiveness of a therapeutic program for overweight and obese adolescents: comprehensive nutrition and physical activity education (Martinez-Gomez et al. 2009). It is a national pilot intervention program aimed at establishing a useful educational program specifically targeted at overweight and obese adolescents (http://www.ame-ab.es/cms/alimentacion-y-salud/proyectos/proyecto-evasyon/).
- The IGENOI study is a two-year family-based lifestyle intervention study involving children and adolescents with abdominal obesity. It is a randomized controlled clinical trial (NCT03147261) in which the control group received standard pediatric recommendations on healthy diet, while the intensive care group was advised to follow a moderately hypocaloric Mediterranean diet. Both groups were encouraged to accumulate an extra time of 200 min of physical activity per week at 60–75% of their maximum heart rate (Ojeda-Rodriguez et al. 2018a, b).

Table 2 Human studies assessing the effect of lifestyle intervention aimed to reduce cardiometabolic risk on TL in adult and pediatric populations

Main findings	Type of study	Sample characteristics	Publication
Adult subjects			
Baseline TL is inversely associated with changes in obesity parameters after the intervention. A reduction in obesity indices when an increase in TL is observed after 5 year of MD intervention	Nutritional program to follow the Mediterranean diet for 5-year (all subjects together)	520 subjects with high cardiovascular risk from the PREDIMED-Navarra study	García-Calzón et al. (2014a)
A greater baseline adherence to MD was linked to longer telomeres in women. No beneficial effect of following an MD compared to low-fat diet in telomere erosion	Nutritional program to follow the Mediterranean diet for 5-year supplemental foods (olive oil or mixed nuts), control group were advice to follow a low-fat diet	520 subjects at high cardiovascular risk from the PREDIMED-Navarra study	García-Calzón et al. (2016)
Increase in the TL over the 4.5 year period both in the intervention and in the control group	Lifestyle intervention (4.5 years of follow-up)	311 adults with impaired glucose tolerance	Hovatta et al. (2012)
Changes in TL were associated to baseline TL. No changes in telomere length in any intervention groups compared to the control group.	12-Month program with a 10% weight loss goal, or an increase in moderate to vigorous exercise, or both, plus Control group (no intervention)	439 postmenopausal overweight or obese women	Mason et al. (2013)
No changes in telomere length in any intervention groups compared to the control group. Weight-loss maintenance of 10% or more was associated with larger telomeres 12 month later	5,5-Month weight loss program (and follow up to month 12) with or without mindfulness training with identical diet-exercise recommendations	194 adult with abdominal obesity	Mason et al. (2018)
Changes in TL were associated to baseline TL. No change in telomere length between intervention groups was observed	6-Month weight loss program with or without exercise prescription	50 adult with abdominal obesity	Svenson et al. (2011)
Weight-loss after a bariatric surgery intervention was associated with larger telomeres	Lifestyle education 6-Month program after bioenteric intragastric balloon	42 obese subjects	Carulli et al. (2016)

(continued)

Table 2 (continued)

Main findings	Type of study	Sample characteristics	Publication
Children and adolescents			
Weight loss intervention is accompanied by an increase in LTL. Initial longer LTL predicts a better weight loss response	Lifestyle Intervention based on dietary and PA recommendations (EVASYON): 2 month of an energy restricted diet and 6 months of follow up	74 adolescents aged 12–16 years with obesity	García-Calzón et al. (2014b)
Longer telomeres at baseline were associated with a higher reduction in glucose and IL-6 serum levels after 2 months of the weight-loss treatment	Lifestyle Intervention based on dietary and PA recommendations (EVASYON): 2 month of an energy restricted diet and 6 months of follow up	66 children aged 12–16 years with abdominal obesity	Garcia-Calzón et al. (2017)
Inverse correlation between TL and obesity traits was observed in children with abdominal obesity. Baseline TL could predict changes in blood glucose levels	Lifestyle Intervention based on dietary and PA recommendations (IGENOI): 2 month of an energy restricted diet and 10 months of follow up	106 children aged 7–16 years with abdominal obesity	Morell-Azanza et al. (2020)
Favorable changes in diet quality indices could contribute to telomere integrity	Lifestyle Intervention based on dietary and PA recommendations (IGENOI): 2 month of an energy restricted diet and 10 months of follow up	87 children aged 7–16 years with abdominal obesity	Ojeda-Rodríguez et al. (2020)
Changes in physical activity had a direct effect on TL	Lifestyle Intervention based on dietary and PA recommendations (IGENOI): 2 month of an energy restricted diet and 10 months of follow up	121 children aged 7–16 years with abdominal obesity	Ojeda-Rodríguez et al. (2021)

Interestingly, we reported that TL is linked to anthropometry changes in adult and adolescents subjects. In the PREDIMED-Navarra study, TL was measured at baseline and after 5 years follow-up in subjects at high cardiovascular risk (García-Calzón et al. 2014a). We ran a multiple regression model to predict changes in adiposity indices at year 5 according to baseline LT. We found that higher LT at baseline significantly predicted greater reductions in body weight, body mass index, and waist circumference adjusted for age, sex, and the corresponding anthropometric variable at baseline. Moreover, an association was also found between changes in LT during the intervention period and anthropometric variables after 5 years of the nutritional intervention based on the MD pattern. The reduction in markers of adiposity, associated with increased LT, was even greater in those with longer telomeres at baseline. Interestingly, the *odds ratio* of remaining obese after

5 years, for those with the longest TL, was 0.27 among those who increased TL, and 0.43 among those who decreased TL during follow-up. In regard to this, a trend was observed: the higher the LT at baseline and the greater the increase in LT, the lower the risk of remaining obese. In the EVASYON study, TL was measured in 74 overweight or obese adolescents at baseline and after 2 or 6 months of follow-up. They significantly lost body weight and had a significant improvement in cardiometabolic parameters (García-Calzón et al. 2014b, 2017). The multiple regression models allow us to predict changes in anthropometric index according to LT at baseline. In adolescent, during the 2-month intensive treatment phase, LT at baseline significantly predicted a greater reduction in body weight and BMI-SDS. Interestingly, males with higher baseline LT also had a greater decrease in body weight and BMI-SDS after 6 months of the multidisciplinary intervention.

It is also noticeable that weight loss in some studies was associated to longer TL (Mason et al. 2018; Carulli et al. 2016; Hovatta et al. 2012; García-Calzón et al. 2014b). Similarly, our group also found that TL was associated with an improvement in glucose levels in lifestyle intervention studies (García-Calzón et al. 2017; Morell-Azanza et al. 2020). On the other hand, several intervention studies indicated that changes in TL are associated to baseline TL (Mason et al. 2013; García-Calzón et al. 2014b).

Furthermore, the association between TL and changes in lifestyle factors in intervention programs aimed to lower cardiometabolic risk was evaluated in several research works. No evidence for an improvement on TL was reported when a healthy lifestyle was compared to that of the control group (Mason et al. 2013, 2018; Hovatta et al. 2012; Svenson et al. 2011, García-Calzón et al. 2016). But, in women of the PREDIMED-Navarra the adherence to Mediterranean diet (14 item questionnaire) was found to be linked to longer telomeres at baseline (García-Calzón et al. 2016). Moreover, in a pediatric population with abdominal obesity favorable changes in diet quality or PA seem to influence telomere integrity in the IGENOI study. Notably, each increase in 1 MET (metabolic equivalent) in the PA level was independently associated with a 26-units increase in TL, measured as the ratio T/S. Also, we observed that the model that account for the METs explained up to 58% of the variability in TL (Ojeda-Rodríguez et al. 2021). It is known that regular physical activity has also been associated with decreased levels of oxidative stress and inflammation, which affect telomere shortening (Himbert et al. 2018).

In spite of the differences in the study design and methodology, limitations, and pitfalls of the studies, it seem that programs to prevent excess weight or associated comorbidities may also contribute to ameliorate telomere attrition. However, more interventions are needed to confirm the benefits of lifestyle changes on telomere dynamics (Erusalimsky 2020). Furthermore, it is worthy to mention that that genetic variant may help to explain different responses to lifestyle interventions. For example, García-Galzón et al. (2015) reported that Ala carrier subjects for the PPARG2 gene (rs1801282) with a high adherence to the MD pattern exhibited increased TL after 5 year follow-up. In addition, effective intervention strategies to delay telomere shortening should examine the complex interaction between environmental factors including health habits and behaviors (lifestyle), and genetic, epigenetic, and other

"omics" markers. The new challenge is to integrate "multi-omics" data together with lifestyle data and to discover new markers (telomere length) to successfully deliver and evaluate individualized interventions as required for Precision Nutrition (Medicine).

TL as a Biomarker in Lifestyle Intervention

Substantial weight loss is able to lower chronic inflammation and adipose tissue oxidative stress and can lead to promote TL conservation and DNA repair, thus reduction telomere attrition. One important finding coming from the intervention studies compiled here is the association between TL and adiposity changes in lifestyle interventions, suggesting that TL may be used as a **biomarker of response** (Welendorf et al. 2019). Thus, this blood biomarker may serve as a noninvasive, low cost, and time-efficient tool to assess effectiveness of lifestyle programs for lowering cardiometabolic risk.

Furthermore positive effects on TL could be also related with a reduction in saturated fat and sugar consumed, as well as with an increase in vitamin and mineral (antioxidant) intake. TL may not only reflect one nutrient but may be associated with a dietary pattern or food group. In this regard, we found a higher risk of short telomeres linked to an inflammatory diet (in the PREDIMED-Navarra study, Am J Clin Nutr. 2015;102(4):897–904) or high consumption of ultraprocessed food (Am J Clin Nutr. 2020;111(6):1259–1266, Alonso-Pedrero et al., 2020) while a lower risk of shorter telomeres was associated with a high adherence to MD in cross-sectional studies with older subjects from the SUN study (Clin Nutr. 2020;39(8):2487–2494, Ojeda-Rodriguez et al. 2020). Findings from our work and others suggest that the MD pattern helps to lower inflammation and oxidative stress and could provide protection for telomeres.

In conclusion, there is a need for the identification of dynamic biomarkers that may predict weight loss and could help in obesity management for the prescription of most suitable – individual – lifestyle changes. However, inconsistent results concerning the benefit of lifestyle intervention on TL suggest the need for more studies – probably devoted to the measurement of ***excessively short telomeres*** – before its clinical application in routine use as biomarker.

Applications to Prognosis or Applications to Other Diseases or Conditions

Applications to Prognosis

In this chapter an association between TL and measures of adiposity is described in adult and adolescent subjects enrolled in lifestyle interventions (García-Calzón et al. 2014a, b). Also, a recent review indicated that interventions involving lifestyle modification components (i.e., diet and exercise) aimed to weight loss may influence TL (Qiao et al. 2020). One important finding coming

from the evidence compiled here is the association between TL and measures of adiposity in lifestyle interventions, suggesting that TL may be used as a ***biomarker of response*** *in adults and adolescent subjects (Welendorf et al. 2019). Thus, this blood biomarker may serve as a noninvasive, low cost marker to assess effectiveness of lifestyle programs aimed to lower cardiometabolic risk.*

Applications of *short telomeres* measurement as a new clinical marker

In this chapter, it is stated that abnormal telomere shortening underlies patients' risk of developing age-related degenerative diseases, including metabolic (diabetes, obesity, etc.) and cardiovascular diseases, and several cancers. Although it is indisputable that telomere shortening is a characteristic associated with these pathologies, the simple quantification of the mean telomere size of a sample is an insufficient surrogate marker, and it is necessary to develop new tools in biomedical research and clinical practice, capable of measuring telomeres and specifically identify those ***excessively short telomeres****, as the true characteristics of diseases related to aging (Vera and Blasco 2012).*

Mini-Dictionary of Terms

- **Canonical sequence:** DNA sequence that reflects the most common choice of bases at each position. In the case of telomeres, this sequence is repeated thousands of times.
- **Cardiometabolic risk:** It describes a person's chances of having a cardiovascular event such as heart attack or stroke when one or more risk factors are present. Some major risk factors include: obesity, high LDL ("bad") cholesterol, high blood fat (triglycerides), low HDL ("good") cholesterol, high blood pressure, diabetes, smoking, and tobacco.
- **-Clinical trial**: It is a formal study carried out according to a prospectively defined protocol that is intended to discover or verify the safety and effectiveness of procedures or interventions in humans (US National Library of Medicine 2019).
- **8-oxo-7,8-dihydroguanine**: The reactive oxygen species most abundant in senescent cells.
- **Fluorescence in situ hybridization (FISH)**: It is a laboratory technique for detecting and locating a specific DNA sequence on a chromosome. A fluorescently labeled DNA probe is capable of specifically detecting its complementary DNA sequence present on the chromosomes of a cell.
- **High-throughput technologies:** Those in which exist automation of experiments such that large-scale repetition becomes feasible. In the case of the molecular biology studies, those kinds of technologies allow sequencing of DNA and RNA, amplification of DNA fragments by PCR, etc., in a rapid and cost-effective manner.
- **Intervention:** It refers to any drug, device, biologic, behavioral modification, nutritional modification, lifestyle modification, or other treatment intended to improve health.

- **Inflammation:** A local response to cellular injury that is marked by capillary dilatation, leukocytic infiltration, redness, heat, pain, swelling, or loss of function and that serves as a mechanism initiating the elimination of elimination of foreign substances and for healing damaged tissue.
- **Mediterranean diet:** It is a generic term based on the traditional eating habits in the 16 countries bordering the Mediterranean Sea. A Mediterranean-style diet typically includes: plenty of fruits, vegetables, bread and other grains, potatoes, beans, nuts, and seeds; olive oil as a primary fat source; and dairy products, eggs, fish, and poultry in low to moderate amount.
- **MET**: One metabolic equivalent is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml O_2 per kg body weight x min.
- **Oxidative stress:** It reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of reactive oxygen species (peroxides and free radicals) that damage all components of the cell, including proteins, lipids, and DNA.
- **Randomized controlled trial:** A study in which the participants are assigned by chance to separate groups that compare different treatments; neither the researchers nor the participants can choose which group. Using chance to assign people to groups means that the groups will be similar and that the treatments they receive can be compared objectively. At the time of the trial, it is not known which treatment is best.
- **Telomeres:** Regions of repeating noncoding nucleotide sequences at the end of each chromosome. Their main function is to protect the ends of the chromosomes from degradation and maintain the integrity of the chromosomes.

Key Facts of TL Measurement

Some techniques used to measure telomeres may include non-canonical sequences from the subtelomeric region, which may overestimate the reliability of the measurement.

Short telomeres are a characteristic of diseases related to senescence and aging, so the inability to detect them represents a significant lack of information

Key Facts of Obesity

Obesity prevalence is rising worldwide being a number 1 problem in public health.

Obesity is accompanied by inflammatory and oxidative stress processes that partly explain the increased cardiometabolic risk and the comorbidities associated with obesity.

Lifestyle changes are useful strategies for obesity prevention and treatment.

Key Facts of Lifestyle Intervention

Lifestyle interventions aimed to lower cardiometabolic risk encourage participants to follow healthy dietary and physical activity recommendations.

There are many types of interventions; an important issue is the number and characteristics of subjects, experimental design, and duration of the follow-up.

Interventions that are able to increase the adherence to Mediterranean diet are successful in reducing cardiometabolic risk.

Summary Points

- The detection of excessively short telomeres emerges as an important new tool in biomedical research and clinical practice.
- Successful lifestyle intervention (those able to reduce cardiometabolic risk) may ameliorate inflammatory and oxidative stress processes ameliorating telomere erosion.
- Research works indicate that telomere length varies by age and sex, but there are limited data to evaluate the clinical relevance of these differences.
- Lifestyle factors that impact telomere length include exercise, smoking, measures of adiposity, alcohol, among others.
- Some studies showed that changes in telomere length are associated with changes in adiposity measures in adolescents and adults after lifestyle interventions.
- Changes in physical activity levels had a direct effect on telomere length, a biomarker of cellular aging and oxidative stress.

References

Alonso-Pedrero L, Ojeda-Rodríguez A, et al. Ultra-processed food consumption and the risk of short telomeres in an elderly population of the Seguimiento Universidad de Navarra (SUN) Project. Am J Clin Nutr. 2020;111:1259–66.

Alonso-Pedrero L, Ojeda-Rodríguez A, Zalba G, Razquin C, Martínez-González MÁ, Bes-Rastrollo M, Marti A. Association between ideal cardiovascular health and telomere length in participants older than 55 years old from the SUN cohort. Rev Esp Cardiol (Engl Ed). 2022;75 (4):308–315. https://doi.org/10.1016/j.rec.2021.04.002. Epub 2021 May 14.PMID: 33994338

Arsenis NC, You T, Ogawa EF, et al. Physical activity and telomere length: impact of aging and potential mechanisms of action. Oncotarget. 2017;8:45008–19.

Aviv A, Hunt SC, Lin J, et al. Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by southern blots and qPCR. Nucleic Acids Res. 2011;39:e134.

Baerlocher GM, Mak J, Tien T, et al. Telomere length measurement by fluorescence in situ hybridization and flow cytometry tips and pitfalls. Cytometry. 2002;47:89–99.

Baerlocher GM, Vulto I, de Jong G, et al. Flow cytometry and fish to measure the average length of telomeres (Flow FISH). Nat Protoc. 2006;1:2365–76.

Baird DM, Rowson J, Wynford-Thomas D, et al. Extensive allelic variation and ultrashort telomeres in senescent human cells. Nat Genet. 2003;33:203–7.

Bendix L, Horn PB, Jensen UB, et al. The load of short telomeres, estimated by a new method, universal STELA, correlates with number of senescent cells. Aging Cell. 2010;9:383–97.

Brown CL, Perrin EM. Obesity prevention and treatment in primary care. Acad Pediatr. 2018;18(7): 736–45.
Burki T. European Commission classifies obesity as a chronic disease. Lancet Diabetes Endocrinol. 2021;9:418.
Canudas S, Becerra-Tomás N, Hernández-Alonso P, et al. Mediterranean diet and telomere length: a systematic review and meta-analysis. Adv Nutr. 2020;11(6):1544–54.
Carulli L, Anzivino C, Baldelli E, et al. Telomere length elongation after weight loss intervention in obese adult. Mol Genet Metab. 2016;118(2):138–42.
Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002;30:e47.
Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 2009;37:1e7.
Chaput JP, Saunders TJ, Carson V. Interactions between sleep, movement and other non-movement behaviours in the pathogenesis of childhood obesity. Obes Rev. 2017;18:7–14.
Dweck A, Maitra R. The advancement of telomere quantification methods. Mol Biol Rep. 2021;48 (7):5621–7.
Erusalimsky JD. Oxidative stress, telomeres and cellular senescence: what non-drug interventions might break the link? Free Radic Biol Med. 2020;150:87–95.
Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. New Engl J Med. 2018;379 (14):1388.
Foster C, Moore JB, Singletary CR, et al. Physical activity and family-based obesity treatment: a review of expert recommendations on physical activity in youth. Clin Obes. 2018;8(1):68–79.
Galiè S, Canudas S, Muralidharan J, et al. Impact of nutrition on telomere health: systematic review of observational cohort studies and randomized clinical trials. Adv Nutr. 2020;11(3):576–601.
García-Calzón S, Marti A. Role of dietary pattern and obesity on telomere homeostasis. In: Marti A, Zalba G, editors. Telomeres, diet and human disease: advances and therapeutic opportunities. Boca Raton, FL: CRC Press; 2017. p. 133–48.
García-Calzón S, Gea A, Razquin C, et al. Longitudinal association of telomere length and obesity indices in an intervention study with a Mediterranean diet: the PREDIMED-NAVARRA trial. Int J Obes (Lond). 2014a;38(2):177–82.
García-Calzón S, Moleres A, Marcos A, et al. Telomere length as a biomarker for adiposity changes after a multidisciplinary intervention in overweight/obese adolescents: the EVASYON study. PLoS One. 2014b;9(2):e89828.
García-Calzón S, Martínez-González MA, Razquin, et al. Pro12Ala polymorphism of the PPARγ2 gene interacts with a mediterranean diet to prevent telomere shortening in the PREDIMED-NAVARRA randomized trial. Circ Cardiovasc Genet. 2015;8(1):91–9.
García-Calzón S, Martínez-González MA, Razquin C, et al. Mediterranean diet and telomere length in high cardiovascular risk subjects from the PREDIMED-NAVARRA study. Clin Nutr. 2016;35 (6):1399–405.
García-Calzón S, Moleres A, Gómez-Martinez S, et al. Association of telomere length with IL-6 levels during an obesity treatment in adolescents: interaction with the-174G/C polymorphism in the IL-6 gene. Pediatr Obes. 2017;12(3):257–63.
Gielen M, Hageman GJ, Antoniou EE, Nordfjall, et al. Body mass index is negatively associated with telomere length: a collaborative cross-sectional meta-analysis of 87 observational studies. Am J Clin Nutr. 2018;108(3):453–75.
Henriksson PH, Henriksson I, Labayen I, et al. Correlates of ideal cardiovascular health in European adolescents: The HELENA study. Nutr Metab Cardiovasc Dis. 2018;28(2):187–94.
Himbert C, Thompson H, Ulrich CM. Effects of intentional weight loss on markers of oxidative stress, DNA repair and telomere length – a systematic review. Obes Facts. 2018;10(6):648–65.
Hovatta I, de Mello VD, Kananen L, et al. Leukocyte telomere length in the Finnish diabetes prevention study. PLoS One. 2012;7(4):e34948.

Hughes AR, Henderson A, Ortiz-Rodriguez V, Artinou ML, Reilly JJ. Habitual physical activity and sedentary behaviour in a clinical sample of obese children. Int J Obes (Lond). 2006;30 (10):1494–500. https://doi.org/10.1038/sj.ijo.0803334. Epub 2006 Apr 11.PMID: 16607385
Kimura M, Stone RC, Hunt SC, et al. Measurement of telomere length by the southern blot analysis of terminal restriction fragment lengths. Nat Protoc. 2010;5:1596–607.
Konstabel KT, Verbestel VV, Moreno LA, et al. Objectively measured physical activity in European children: The IDEFICS study. Int J Obes. 2014;38(S2):S135–43.
Lai T-P, Wright WE, Shay JW. Comparison of telomere length measurement methods. Philos Trans R Soc B. 2018;373:20160451.
Lanigan J. Prevention of overweight and obesity in early life. Proc Nutr Soc. 2018;77(3):247–56.
Lansdorp PM, Verwoerd NP, van de Rijke FM, et al. Heterogeneity in telomere length of human chromosomes. Hum Mol Genet. 1996;5:685–91.
Lauzon W, Sanchez Dardon J, Cameron DW, et al. Flow cytometric measurement of telomere length. Cytometry. 2000;42:159–64.
Lindrose AR, McLester-Davis LWY, Tristano RI, et al. Method comparison studies of telomere length measurement using QPCR approaches: a critical appraisal of the literature. PLoS ONE. 2021;16(1):e0245582.
Luo Y, Viswanathan R, Hande MP, et al. Massively parallel single-molecule telomere length measurement with digital real-time PCR. Sci Adv. 2020;6(34):eabb7944.
Luque V, Escribano J, Closa-Monasterolo J, et al. Unhealthy dietary patterns established in infancy track to mid-childhood: The EU childhood obesity project. J Nutr. 2018;148(5):752–9.
Marti A, Aguilera CM, editors. Obesity: oxidative stress and diettary antioxidants. 1st ed. London: Academic Press; 2018. p. 1–304.
Marti A, Zalba G, editors. Telomeres, diet and human disease: advances and therapeutic opportunities. 1st ed. CRP Press; 2017. p. 1–186.
Marti A, Echeverría R, Morell-Azanza L, et al. Telomeres and diet quality. Nutr Hosp. 2017;34(5): 1226–45.
Martinez-Gomez D, Gomez-Martinez S, Puertollano MA, Nova E, Wärnberg J, Veiga OL, Martí A, Campoy C, Garagorri JM, Azcona C, Vaquero MP, Redondo-Figuero C, Delgado M, Martínez JA, Garcia-Fuentes M, Moreno LA, Marcos A. Design and evaluation of a treatment programme for Spanish adolescents with overweight and obesity. EVASYON Study Group. BMC Public Health. 2009;9:414. https://doi.org/10.1186/1471-2458-9-414. PMID: 19912668
Mason C, Risques RA, Xiao L, et al. Independent and combined effects of dietary weight loss and exercise on leukocyte telomere length in postmenopausal women. Obesity (Silver Spring). 2013;21(12):E549–54.
Mason AE, Hecht FH, Daubenmier JJ, et al. Weight loss maintenance and cellular aging in the supporting health through nutrition and exercise study. Psychosom Med. 2018;80(7):609–19.
Montpetit AJ, Alhareeri AA, Montpetit M, et al. Telomere length: a review of methods for measurement. Nurs Res. 2014;63:289–99.
Morell-Azanza L, Ojeda-Rodríguez A, Azcona-SanJulián MC, et al. Associations of telomere length with anthropometric and glucose changes after a lifestyle intervention in abdominal obese children. Nutr Metab Cardiovasc Dis. 2020;30(4):694–700.
Ojeda-Rodríguez A, Zazpe Z, Morell-Azanza L, et al. Improved diet quality and nutrient adequacy in children and adolescents with abdominal obesity after a lifestyle intervention. Nutrients. 2018a;10(10):1500.
Ojeda-Rodríguez A, Morell-Azanza L, Alonso-Pedrero L, Marti A. Aging, telomere integrity, and antioxidant food. In: Marti A, Aguilera CM, editors. Obesity. Oxidative stress and dietary antioxidants. London: Elsevier; 2018b. p. 241–61.
Ojeda-Rodríguez A, Morell-Azanza L, Zalba G, et al. Associations of telomere length with two dietary quality indices after a lifestyle intervention in children with abdominal obesity: a randomized controlled trial. Pediatr Obes. 2020;15(11):e12661.

Ojeda-Rodríguez A, Morell-Azanza L, Martín-Calvo N, et al. Association between favourable changes in objectively measured physical activity and telomere length after a lifestyle intervention in pediatric patients with abdominal obesity. Appl Physiol Nutr Metab. 2021;46(3):205–12.

Pejenaute A, Zalba G. Measurement of telomere length. In: Marti A, Zalba G, editors. Telomeres, diet and human disease: advances and therapeutic opportunities. Boca Raton: CRC Press; 2017. p. 39–54.

Poitras VJ, Gray CE, Borghese MM, et al. Relationships between objectively measured physical activity and health indicators in school-aged children and youth. Med Sci Sports Exercise. 2016;48:235–6.

Qiao S, Jiang Y, Li X. The impact of health promotion interventions on telomere length: a systematic review. Am J Health Promot. 2020;34(6):633–47.

Rufer N, Dragowska W, Thornbury G, et al. Telomere length dynamics in human lymphocyte subpopulations measured by flow cytometry. Nat Biotechnol. 1998;16:743–7.

Svenson U, Nordfjäll K, Baird D, et al. Blood cell telomere length is a dynamic feature. PLoS One. 2011;6(6):e21485.

Tognon GA, Lanfer HA, Moreno LA, et al. Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study. Nutr Metabol Card Dis. 2014;24(2):205–13.

Van Hoek E, Feskens E, Bouwman LI, et al. Effective interventions in overweight or obese young children: systematic review and meta-analysis. Childhood Obes. 2016;10(6):448–60.

Velazquez-Lopez L, Santiago-Diaz G, Nava-Hernandez J, et al. Mediterranean-style diet reduces metabolic syndrome components in obese children and adolescents with obesity. BMC Pediatr. 2014;14(1):175.

Vera E, Blasco MA. Beyond average: potential for measurement of short telomeres. Aging (Albany NY). 2012;4:379–92.

Welendorf C, Nicoletti CF, de Souza Pinhel MA. Obesity, weight loss, and influence on telomere length: new insights for personalized nutrition. Nutrition. 2019;66:115–21.

WHO (2021) Obesity and overweight: key facts. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (available on May 20, 2022)

Wilfley DE, Saelens BE, Stein RI, et al. Dose, content, and mediators of family-based treatment for childhood obesity a multisite randomized clinical trial. JAMA Pediatr. 2017;171(12):1151–9.

What is a clinical study? National Library of Medicine, US National Institutes of Health. 2019. https://clinicaltrials.gov/ct2/aboutstudies/learn#WhatIs. (Accessed on May 20, 2022).