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Telomere Length and Accelerated Biological Aging in the China Suboptimal Health Cohort: A Case–Control Study

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Abstract

Suboptimal health status (SHS) has been linked to cardiovascular risk factors, psychosocial stress, and unhealthy lifestyle. These factors also contribute to the shortening of telomere length (TL). A case–control study was conducted to examine the association between subjective health measures of SHS from the behavior perspective and also objective measures of TL at molecular level. SHS (cases = 294) was matched by age, sex, and body mass index with ideal health (controls = 294) using a propensity score matching method. Suboptimal health status questionnaire-25 (SHSQ-25) was used in the community-based health survey. A quantitative polymerase chain reaction was used to measure relative telomere length (RTL). Shorter RTL was found among the SHS group compared to the ideal health group ($p < 0.05$). SHS was almost four times likely to be in the first quartile (odds ratio [OR] = 3.81; 95% confidence interval [CI] 2.21–6.56), almost thrice in second quartile (OR = 2.84; 95% CI 1.65–4.90), and almost twice likely to be in the third quartile (OR = 1.71; 95% CI 1.00–2.94) compared to the fourth quartile (the longest) of RTL after adjusting for socioeconomic, dietary intake, anthropometric, blood pressure, and biochemistry variables ($p < 0.05$). Notably, SHS score was negatively correlated with RTL ($r = -0.218$, $p < 0.05$). Our study confirms an association between SHS and short RTL. Combination of subjective (SHS) and objective (RTL) measures is a novel tool for health aging investigation. Therefore, SHSQ-25 could be used as a screening tool for measuring biological aging in low-income countries at community level where the expensive technique for RTL measurement is not applicable.

Keywords: suboptimal health status, relative telomere length, and China suboptimal health cohort study

Introduction

Suboptimal health status (SHS) is a physical state between health and disease, characterized by: (1) ambiguous health complaints, (2) loss of vitality, (3) chronic fatigue, and (4) low energy levels within a period of 3 months (Wang and Yan, 2012; Wang et al., 2014, 2016). It is regarded as a subclinical and reversible stage of chronic health condition (Bi et al., 2014; Kupaev et al., 2016; Tian et al., 2016; Wang and Yan, 2012; Wang et al., 2014, 2016; Yan et al., 2009).

SHS has been internationally recognized as a tool for early detection of chronic diseases (Bi et al., 2014; Kupaev et al., 2016; Wang et al., 2014, 2016; Yan et al., 2009). It is also difficult to be diagnosed because of vague changes in function and no obvious clinical symptoms of organ disease (Wang and Yan, 2012; Yan et al., 2012). The etiology and mechanism of development in SHS remain unknown (Wang and Yan, 2012; Wang et al., 2016; Yan et al., 2012).

Our team created a comprehensive Suboptimal Health Status Questionnaire-25 (SHSQ-25) (Supplementary Data) and validated it in different populations, including Chinese,

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African (ongoing), and European (Kupaev et al., 2016; Tian et al., 2016; Wang and Yan, 2012; Wang et al., 2016; Yan et al., 2012). Our previous studies have shown an association between SHS with cardiovascular risk factors, endothelial dysfunction, chronic psychosocial stress, and unhealthy lifestyle factors (Bi et al., 2014; Kupaev et al., 2016; Yan et al., 2009, 2015). These factors also contribute to the shortening of telomere length (TL), thus accelerating the development of age-related diseases (Mirabello et al., 2009; Osler et al., 2016; Peng et al., 2017; Rehkopf et al., 2016; Shalev et al., 2013; Sun et al., 2012).

Telomeres are nucleoprotein structures that cap the ends of the chromosome (O'Callaghan and Fenech, 2011). In humans, telomeres are double-strand nucleotide repeats of TTAGGG sequence which protect the chromosome from degradation (Hsieh et al., 2016). In the somatic cell, telomeres reduce in length with each cell division (Du et al., 2015). The progressive shortening of telomeres to a critical length eventually leads to cell senescence, affecting health and life span (Hsieh et al., 2016). Environmental factors, such as unhealthy lifestyle and psychological and physiological stress, usually accelerate telomere erosion (Mirabello et al., 2009; O'Callaghan and Fenech, 2011).

It has been previously demonstrated that people with shorter telomeres are likely to have chronic diseases, such as diabetes (Willeit et al., 2014; Zhao et al., 2014), cardiovascular diseases (Peng et al., 2017; Rehkopf et al., 2016), lung diseases (Rode et al., 2012), impaired immune function (Damjanovic et al., 2007), and certain types of cancer (Du et al., 2015). In contrast, longer TL is linked to better health and protects from age-related diseases (Shalev et al., 2013).

Studies have proposed that telomere erosion is modifiable and that the variation present in the telomere erosion rate is independent of chronological age (Crous-Bou et al., 2014; Sun et al., 2012). Moreover, several studies have proposed TL as a biomarker for biological aging (Bekaert et al., 2007; Hamad et al., 2016; Peng et al., 2017). Therefore, identifying the telomere attrition high-risked group may be helpful in the prevention of age-related chronic diseases which is the main focus of the China suboptimal health cohort study (COACS) (Wang et al., 2016). In this study, we aimed to examine the association between SHS and short relative telomere length (RTL) in a real-life community-based COACS.

Materials and Methods

Study population and design

A retrospective case–control study was conducted as part of the baseline survey of the COACS, a prospective community-based cohort study, with participants free from any diseases at the baseline screening, aged 18–67 years at the enrolment (Wang et al., 2016). The COACS was established in 2013, and the participants are followed up every year (Wang et al., 2016). A propensity score matching was performed to match a subset of 294 SHS cases (SHS score ≥35) to a subset of 294 ideal healthy controls (SHS score <35) by age, gender, and body mass index (BMI). The score was calculated from SHSQ-25 according to 25 items under five domains of (1) fatigue, (2) the cardiovascular system, (3) the digestive tract, (4) the immune system, and (5) mental status (Wang and Yan, 2012; Wang et al., 2016; Yan et al., 2009). A score ≥35 represents a SHS and <35 represent an ideal health (Wang and Yan, 2012; Wang et al., 2016; Yan et al., 2009).

DNA extraction, quantification, and dilution

DNA was extracted from the frozen whole blood samples using Blood Genome DNA Extraction Kits according to the manufacturer's protocol (BioTeke, Beijing, China). All DNA samples were stored in freezers at −80°C until use. DNA samples were thawed at 4°C on the same day of quantitative polymerase chain reaction (qPCR) analysis. Concentration and purity of each sample were measured twice using microplate spectrophotometer and then the average was taken. All samples were diluted to 5 ng/μL.

RTL measurement by qPCR

RTL was measured in genomic DNA using a qPCR, a method developed by Cawthon (2002) and modified by Epel et al. (2004) and Lapham et al. (2015). Briefly, RTL was measured by the ratio of telomere product (T) to a single-copy reference gene product (S) and expressed as a (T/S) ratio in individual samples relative to a standard DNA (Cawthon, 2002; Lapham et al., 2015). The standard DNA was pooled from five ideal healthy individuals chosen from the COACS 2016 follow-up, prepared in 13.5 ng/μL concentration as a single batch liquated and stored at −80°C. Twofold serial five time dilution was taken from the standard DNA resulting into sixth dilution point to generate a standard curve with concentration ranging from 13.5 to 0.42 ng/μL.

All samples were processed in triplicate in separate qPCRs, in which telomere and reference gene (human beta-globin) for each sample were placed in the same position in a 96-well plate format. They were carried out on an Applied Biosystems QuantStudio 5 Real-Time PCR System. DNA samples were run in 20 μL reactions containing 10 μL SYBR Green Master Mix (Applied Biosystems), 3 μL of DNA ($\sim$5 ng/μL), and primers as follows: telomere primers were tel1b [5′CGGTTT(GTTTGG)$_5$GTT3′] and tel2b [5′-GGC TTG(CCTTAC)$_5$CCT-3′], with respective final concentrations of 900 and 250 nM (Lapham et al., 2015); reference gene primes were hbg1 [5′GCTTCTGACACAACTGTGTT CACTAGC-3′] and hbg2 [5′CACCAACTTCATCCACG TTCACC-3′], with respective final concentrations of 700 and 300 nM (Lapham et al., 2015).

The thermal cycling profile for telomere: denature at 95°C for 10 min for 1 cycle, denature at 95°C for 15 sec, anneal/extend at 56°C for 60 sec, with fluorescence data collection, 30 cycles, and for human beta-globin: denature at 96°C for 10 min for 1 cycle, denature at 95°C for 15 sec, anneal at 60°C for 60 sec, with fluorescence data collection, 35 cycles. Then melt curve was performed at the end of each run. Each qPCR plate included samples, the standard reference DNA, non-template control (NTC), and positive control. One sample from the standard reference DNA was used as positive control and run on every plate to calculate an interplate coefficient of variation (CV).

qPCR quality control

Six criteria were included in qPCR quality control namely (1) the efficiency of standard curve for both T and S fell within the range of 90–105% and linearity of $r^2 \geq 0.990$ (Bustin et al.,

2009); (2) no amplification in NTC; (3) Melting curve was evaluated after each run for nonspecific products or primer-dimer formation; (4) replicates for both T and S were assessed for variation; samples were accepted only if Ct standard deviation (SD) <0.5 (95.3% of all samples contained no outliers), then the CV was calculated from the Ct-value of each replicate, CV ranging from 0.02% to 2.89% for telomere, 0.01% to 2.12% for reference gene; (5) interplate CV was calculated from T/S of the positive control and it was 7.8%; and (6) any sample amplified outside the standard curve was rerun. Any assay or sample that failed in one of these criteria was rerun. In case that a sample failed in the second test, it was rejected and excluded from the further analysis.

Covariates

In this study, different types of covariates such as demographic variables (age, sex, marital status, education level, and family income), dietary intake (milk, vegetable, fruit, meat, eggs, aquatic, soy, and salt), alcohol drinking, smoking history, physical activity, and sleeping hours per night were included. In addition, anthropometric measurements (BMI, hip circumference, and abdominal circumference), blood pressure, and biochemistry measurements (fasting plasma glucose, triglyceride, low density lipoprotein, very low density lipoprotein (VLDL), total cholesterol, alkaline phosphates, and total bilirubin) were also included. Methods of collecting these data have been described previously (Wang et al., 2016).

Statistical analyses

Parametric continuous variables were presented as a mean ± SD and analyzed by independent *t*-test, while nonparametric continuous variables were presented as a median (interquartile range) and analyzed by Mann–Whitney *U* test. The chi-square test was used to examine differences in categorical variables between the cases (SHS score ≥35) and controls (SHS score <35). Spearman correlation analysis was used to determine the relationship between RTL and SHS score. Logistic regression model was used to determine the link between SHS and RTL quartiles, and the results were presented as odds ratio (OR) with 95% confidence interval (CI) after adjustment for different covariates. All statistical tests were two sided, and $p < 0.05$ was considered significant. All statistical analyses were carried out using IBM SPSS Statistics software (version 21.0 from Armonk, NY: IBM Corp, USA).

Ethical clearance

The Ethics Committees of the Staff Hospital of *Jidong* oil field of Chinese National Petroleum, *Beijing Tiantan* Hospital, and Capital Medical University approved the study. The ethical guidelines of the Helsinki Declaration were also followed.

Results

The mean age of study population was 36.63 ± 9.92 years; almost two-thirds (63.95%) of them were female and the mean BMI was 23.12 ± 3.51 kg/m^2. Regarding marital status, ''never married'' were significantly fewer in the SHS compared to the ideal healthy group, whereas widowed or divorced were significantly more frequent among the SHS group compared to the ideal healthy group ($p < 0.05$). The proportion of SHS was significantly high among those with shorter sleep duration at night, anxiety, depression, smoking, and heavy drinkers compared to the ideal healthy group ($p < 0.05$). In addition, they also had shorter median RTL compared to the ideal healthy group ($p < 0.05$).

The proportion of SHS was significantly high (60.88%) in the lower (first and second) quartiles, whereas for the ideal health group was high (60.88%) in the upper (third and fourth) quartiles of RTL measures ($p < 0.05$) as shown in Table 1. The SHS group had lower systolic blood pressure, as well as consumed less milk, fruits, vegetable, aquatic products, eggs, and soy than the ideal healthy group ($p < 0.05$). A spearman's correlation coefficient result shows that SHS score was negatively correlated with RTL ($r = -0.218$, $p < 0.05$).

Table 2 shows that the SHS group was almost four times more likely to be in the first quartile (OR = 3.81; 95% CI 2.21–6.56), almost thrice more likely to be in the second quartile (OR = 2.84; 95% CI 1.65–4.90), and almost twice more likely to be in the third quartile (OR = 1.71; 95% CI 1.00–2.94) compared to the fourth quartile (the longest RTL) after adjusting for socioeconomic variables, health-related variables, dietary intake, anthropometric measurements, blood pressure, and biochemistry measurements ($p < 0.05$).

Table 3 shows that almost two-thirds of males (63.21%) were physically inactive and were more likely to have shorter RTL compared to physically active males (OR = 2.12; 95% CI 1.02–4.40), whereas more than two-thirds (69.42%) of females slept for 7–8 h per night and they were less likely to have shorter RTL compared to those sleeping >8 h per day (OR = 0.28; 95% CI 0.09–0.86). The participants with good memory were less likely to have shorter RTL among male (OR = 0.48; 95% CI 0.25–0.95) and female (OR = 0.55; 95% CI 0.33–0.89) compared to those with memory loss.

Discussion

SHS provides opportunity for detecting subclinical stage of chronic disease to provide targeted preventive measures before the onset of the disease (Wang et al., 2014). Our previous studies suggest that the SHS instrument, SHSQ-25, is suitable for early detection of chronic diseases; thus can be used as a new tool for predictive, preventive, and personalized medicine (Kupaev et al., 2016; Wang et al., 2014, 2016). In this study, we examined the association between SHS and RTL, to apply it in investigating biological aging. To the best of our knowledge, this is the first study to assess this association.

We found that the SHS group had significantly shorter RTL compared to the ideal health group ($p < 0.05$). SHS was more likely to be in the shorter quartiles compared to longest quartile of RTL after adjusting for socioeconomic variables, health related variables, dietary intake, anthropometric measurements, blood pressure, and biochemistry measurements ($p < 0.05$). Notably, SHS score was negatively correlated with RTL ($r = -0.218$, $p < 0.05$). Our findings are in agreement with studies that showed the benefits of good health status on TL (Peng et al., 2017; Sun et al., 2012; Terry et al., 2008). Longer TL was found to be associated with better self-reported health status and Years of Health Life

Table 1. Characteristics of Study Population According to Suboptimal Health Status Classification

Variables	*Total*	*Healthy* (n = *294*)	*SHS* (n = *294*)	p
Age (years)	36.63 ± 9.92	36.58 ± 10.57	36.68 ± 9.24	0.781[a]
Gender *n* (%)				
Male	212 (36.05)	106 (36.05)	106 (36.05)	1.000[b]
Female	376 (63.95)	188 (63.95)	188 (63.95)	
BMI (kg/m^2)	23.12 ± 3.51	23.15 ± 3.33	23.10 ± 3.69	0.861[a]
Physical activity *n* (%)				
Inactive	450 (76.53)	218 (74.15)	232 (78.91)	0.344[b]
Moderately	70 (11.90)	37 (12.58)	33 (11.22)	
Very active	68 (11.57)	39 (13.27)	29 (9.87)	
Education level *n* (%)				
Illiteracy or compulsory	43 (7.31)	23 (7.82)	20 (6.80)	0.702[b]
High school	116 (19.73)	61 (20.75)	55 (18.71)	
College or higher	429 (72.96)	210 (71.43)	219 (74.49)	
Marital status *n* (%)				
Never married	56 (9.52)	40 (13.61)	16 (5.44)	<0.001[b]
Married	516 (87.76)	252 (85.71)	264 (89.80)	
Widowed or divorced	16 (2.72)	2 (0.68)	14 (4.76)	
Family income *n* (%)				
≤¥3000	221 (37.58)	105 (35.72)	116 (39.46)	0.642[b]
¥3001–5000	321 (54.60)	165 (56.12)	156 (53.06)	
>¥5000	46 (7.82)	24 (8.16)	22 (7.48)	
Night sleeping hours *n* (%)				
≤6 h per day	154 (26.19)	55 (18.71)	99 (33.67)	<0.001[b]
7–8 h per day	409 (69.56)	225 (76.53)	184 (62.59)	
≥9 h per day	25 (4.25)	14 (4.76)	11 (3.74)	
Smoking *n* (%)				
Never	464 (78.91)	239 (81.29)	225 (76.53)	0.363[b]
Current	122 (20.75)	54 (18.37)	68 (23.13)	
Former	2 (0.34)	1 (0.34)	1 (0.34)	
Drinking history *n* (%)				
Never	432 (73.47)	231 (78.57)	201 (68.37)	0.018[b]
Moderate	92 (15.65)	39 (13.27)	53 (18.03)	
Heavy	64 (10.88)	24 (8.16)	40 (13.60)	
RTL (median and IQR)	0.91 (0.75–1.06)	0.98 (0.82–1.13)	0.86 (0.71–1.00)	<0.001[c]
RTL quartiles *n* (%)				
Q1	147 (25.00)	51 (17.35)	96 (32.65)	<0.001[b]
Q2	147 (25.00)	64 (21.77)	83 (28.23)	
Q3	147 (25.00)	79 (26.87)	68 (23.13)	
Q4	147 (25.00)	100 (34.01)	47 (15.99)	

Descriptive are mean ± SD or median (interquartile range) where appropriate.
[a]Independent samples *t*-test.
[b]A chi-squared test.
[c]Mann–Whitney *U* test.
¥, Chinese Yuan; BMI, body mass index; SD, standard deviation; SHS, suboptimal health status; RTL, relative telomere length.

among the elderly individuals in a U.S. population (Njajou et al., 2009).

In another study, healthy centenarians were found to have longer TL compared to poor health centenarians in the United States and Canada (Terry et al., 2008). Peng et al. (2017) demonstrated the link between longer TL and ideal cardiovascular health (CVH) indices defined by four behavioral factors (smoking, physical activity, diet, and BMI) and three health factors (blood pressure, cholesterol, and fasting glucose) (Peng et al., 2017). In addition, longer TL was associated with adherence to optimal healthy lifestyle practices among the middle-aged U.S. women (Sun et al., 2012). On the contrary, a recent study in New Zealand population found no link between TL and health status (Appleby et al., 2017). The authors attributed this to unsuitable study design, insufficient study population age for the aging research, and inappropriate questionnaire for assessment of healthy individual (Appleby et al., 2017).

In this study, we found that physically inactive males were more likely to have short RTL ($p < 0.05$); this is consistent with other studies that have shown positive correlation between regular physical activities and TL (Latifovic et al., 2016; Zhu et al., 2011). We also found that females sleeping between 7 and 8 h per night were less likely to have short RTL compared to those sleeping ≥9 h per night ($p < 0.05$). Short or long sleep duration has been previously associated with short

Table 2. Association Between Relative Telomere Length Quartiles With Suboptimal Health

	Quartiles			
	Q1 *OR (95% CI)*	*Q2* *OR (95% CI)*	*Q3* *OR (95% CI)*	*Q4*
Model 1	4.01 (2.47–6.51)***	2.76 (1.71–4.44)***	1.83 (1.14–2.94)*	Ref
Model 2	4.07 (2.50–6.62)***	2.83 (1.74–4.55)***	1.85 (1.15–2.97)*	Ref
Model 3	3.91 (2.33–6.55)***	2.91 (1.75–4.87)***	1.75 (1.05–2.90)*	Ref
Model 4	4.07 (2.40–6.89)***	3.04 (1.80–5.13)***	1.83 (1.09–3.07)*	Ref
Model 5	3.81 (2.21–6.56)***	2.84 (1.65–4.90)***	1.71 (1.00–2.94)*	Ref

Asterisks indicate level of statistical significance: *≤0.05; *** ≤0.001. Model 1: Crude mode no adjustment. Model 2: adjusts for: age and gender. Model 3: Model 2 plus BMI, marital status, education levels, family income, physical exercises, night sleeping per hours, smoking, drinking, and hip and abdominal circumference. Model 4: Model 3 plus systolic blood pressure, diastolic blood pressure, fasting plasma glucose, triglyceride, low density lipoprotein, total cholesterol, alkaline phosphates, total bilirubin, and very low density lipoprotein. Model 5: Model 4 plus food consumption (milk, vegetable, fruit, aquatics, sugary, nuts and eggs, and salt intake).
CI, confidence interval; OR, odds ratio; Q, quintile.

TL (Jackowska et al., 2012; Liang et al., 2011). This is because adequate sleeping duration has a better health outcome resulting in a maintained TL; moreover, long sleep duration is also an indicator of depression and reduces physical activities (Stenholm et al., 2010; Zhai et al., 2015).

Previously, a study demonstrated the link between long sleep duration and increased risk of depression (Zhai et al., 2015), while another study indicated that long sleep duration (≥9 h) was more likely to decrease physical activity among the aging women compared with normal sleep duration (7–8 h) (Stenholm et al., 2010). Interestingly, we found that memory status was linked to RTL; participants with good memory were less likely to have short RTL ($p < 0.05$). This is in agreement with studies which illustrated that short TL was associated with poorer cognitive function, including memory assessment (Cohen-Manheim et al., 2016; Valdes et al., 2010).

None of the other SHS risk factors independently showed an association with RTL. This finding indicates that the observed association was due to joint effects of SHS risk factors. Peng et al. (2017) also failed to find significant associations of TL with each cardiovascular factor (diet, physical activity, and smoking); however they confirmed the benefit of achieving four or more ideal CVH indices on TL.

Shorter TL is not only genetically determined but also environmentally influenced (Nawrot et al., 2004; Theall et al., 2013). This has been proved by observed difference in the TL among the siblings formed from the same zygote (De Vusser et al., 2015; Kawanishi and Oikawa, 2004). Moreover, environmental factors, such as unhealthy lifestyle and psychological and physiological stress, usually accelerate telomere erosion (Mirabello et al., 2009; O'Callaghan and Fenech, 2011). Telomere shortening has been shown to be the main pathway by which oxidative stress accelerates biological aging and age-related diseases (Chen et al., 2014; Fyhrquist et al., 2013). Therefore, the observed association between SHS and short TL in our study reveals that SHSQ-25 would be ideal tool for screening of biological aging.

Strength and limitations

The study is strengthened by a well-characterized study population COACS, which include their detailed information on sociodemographic lifestyle factors, anthropometric measurement, cardiovascular risk factors, mental health, dietary assessment, and biochemistry measurement allowing us to control for a wide range of potentially confounding factors compared to the other studies. However, these findings should be considered with the following limitations: First, the study design did not enable us to establish a temporal association between RTL and SHS; it requires a longitudinal prospective study with repeated measurements of RTL. Second,

Table 3. Factors Associated with Short Relative Telomere Length Among Males And Females

	Male			*Female*		
Variables	n *(%)*	*OR (95% CI)*	p	n *(%)*	*OR (95% CI)*	p
Physical activity						
Inactive	134 (63.21)	2.12 (1.02–4.40)	0.045	316 (84.04)	0.81 (0.35–1.88)	0.625
Moderately	34 (16.03)	1.88 (0.74–4.80)	0.186	36 (9.58)	1.00 (0.35–2.85)	1.00
Very active	44 (20.76)	Ref		24 (6.38)	Ref	
Night sleeping hours/day						
≤6 h per day	57 (26.89)	0.66 (0.12–3.54)	0.625	96 (25.53)	0.36 (0.11–1.16)	0.086
7–8 h per day	149 (70.28)	0.79 (0.16–4.06)	0.780	261 (69.42)	0.28 (0.09–0.86)	0.027
>8 h per day	6 (2.83)	Ref		19 (5.05)	Ref	
Memory loss						
No	58 (27.36)	0.48 (0.25–0.95)	0.034	99 (26.33)	0.55 (0.33–0.89)	0.016
Few	44 (20.75)	0.90 (0.44–1.81)	0.759	89 (23.67)	1.09 (0.65–1.82)	0.740
Yes	110 (51.89)	Ref		188 (50.00)	Ref	

the study lacks information on genetic contribution to TL. Finally, COACS includes only Chinese ethnic group, and telomere dynamics may differ between different populations; therefore, results might not be generalizable.

Conclusions

SHS is significantly associated with short RTL. Lack of physical activities among males, sleeping more than normal hours at night among females, and memory loss are associated to short RTL. The combination of subjective (SHS) and objective (RTL) measure is a novel tool for biological aging investigation. Therefore, SHSQ-25 could be ideal instrument for biological aging at the community level in low- and middle-income countries where the expensive technique for TL measurement is not applicable.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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Abbreviations Used

BMI = body mass index
COACS = China suboptimal health cohort study
CV = coefficient of variation
CVH = cardiovascular health
NTC = nontemplate control
OR = odds ratio
RTL = relative telomere length
SD = standard deviation
SHS = suboptimal health status
SHSQ-25 = Suboptimal Health Status Questionnaire-25 (SHSQ-25)
TL = telomere length