Original Article

Telomere Length in Various Age Groups of Normal-Body Weight Thais and Obese Thais

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Abstract

Introduction: Telomere is non-coding nucleoprotein at the terminal of chromosomes that shortens during DNA replication as a cause of aging. In obese people, excess body fat is a key role in inducing chronic inflammation which accelerates telomere shortening. This study aimed to determine the difference in telomere length between normal body weight and obese Thais males aged 21-82 years.

Methods: This study was a cross-sectional design including 39 normal body weight and 41 obese subjects. Nutritional status was assessed using body composition and blood biochemistry. General information and health information were obtained by using questionnaires. Telomere length was measured using the monochromatic multiplex real-time quantitative PCR (MMqPCR) and reported in T/S ratio.

Results: In both normal weight and obese groups, the mean telomere length was found to be shortest in the oldest group and tended to be longer in normal weight than obese group in other age groups (aged 21-40 years: 1.19 ± 0.12 vs 1.12 ± 0.07, aged 41-60 years: 1.08 ± 0.12 vs 1.06 ± 0.10, and aged 61-82 years: 0.96 ± 0.06 vs 0.90 ± 0.07). Mean telomere length was found to be shorter in subjects with increased visceral fat, high fasting plasma glucose, BUN, and lack of exercise.

Conclusions: The findings among Thai subjects in different age groups revealed that telomere length significantly shortens with age and was found shorter in obese subjects in the same age groups. This may deteriorate the functions of various organ systems that would lead to complications of obesity.

Keywords: Telomere length, Obesity, Body weight, Age groups, Nutritional status

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Introduction

Telomere is a repetitive nucleotide sequence of DNA, located at the terminal ends of chromosomes which the sequence of nucleotides repeats, consisting of TTAGGG, in human.[1] The function of telomere is preventing chromosome ends from degradation, fusion, protecting the cell from treating chromosome ends as broken chromosomes, likened to the plastic protective tips on shoelaces, thus can be called a chromosome cap.[2] Consequently, telomere shortening and dysfunction have been offered as a biomarker of human aging. However, several studies have involved telomere length and accelerated telomere attrition which is especially an early onset risk factor for age-related diseases is that they reflect the cumulative burden of oxidative stress and inflammation occurring over the life course.[3,4]

Obesity is defined as abnormal or excessive body fat accumulation, which can be assessed from percentage of body fat that presents risks to health.[5] Thus, obesity is only classified by excess body fat or overweight with excess body fat. It is well known that obesity is continuously increasing systemic inflammation and oxidative stress which in turn affects cell metabolism, leading to attrition of telomere length.[6]

In Thailand, there is limited published studies on the association of telomere length in different age groups to our knowledge regarding normal weight and obese subjects. Thus, this study is preliminary research that will be a big step for raising the awareness of obesity which then would lead to more effective prevention and treatment to prevent DNA damage and DNA repair mechanisms from oxidative stress and inflammation caused by obesity. Moreover, tackling obesity could be a starting point to delay telomere shortening and to decrease the prevalence of age-related diseases. The objectives of this study were to determine the difference in telomere length between normal body weight and obese Thais males aged 21-82 years, and to study the relationship between telomere length and nutritional status.

Methods

The study was cross-sectional that studied population including males aged 21-82 years in Thai, separated into normal weight and obese groups.

The sample size consideration was based on the study of Hertzog et al.[7] which recommended that an appropriate sample size of a pilot study comparing groups should be at least 35-40 subjects per group.

The inclusion criteria of subjects was Thai males aged 21-82 years who were willing to participate in the study by completing the informed consent form. All subjects were categorized by percentage of body fat and divided into 2 groups including normal weight group (n = 39) and obese group (n = 41). The exclusion criteria were those with anemia, kidney disease, liver disease, or thyroid disease from the screening of blood examination.

Parameter to Be Assessed

All of the parameters were completely collected at the beginning of the study.

- General and health information

General and health information including underlying diseases, personal habits, and dietary behaviors, were collected with questionnaires.

- Body composition assessment

Body composition parameters, including height, body weight (kg), body mass index (BMI: kg/m^2), body fat (% of body weight), fat mass (kg), fat free mass (FFM: kg), muscle mass (kg), total body water (%), bone mass (kg), and visceral fat, were assessed using a Tanita BC-420MA segmental body composition analyzer Tanita Corporation, Tokyo, Japan.[8]

- Biochemical assessment

Biochemical measurements were collected after 8-12 hours of fasting (15 ml). To test for the following blood chemistry levels; hemoglobin, fasting plasma glucose (FPG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), uric acid, creatinine, and blood urea nitrogen (BUN) were analyzed by automated blood BS-400 Chemistry Analyser, Mindray Bio-Medical Electronics Co.Ltd.[9] For telomere length measurement, 5 ml of blood sample was divided to put immediately in vacutainer tubes containing EDTA.

Operational Definitions

- Body fat status

Body fat status was categorized according to the gender and age-specific distribution of body fat.[10] For aged 20-39 years, normal body weight and over or excess body fat were defined by percentage of body fat 8.0-19.9%, and 20.0% or greater, respectively. For aged 40-59 years, normal body weight and over or excess body fat were defined by the percentage of body fat 11.0-21.9%, and 22.0% or greater, respectively. For aged 60 years and more, normal body weight and over or excess body fat were defined by the percentage of body fat 13.0-24.9%, and 25.0% or greater, respectively.

- Body weight status

Classification of weight status according to BMI in Asian Adults.[11] Normal weight was defined as a BMI 18.5-22.9 kg/m^2, overweight was defined as a BMI 23.0-24.9 kg/m^2, obese I was defined as a BMI 25.0-29.9 kg/m^2, and obese II was defined as a BMI $\geq$ 30.0 kg/m^2.

- Visceral fat rating

A rating between 1-12 was indicated as a healthy level and a rating between 13-59 was indicated as an excess level of visceral fat.[12]

Telomere Length Measurement

Briefly, genomic DNA was extracted from whole blood samples the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instruction for DNA purification from buffy coat. The telomere length was measured using the monochromatic multiplex real-time quantitative PCR (MMqPCR) technique for relative telomere length, originally developed by R.M. Cawthon.[13] The relative telomere length was calculated by dividing telomere fluorescent signals (T) normalized to the copy number of a single-copy nuclear gene (S), T/S ratio. The ratio of T/S for each sample was calculated from the CT values using the following formula: T/S ratio = $2^{-(\Delta\Delta CT)}$, Where $\Delta\Delta CT = \Delta CT$ sample − ΔCT reference curve. All samples were measured in duplicate, the average T/S ratio is an indicator of telomere length; a lower T/S ratio reflects shorter telomere length.

The conversion from T/S ratio to base pair is calculated based on a comparison of telomeric restriction fragment (TRF) length from Southern blot analysis (gold standard). The qPCR method has been validated against the TRF assay with high correlation.[14] For human peripheral cells, this method was shown to be highly repeatable and accurate. This assay is therefore suitable for epidemiological studies.[15,16]

Ethical Approval and Permission

This study, including the protocol and the consent forms for all subjects, was approved by the Ethics Committee at the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Written informed consent documents were obtained from the subjects and their legal guardians.

Statistical Analysis

Statistical analysis was performed using SPSS statistics version 19.0. General information data, health information data, nutritional status, and telomere length measurement were expressed as the mean (± standard deviation, SD) or percentage. The differences of mean values among groups were determined by independent *t*-test and one-way ANOVA in body composition, biochemical data, and telomere length. The association of telomere length with body composition and biochemical data was analyzed using Pearson's correlation. Stepwise multiple linear regression was constructed to evaluate the relationship between predictor variables and telomere length. *P*-values < .05 were considered statistically significant.

Results

The average age in each age group of normal weight males (NM) and obese males (OM) were not statistically different as shown in Table 1. Twenty-three subjects (60.5%) in obese group had underlying diseases. Alcohol consumption was found in 51.0% of NM, and 59.0% of OM. Non-smoker subjects were 52.8%, and 47.2% in NM and OM, respectively. Only two OM smoked > 10 cigarettes/day. Duration of smoking, one NM, two OM, and one OM had been smoking for 40, 30-36, and 7 years, respectively. Most of the subjects exercised 3-5 times/week, 30-60 minutes/day. NM and OM did not drink sugar-sweetened beverages (SSBs) were 20.5% and 14.6%. OM who drank SSBs 1-3 glasses/week and > 3 glasses/week were 41.5% and 43.9%, respectively.

Table 1 Characteristics of subjects

Parameters	NM (n= 39)	OM (n= 41)	Total (n= 80)	*P*-value
Age (years), mean ± SD (n)				
21-40	31.0 ± 4.7 (13)	32.0 ± 5.6 (15)	31.5 ± 5.1 (28)	.59
41-60	51.6 ± 3.8 (14)	50.3 ± 5.7 (15)	50.9 ± 4.8 (29)	.48
61-82	68.9 ± 5.0 (12)	71.8 ± 6.1 (11)	70.3 ± 5.6 (23)	.26
Body composition, mean ± SD				
Height (cm)	170.2 ± 5.1	167.7 ± 5.7	168.9 ± 5.6	<. 05
Weight (kg)	65.2 ± 6.7	80.0 ± 15.5	72.8 ± 14.1	<. 0001
BMI (kg/m^2)	22.5 ± 2.1	28.3 ± 5.0	25.5 ± 4.8	<. 0001
Body fat (%bw)	17.8 ± 4.1	27.0 ± 5.3	22.5 ± 6.6	<. 0001
Fat mass (kg)	11.8 ± 3.5	22.2 ± 9.1	17.1 ± 8.7	<. 0001
Visceral fat	9.3 ± 4.3	14.7 ± 3.8	12.1 ± 4.8	<. 0001
Underlying diseases, n (%)				
No	24 (61.5)	18 (43.9)	42 (52.5)	
Yes	15 (38.5)	23 (56.1)	38 (47.5)	
Alcoholic beverage, n (%)				
Non-drinker	32 (82.1)	31 (75.6)	63 (78.8)	
Drinker	7 (17.9)	10 (24.4)	17 (21.3)	
Smoking cigarettes, n (%)				
Non-smoker	37 (94.9)	33 (80.5)	70 (87.5)	
Smoker	1 (2.6)	3 (7.3)	4 (5.0)	
Ex-smoker	1 (2.6)	5 (12.2)	6 (7.5)	
Exercise, n (%)				
Yes	34 (87.2)	33 (80.5)	67 (83.8)	
No	5 (12.8)	8 (19.5)	13 (16.3)	
Frequency of drinking SSBs, n (%)				
No	8 (20.5)	6 (14.6)	14 (17.5)	
1-3 glasses/week	15 (38.5)	17 (41.5)	32 (40.0)	
More than 3 glasses/week	16 (41.0)	18 (43.9)	34 (42.5)	

NM = Normal male, OM = Obese male
SSBs = Sugar-sweetened beverages

The mean telomere length of total subjects aged 61-82 years, had significantly shorter than those in aged 21-40 and 41-60 years. In subjects aged 41-60 years, telomere length was also significantly shorter than those in aged 21-40 years. In both normal weight and obese groups, telomere length of subjects aged 21-40 years was significantly longer than those in aged 41-60 and 61-82 years. In the same way, telomere length in aged 41-60 years was significantly longer than those in aged 61-82 years. When comparison was made between normal weight and obese group, OM aged 61-82 years had significantly shorter telomere length than those in NM. The mean telomere length in aged 21-40 and 41-60 years tended to be shorter than NM, but did not reach statistical significance (Table 2).

Table 3 showed the mean telomere length of normal weight and obese group in various factors. We found that subjects with normal BMI had longer telomere than those in overweight, obese I, and obese II, especially in NM, the mean telomere length was significantly longer than those in obese I. Similarly, the mean telomere length of both groups with fat mass index range ≤ 50th percentiles

Table 2 Mean (± SD) and range of relative telomere length classified by age groups and body weight status

Age (years)	T/S ratio (range)					
	NM	n	OM	n	Total	n
21-40	1.19 ± 0.12 (1.04 - 1.37)	13	1.12 ± 0.13 (0.89 - 1.34)	15	1.15 ± 0.13 (0.89 - 1.37)	28
41-60	1.08 ± 0.12^{b2} (0.84 - 1.25)	14	1.06 ± 0.10 (0.89 - 1.29)	15	1.07 ± 0.11^{b2} (0.84 - 1.29)	29
61-82	0.96 ± 0.06^{b1c2} (0.88 - 1.04)	12	0.90 ± 0.07^{a1b1c1} (0.81 - 0.99)	11	0.93 ± 0.07^{b1c1} (0.81 - 1.04)	23

NM = Normal male, OM = Obese male
Significant difference from NM, ^{a1} $P < .05$
Significant difference from 21-40 years within body weight status, ^{b1} $P < .0001$, ^{b2} $P < .05$
Significant difference from 41-60 years within body weight status, ^{c1} $P < .0001$, ^{c2} $P < .005$, ^{c3} $P < .05$

Table 3 Mean (± SD) of relative telomere length classified by body weight status and various parameters

Parameters	T/S ratio			
	n (%)	NM	n (%)	OM
BMI (kg/m^2)				
Normal	25 (64.1)	1.12 ± 0.12	2 (4.9)	1.20 ± 0.12
Overweight	9 (23.0)	1.05 ± 0.14	8 (19.5)	1.08 ± 0.15
Obese I	5 (12.9)	0.95 ± 0.12^{b2}	23 (56.1)	1.00 ± 0.13
Obese II	-	-	8 (19.5)	1.05 ± 0.14
Fat mass index				
≤ 50th percentiles	33 (84.6)	1.10 ± 0.13	8 (19.5)	1.13 ± 0.16
< 50th - ≤ 75th percentiles	6 (15.4)	0.96 ± 0.11^{d1}	14 (34.1)	1.03 ± 0.11
>75th percentiles	-	-	19 (46.4)	1.00 ± 0.14^{d1}
Underlying diseases				
No	24 (61.5)	1.11 ± 0.13	18 (43.9)	1.12 ± 0.13
Yes	15 (38.5)	1.03 ± 0.12	23 (56.1)	0.97 ± 0.11^{a1c1}
Alcohol consumption				
Non-drinker	32 (82.1)	1.09 ± 0.13	31 (75.6)	1.05 ± 0.14
Drinker	7 (17.9)	1.10 ± 0.17	10 (24.4)	0.99 ± 0.14^{c1}
Exercise				
Yes	34 (87.2)	1.08 ± 0.14	33 (80.5)	1.05 ± 0.15
No	5 (12.8)	1.08 ± 0.13	8 (19.5)	1.01 ± 0.08
Duration of exercise				
30-60 minutes/day	27 (79.4)	1.08 ± 0.13	27 (81.8)	1.04 ± 0.14
> 60 minutes/day	7 (20.6)	1.07 ± 0.17	6 (18.2)	1.07 ± 0.19
Visceral fat				
Normal level	6 (66.7)	1.14 ± 0.13	13 (31.7)	0.97 ± 0.08^{a1}
Excessive level	13 (33.3)	1.11 ± 0.17^{b2}	28 (68.3)	1.00 ± 0.11^{a1}
FPG				
Normal	30 (77.0)	1.09 ± 0.14	27 (65.8)	1.08 ± 0.14
IFG	9 (23.0)	1.04 ± 0.13	12 (34.2)	0.96 ± 0.10^{b1}
BUN, Creatinine				
Normal	39 (100.0)	1.08 ± 0.13	41 (100.0)	1.04 ± 0.14

NM = Normal male, OM = Obese male
Significant difference from NM, ^{a1} $P < .05$
Significant difference from normal, ^{b1} $P < .005$, ^{b2} $P < .05$
Significant difference from no and non-drinker, ^{c1} $P < .05$
Significant difference from ≤ 50th percentiles, ^{d1} $P < .05$

was significantly longer than those in < 50th - ≤ 75th percentiles of NM and > 75th percentiles of OM.

The mean telomere length in subjects who had underlying diseases tended to be shorter than those who did not have underlying disease. When we compared the mean telomere length between normal weight and obese group, OM who had underlying diseases had significantly shorter than those in NM. Among drinkers, we found that the mean telomere length in obese group was significantly shorter than non-drinkers. The mean telomere length of subjects who did not exercise was shorter than subjects who regularly exercised in both groups.

Visceral fat rating was observed in obese group, the mean telomere length was significantly shorter than those in NM. Moreover, we found that differences of telomere length were found to be significantly longer in subjects with normal level of visceral fat rating than those with excessive level in normal weight group. Subjects who had normal FPG was found the mean telomere length longer than those in IFG. Kidney function in both normal weight and obese groups was in the normal range.

As for relationship between telomere length and various factors, this study found the negative association between relative telomere length and age (r = -0.630, P < .0001), BMI (r = -0.277, P < .05), percentage of body fat (r = -0.316, P < .005), visceral fat (r = -0.476, P < .0001), FPG (r = -0.371, P < .005), and BUN (r = -0.300, P < .01) were maintained in all subjects.

Stepwise multiple regression analysis was performed to predict independent associations between telomere length and various factors. Age, BMI, percentage of body fat, fat mass, visceral fat, FPG, TG, TC, HDL-C, LDL-C, and BUN were analyzed as the independent variables in multiple linear regression models for telomere length. The regression model, we found significant predictors that effect on telomere length including age (β= -0.601, P < .0001) and percentage of body fat (β= -0.245, P < .01).

Discussion

Telomere length has been proposed as a candidate biomarker of aging because telomere shortening leads to senescence and apoptosis that affect health and lifespans. In general, telomere length varies throughout a span of life, which progressively shortens with age. The present study was conducted in normal weight and obese only Thai male population in various age groups. We found that the shortest telomere length was observed in subjects aged 61-82 years while the longest telomere length was found in subjects aged 21-40 years in both normal weight and obese groups. Consistent with other studies in the Thai population, the mean telomere length was found statistically different among age groups and decreased with increasing age.[17,18] Those results were also in line with our findings. It is well known that telomerase is a cellular reverse transcriptase that synthesizes tandem repeats of the telomeric DNA sequence.[19] Oxidative stress has been found to increase in elderly, probably due to an unregulated generation of free radicals caused by mitochondrial senescence and a reduction in antioxidant defenses.[20] These evidences supported our findings, age estimation in biological evidence is one of the features that determine a person identity.

Excessive and abnormal accumulation of body fat induced oxidative stress and inflammation leading to imposing devastating health and financial tolls on individuals and society.[21] We found that percentage of body fat is a predictor variable with telomere length. The result showed that the mean telomere length in obese males was found to be significantly shorter than those in normal weight group aged 61-82 years. Nevertheless, the trend of telomere length of normal weight males in other age groups appeared to be shorter than obese group, but did not reach statistically significance. Most of the previous studies reported that telomere length in overweight and obese subjects was found significantly shorter than normal weight subjects.[22,23] Those studies reported a negative correlation between telomere length and obesity, and pointed out the increased inflammatory processes that accompanied with excess body weight. Oxidative damage of cellular structures including DNA, proteins, and lipids was deregulated by reactive oxygen species production.[24] Among of basic four DNA bases, guanine has the lowest redox potential that makes it the major target of oxidation. Telomere length has been considered 'hot zones' for oxidative damage. Because of the G-rich nature of TTAGGG repeats renders, telomere length is highly sensitive to oxidative stress damage because it has the lowest redox potential and high content of guanines within the telomere sequences.

Visceral fat rating was associated with shorter telomere in both normal weight and obese groups, but it was not analyzed as a predictor variable with telomere length. Hunter GR et al.[25] identified an important age-related factor that contributes to the increasing visceral adiposity which showed that fat distribution may be as important as overall obesity in increasing risk of type 2 diabetes and cardiovascular diseases. The mechanism that leads to increase oxidative stress caused by overweight and obesity. Heightened production of adipokines is likely more related to central obesity that leads to increase visceral fat accumulation which was known to correlate with metabolic diseases[26] and telomere shortening.[27]

Following the present study, we revealed that the FPG level in obese subjects was higher than normal weight subjects possibly due to the effect of adipose tissue that is an endocrine organ that influences glucose metabolism. Thus, exceeding the storage adipose tissue that results in lipotoxicity, a condition characterized by fatty acid infiltration of insulin target tissues that leads to insulin resistance.[28] Consistent with this research, the mean telomere length in normal FPG subjects was longer than those who had IFG in both normal weight and obese groups. Increasing evidence showed that telomere length was shorter in age-related diseases, especially diabetes. Ma et al.[29] determined whether the shorter of telomere occurs in type 1 and type 2 diabetes, and explored the effect of antioxidant status on the telomere length, and suggested that relationship between diabetes mellitus and telomere length was influenced by age and BMI. Consequently, telomere length was closely associated with plasma glucose status in diabetes patients and increased oxidative stress that leads to accelerated telomere shortening.[30]

Age has a particularly negative impact on the kidneys, and their deterioration contributes directly to the etiology of many other age-related diseases. In this study, the mean BUN level in subjects aged 61-82 years was significantly higher than that in other age groups. The correlation coefficient confirmed that telomere length was negatively correlated with BUN. In agreement with Percy et al.[31] which suggested that increased oxidative stress and free radical damage seen in the elderly can also contribute to an increase in BUN, and is a major cause of age-related tissue atrophy, which results in a leading to a failure to protect the DNA in cells.

Subjects who had regularly exercised tended to be longer telomere length than those who lacked exercise but did not show a significant difference. The mean telomere length of subjects who performed more than 60 minutes of exercises was found to be longer than those with 30-60 minutes of exercise. These findings were consistent with the study of Puterman et al.[32] which suggested that about 75 minutes of vigorous exercise per week, based on Centers for Disease Control recommendations (CDC), was found to be associated with increased oxidative damage. Despite vigorous physical activity, exercisers had longer telomere length, and it appeared to prevent subjects from psychological stress.

Our study suggested the role of body weight status in linking life expectancy to telomere erosion. Overall, tackling obesity, healthy lifestyle behaviors, reductions in oxidative, and lifestyle-related stress appear to be important factors in reducing cellular aging and the deleterious effects that deteriorate the telomere length.

Recommendation

To our knowledge, this is a preliminary study to show telomere length among normal weight and obese Thai males in various age groups. Further studies with the same strict criterion population, genders, more sample size in each age group, and longitudinal research are necessary to study the mechanism of shortening of telomere length, and its effects on well-being and diet-related chronic diseases.

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