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Role of arterial telomere dysfunction in hypertension: relative contributions of telomere shortening and telomere uncapping

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Abstract

Objective—Telomere shortening in arteries could lead to telomere uncapping and cellular senescence, which in turn could promote the development of hypertension.

Methods and results—To assess the novel role of arterial telomere dysfunction in hypertension, we compared mean telomere length (qPCR), telomere uncapping (serine 139 phosphorylated histone γ-H2A.X (γ-H2) localized to telomeres: ChIP), and tumor suppressor protein p53 (P53)/cyclin-dependent kinase inhibitor 1A (P21)-induced senescence (P53 bound to *P21* gene promoter: ChIP) in arteries from 55 age-matched hypertensive and nonhypertensive individuals. Arterial mean telomere length was not different in hypertensive patients compared with nonhypertensive individuals ($P = 0.29$). Arterial telomere uncapping and P53/P21- induced senescence were two-fold greater in hypertensive patients compared with nonhypertensive individuals ($P = 0.04$ and $P = 0.02$, respectively). Arterial mean telomere length was not

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Conflicts of interest
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associated with telomere uncapping or P53/P21-induced senescence (r=– 0.02, $P = 0.44$ and $r = 0.01$, $P = 0.50$, respectively), but telomere uncapping was a highly influential covariate for the hypertension group difference in P53/P21-induced senescence ($r = 0.62$, $P < 0.001$, $\eta_p^2 = 0.35$). Finally, telomere uncapping was a significant predictor of hypertension status ($P = 0.03$), whereas mean telomere length was not ($P = 0.68$).

Conclusion—Collectively, these findings demonstrate that arterial telomere uncapping and P53/P21-induced senescence are linked to hypertension independently of mean telomere length, and telomere uncapping influences hypertension status more than mean telomere length.

Keywords

arteries; cellular senescence; hypertension; telomere

INTRODUCTION

Arterial telomere dysfunction may contribute to the pathogenesis of hypertension by inducing cellular senescence. Telomeres are terminal sequences of TTAGGG repeats that make up the natural ends of chromosomes [1,2]. Telomeres form specialized structures that protect chromosome ends from being recognized as dsDNA breaks and initiating a dsDNA break response [3–5], which can induce cellular senescence through tumor suppressor protein p53 (P53)-dependent expression of cyclin-dependent kinase inhibitor 1A (P21) [5,6]. In-vitro studies in various human cell types have shown that breakdown of telomere structure, referred to as telomere uncapping, leads to P53 activation and P53/P21-induced senescence [5,6]. Replication and genotoxic stress-mediated telomere shortening beyond a critical telomere length may lead to uncapping in human cells [5–8]. Following in-vitro telomere uncapping in human cells, phosphorylation of histone γ-H2A.X at serine 139 (γ-H2) occurs at telomeric chromatin to aid in the dsDNA break response and initiation of P53/P21-induced senescence [5,6]. Importantly, cellular senescence has been implicated in the etiology of chronic diseases [9,10], including cardiovascular diseases (CVDs) [11,12].

Although the role of arterial telomere dysfunction in hypertension has not been assessed, telomere shortening in white blood cells (WBCs) has been linked to hyper-tension [13,14], and telomere shortening in arteries has been associated with abdominal aortic aneurysm [15] and atherosclerotic plaque development [16,17]. Telomere shortening in arteries could lead to telomere uncapping and P53/P21-induced senescence, which in turn could promote the development of hypertension. Interestingly, some studies have suggested that arterial telomere uncap-ping is more closely linked to P53/P21-induced senescence than telomere shortening in human cells [18,19], including arterial tissues [20]. Insight into the relative contributions of arterial telomere shortening and telomere uncapping to the etiology of hypertension will lead to a more complete understanding of the potential role of telomere dysfunction in hypertension. This in turn could lead to novel therapeutic strategies that target telomere dysfunction to delay onset, attenuate severity, or even reverse hypertension.

Therefore, an important unexplored hypothesis is that arterial telomere dysfunction and P53/P21-induced senescence are associated with hypertension. To test this hypothesis, we compared mean telomere length, telomere uncapping (γ-H2 localized to telomeres), and

P53/P21-induced senescence (P53 bound to P21 gene promoter) in arteries from an age-matched sample of hypertensive and nonhypertensive individuals. Next, we used logistic regression to compare the influence of arterial mean telomere length and telomere uncapping on hypertension status in these individuals.

METHODS

Arterial biopsy collection and general sample processing

Arterial biopsies were excised from patients undergoing a prophylactic procedure for melanoma-associated sentinel lymph node biopsy at the Huntsman Cancer Hospital, University of Utah. A heterogeneous sample (*n*) of 55 age-matched individuals (34 men and 21 women) consented to donate arterial biopsies for the study. A comprehensive outline of biometric, physiological, and medical characteristics for all participants enrolled in the study was collected (Table 1). Participants were classified as hypertensive or nonhypertensive according to prior hypertension diagnosis reported in medical history. Both medical histories and prescription medication use were noted, and participants with prior diagnosis of CVD other than hypertension or metastatic melanoma were excluded. Participant blood pressures were measured and recorded during physician consultations according to standard clinical blood pressure measurement guidelines [21]. Participants with high lactate dehydrogenase (LDH) blood values were excluded from the study, as blood LDH levels are considered a strong indicator of melanoma metastasis when outside the normal range [22,23]. Thus, all participants were within our institutionally specified normal range of 313–618 U/l, and LDH levels were not different between groups ($P = 0.32$) nor correlated with any outcomes (all $P \geq 0.08$). No participants included in this study had received chemotherapy, as this criterion was a contraindication for surgery. The Institutional Review Boards of the University of Utah and the Salt Lake City Veteran's Affairs Medical Center approved all protocols, and written informed consent was obtained from all participants prior to biopsy collection.

The arterial biopsies consisted of skeletal muscle feed arteries excised from the inguinal (e.g. hip adductors or quadriceps femoris) or axillary regions (e.g. serratus anterior or latissimus dorsi) and were free of melanoma cells [24]. Arterial biopsies were identified as skeletal muscle feed arteries by entry into muscle bed, gross anatomy, coloration, and pulsatile bleed pattern [24]. There were no differences in our outcomes between arteries from inguinal and axillary regions (all $P < 0.10$), and no interactions between the effects of biopsy ource and group were found in any outcomes (all $P < 0.16$). Arterial biopsies were cleaned of adipose and connective tissue, and washed to remove residual blood cells. The average size of each artery was 2 mm in length, approximately 0.5 mm in luminal diameter, and approximately 10–20 mg in mass. Cleaned arteries were then snap frozen in liquid nitrogen and stored at −08°C prior to performing the following outcomes. All samples were assayed in triplicate, and replicate means were used for analysis.

Mean telomere length

A sequence-independent multiplex qPCR technique using a SYBR Green master mix with 0.625U AmpliTaq Gold 360 DNA polymerase (Life Technologies Corporation, Grand Island, New York, USA) was utilized to determine mean telomere length as described by

Cawthon [25]. Telomeric DNA (T) SQs and albumin SQs, used as single copy gene (S) to control tissue concentration in samples, were generated by standard curve and mean telomere length was expressed as the T/S ratio. Mean telomere lengths and telomere ranges were generated by converting T/S ratios to bp of DNA using the formula: bp = 3330 (T/S) þ 3730, derived by Cawthon [25].

Telomere uncapping

ChIP was used to determine the amount of γ-H2 (Santa Cruz Biotechnology Inc.) localized to telomeres. ChIPs were performed as previously described [20] and analyzed via qPCR for telomere content as described by Cawthon [25]. Final values were expressed as the ratio of background corrected starting quantity (SQ) of telomeric DNA enriched by ChIP to telomeric DNA SQ in INPUT fraction. INPUTs represented 50% of telomeric DNA present in corresponding ChIP and were used to control for tissue concentration in samples [ex: (γ-H2 — SQ background SQ)/INPUT SQ = final value].

P53/P21-induced senescence

ChIPs were performed to assess P53 bound to *P21* gene promoter (EMD Millipore Corporation) as previously described [20], using a sequence-independent qPCR assay with FastStart SYBR Green Master (Roche Diagnostics Corporation, Roche Applied Science).

Data analysis

Outcome measures were defined as mean telomere length, γ-H2 localized to telomeres, P53 bound to P21 gene promoter, and hypertension status. Independent samples *t*-tests were performed to assess group differences in all outcomes. Analysis of variance tests were performed with least significance difference post-hoc tests to assess tertile differences in all outcomes. The Pearson correlation coefficient (*r*) was used to assess correlations between each outcome and between outcomes and participant characteristics. To assess group differences in participant characteristics, independent samples *t*-tests or χ^2 tests were performed. Analysis of covariance tests with least significance difference post-hoc tests were performed with all continuous variable participant characteristics with correlated outcomes to determine the influence of covariates on any group differences in outcomes. All covariates were tested for homogeneity across groups within outcomes ($P = 0.67$) and covariate effect size was assessed using partial eta squared (η_P^2).

Logistic regression

Forward and backward likelihood ratio logistic regression analyses were conducted to assess the influence of mean telomere length, γ-H2 localized to telomeres, and BMI on hypertension status (test statistic: Δ in 2 log likelihood). Logistic regression model goodness-of-fit was determined by comparing the significant predictors of hypertension status identified by forward and backward likelihood ratio logistic regression analyses (good model of data: same predictors). Additionally, the Hosmer–Lemeshow (good model of data: significance value ≥ 0.05) and Nagelkerke's R^2 (pseudo R^2) statistics were used to assess logistic regression model goodness of fit. Significance was set at $P < 0.05$.

RESULTS

Participant characteristics

Approximately, 83% of hypertensive patients used one or more prescription medications to control blood pressure (n = 24; Table 1). By definition, no nonhypertensive individuals used prescription blood pressure medications. BMI was greater in hypertensive patients compared with nonhypertensive individuals (P = 0.01; Table 1), whereas sex, SBP, DBP, and pulse pressure were not different between groups (all $P \geq 0.09$; Table 1).

Arterial telomere dysfunction, P53/P21-induced senescence, and hypertension

Arterial mean telomere length was not different between hypertensive and nonhypertensive individuals (P = 0.29; Fig. 1). Arterial γ-H2 localized to telomeres was =nearly two-fold greater in hypertensive patients compared with nonhypertensive individuals (P = 0.04; Fig. 1). Correspondingly, arterial P53 bound to *P*21 gene promoter was over two-fold greater in hypertensive patients compared with nonhypertensive individuals (P = 0.02; Fig. 1). No participant characteristics were correlated with γ-H2 localized to telomeres or P53 bound to *P21* gene promoter (all $P \geq 0.09$).

Influence of telomere dysfunction on P53/P21-induced senescence

Mean telomere length was not correlated with γ-H2 localized to telomeres or P53 bound to *P21* gene promoter (r=− 0.02, P = 0.44 and r=0.01, P, respectively). Additionally, were there no differences in P53 bound to *P21* gene promoter between participants in the shortest (7.8–9.9 kb), median (10.6–12.4 kb), and longest (12.5– 17.2 kb) tertiles of mean telomere length (all $P \geq 0.08$; Fig. 2). γ-H2 localized to telomeres demonstrated a strong positive correlation with P53 bound to *P21* gene promoter (r = 0.62, $P < 0.001$). Likewise, there was almost six-fold greater P53 bound to *P21* gene promoter among individuals in the highest tertile of γ-H2 localized to telomeres compared with those from the lowest tertile ($P < 0.01$; Fig. 2). Analysis of covariance results indicated that γ-H2 localized to telomeres had a large effect on the P53 bound to P21 gene promoter group difference ($\eta_P^2 = 0.35$), accounting for 35% of the total hypertension-related variance in this P53/P21-induced senescence marker. Controlling for the influence of γ-H2 localized to telomeres, the adjusted group difference in P53 bound to P21 gene promoter was no longer significant (ΔP 0.04–0.24).

Influence of telomere dysfunction and BMI on hypertension status

Logistic regression results indicated that γ-H2 localized to telomeres and BMI were both significant predictors of hypertension status (P = 0.03 and 0.01, respectively; Table 2), whereas mean telomere length was not (P = 0.68; Table 2). Together, γ-H2 localized to telomeres and BMI explained 25% of the variance in hypertension status (pseudo $R^2 = 0.25$; Table 2) and correctly predicted hypertension status in over 67% of hypertensive patients (Supplemental Digital Content; Table S1, http://links.lww.com/HJH/A345. Logistic regression group classification table). Backward and forward likelihood ratio logistic regression analyses produced the same results (Table 2 and S1, http://links.lww.com/HJH/A345), and generated identical Hosmer–Lemeshow test results (both P = 0.78; Table 2).

DISCUSSION

The key novel findings of the current study are as follows. Arterial mean telomere length was not different in hypertensive patients compared with nonhypertensive individuals. Arterial telomere uncapping and P53/P21-induced senescence were greater in hypertensive patients compared with nonhypertensive individuals. Arterial mean telomere length was not associated with telomere un-capping or P53/P21-induced senescence, but telomere uncapping was a highly influential covariate for the group difference in P53/P21-induced senescence. Finally, telo-mere uncapping was a significant predictor of hypertension status, whereas mean telomere length was not. Collectively, these findings demonstrate that arterial telomere uncapping and P53/P21-induced senescence are linked to hyper-tension independently of mean telomere length, and telo-mere uncapping influences hypertension status more than mean telomere length.

Arterial mean telomere length and hypertension

Arterial telomere shortening could play a role in hyper-tension by leading to telomere uncapping and P53/P21-induced senescence. Here we showed no difference in mean telomere length in arteries from hypertensive patients compared with those from nonhypertensive individuals. Furthermore, mean telomere length was not correlated with telomere uncapping or P53/P21-induced senescence. Damage to telomeric DNA caused by mechanical stress [26] on arterial cells from elevations in SBP or reactive oxygen species [7,8] that accumulate in the context of hypertension [27,28] could lead to telomere uncapping independent of telomere shortening. This might explain the lack of associations between mean telomere length and telomere uncapping or P53/P21 senescence. Although its role in hypertension has not previously been assessed, arterial telomere shortening has been associated with chronic obstructive pulmonary disease [12], abdominal aortic aneurysm [15], and atherosclerosis [16,17]. These data demonstrate that arterial mean telomere length is not associated with hypertension, telomere uncapping, or P53/P21-induced senescence, which suggests that arterial telomere shortening likely does not contribute to the pathogenesis of hypertension.

Telomere shortening in WBCs has been correlated with hypertension in medicated individuals [13,14], increased pulse pressure in men [29], and pulmonary hypertension severity [12]. Arterial mean telomere length should be more relevant to the etiology of hypertension than that of WBCs, but the accessibility of blood makes WBCs a preferred source of tissue for researchers interested in the role of telomere dysfunction in CVD. Tissue-specific differences in mean telomere length and rates of telomere shortening may account for the difference in our findings from those in studies with WBCs. Therefore, these results cast doubt on the biological relevance of telomere shortening in WBCs to hypertension.

Arterial telomere uncapping and hypertension

Arterial telomere uncapping could play a role in hyper-tension by leading to P53/P21-induced senescence. We showed that telomere uncapping and P53/P21 senescence were greater in arteries from hypertensive patients compared with those from nonhypertensive

individuals, and these differences were independent of mean telomere length. We also reported that telomere uncapping demonstrated a strong positive correlation with P53/P21 senescence. Interestingly, telomere uncapping was a highly influential covariate for the group difference in P53/P21 senescence, accounting for about 35% of the variance in this outcome. Prior to these findings, the role of telomere uncapping in hypertension was entirely unknown. The only previous study to measure arterial telomere uncapping reported greater γ-H2 localized to telomeres with advancing age, which was positively correlated with P53/P21 senescence independent of telomere shortening [20]. Arterial P53/P21-induced senescence has been linked to atherosclerosis [11] and chronic obstructive pulmonary disease [12], but its association with hypertension was also previously unexplored. These results demonstrate that arterial telomere uncapping and P53/P21-induced senescence are associated with hypertension and establish the link between telomere uncapping and P53/P21-induced senescence. Thus, arterial telomere uncapping may contribute to the etiology of hypertension by leading to P53/P21-induced senescence.

Influence of telomere dysfunction on hypertension status

If arterial telomere dysfunction indeed plays a role in hypertension, then mean telomere length or telomere uncapping should influence hypertension status. Utilizing logistic regression analyses, we showed that telomere uncapping and BMI were significant predictors of hyper-tension status, whereas mean telomere length was not. Both telomere uncapping and BMI correctly predicted hypertension status in over two thirds of hypertensive patients and explained one-quarter of the variance in hypertension status. BMI was included in the logistic regression models because it was higher in hypertensive patients compared with nonhypertensive individuals and a well established CVD risk factor. Thus, we felt it was necessary to account for the influence of BMI on hypertension status in our logistic regression model. These findings demonstrate that telomere uncapping influences hyper-tension status more than mean telomere length, which suggests that in arteries, telomere uncapping may contribute more to the development of hypertension than mean telomere length.

Influence of participant characteristics on outcomes

Participant characteristics that include conventional CVD risk factors or prior CVD diagnoses could influence our hypertension-associated outcomes and hypertension status. To control for these potential confounds, we matched our participants for age and excluded individuals with a prior diagnosis of CVDs other than hypertension. Nonetheless, our hypertensive patients had higher BMI than nonhypertensive individuals. BMI was included in the logistic regression models to account for this group difference, as described above. Importantly, no participant characteristics were correlated with telomere uncapping or P53/P21-induced senescence. Thus, groupdifferences in these outcomes were independent of participant characteristics.

Most hypertensive patients used one or more prescription medications to control their blood pressure, and none of our nonhypertensive individuals took prescription blood pressure medications. Whereas our hypertensive patients' mean SBP was clinically hypertensive, SBP was not different between hypertensive and nonhypertensive individuals. This was not

unexpected, as most of our hypertensive patients used blood pressure medications, and the mean age of both hypertensive and nonhypertensive individuals was nearly 64 years of age. To avoid misidentification of hypertensive individuals as nonhypertensive, we based our group classification on prior diagnosis of hypertension rather than SBP measured during physician consultations. Thus, the lack of group difference in SBP did not affect our outcomes. Collectively, these experimental controls and analyses account for the influence of CVD risk factors and CVDs on our hypertension-associated outcomes and hypertension status.

In conclusion, the goal of this study was to elucidate the potential role of arterial telomere dysfunction in hyper-tension. Our findings demonstrate that arterial telomere uncapping and P53/P21-induced senescence were linked to hypertension independent of mean telomere length. We also reported that telomere uncapping had greater influence on hypertension status than mean telomere length. These results establish the framework for more mechanistic studies aimed at determining the causal role of arterial telomere dysfunction in hypertension. Furthermore, these results provide insight into the relative contributions of arterial telomere shortening and telomere uncapping to the etiology of hypertension and a more complete understanding of the role of telomere dysfunction in CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

All experiments were performed in the Translational Vascular Physiology Laboratory at the University of Utah and Veteran's Affairs Medical Center-Salt Lake City, Geriatric Research Education and Clinical Center. G.M. and A.J.D. contributed to all aspects of the study, including the conception and design of the experiments, collection, analysis, and interpretation of data, and drafting and revision of the article. S.J.I., L.A.L., R.M.C., R.H.I.A., R.D.N., A.E.W., and R.S.R. contributed to the collection and analysis of data as well as revision of the article. Additionally, R.M.C. contributed reagents and analytical tools that were essential to completing this study.

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Abbreviations

COPD	chronic obstructive pulmonary disease
CVDs	cardiovascular diseases
LDH	lactate dehydrogenase
LSD	least significance difference
P21	cyclin-dependent kinase inhibitor 1A
P53	tumor suppressor protein p53
pseudo R^2	Nagelkerke's R^2
r	Pearson correlation coefficient

S	single copy gene
T	telomeric DNA
WBCs	white blood cells
γ-H2	phosphorylated histone γ-H2A.X at serine 139
η_p^2	partial eta squared

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Reviewers' Summary Evaluations

Reviewer 1 In this study the authors compared mean telomere length, telomere uncapping and senescence of 55 age-matched hypertensives to nonhypertensives. Telomere shortening is thought to lead to cellular senescence via telomere uncapping and may play a role in promoting the development of hypertension. The authors found a significant link between telomere uncapping, p53 induced senescence and hypertension. Surprisingly, mean telomere length was not associated with telomere uncapping, senescence, or hypertension. So far, association of telomere shortening with cardiovascular disease has been assessed in white blood cells only. This study casts some doubt on the biological relevance of these previous findings.

Reviewer 2 Compliments to the authors for this very interesting paper assessing a novel role of arterial telomere dysfunction in hypertension. The paper is very technical and probably addressed to more than specialist readers. The main finding is that telomere uncapping is a significant predictor of hypertension status, while mean telomere length is not. Arterial telomere uncapping is therefore linked to hyper-tension independent of mean telomere length. The paper represents a very strong and significant link between pre-clinical and clinical hypertension, what is going to represent one of the scientific challenges of the next decades.

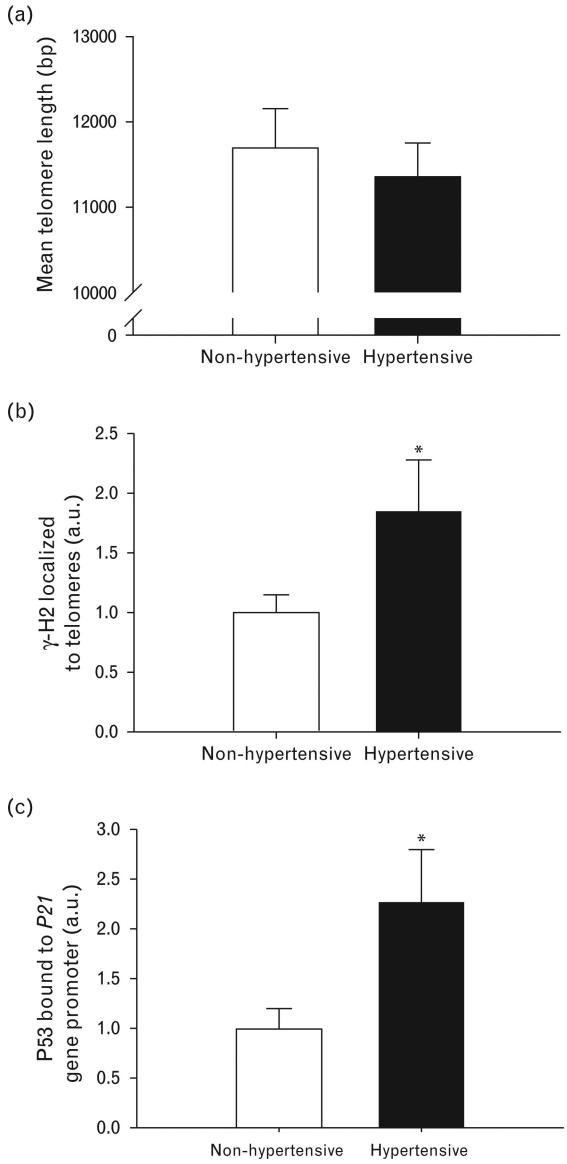

FIGURE 1.

Arterial telomere dysfunction, P53/P21-induced senescence, and hyper-tension. (a) Mean telomere length, (b) γ-H2 localized to telomeres, and (c) P53 bound to P21 gene promoter across groups (both $P < 0.04$). Data presented are mean ± SEM normalized to control group. γ-H2, p-histone γ-H2A.X (ser139); P53, tumor suppressor protein p53; *P21*, cyclin-dependent kinase inhibitor 1A.

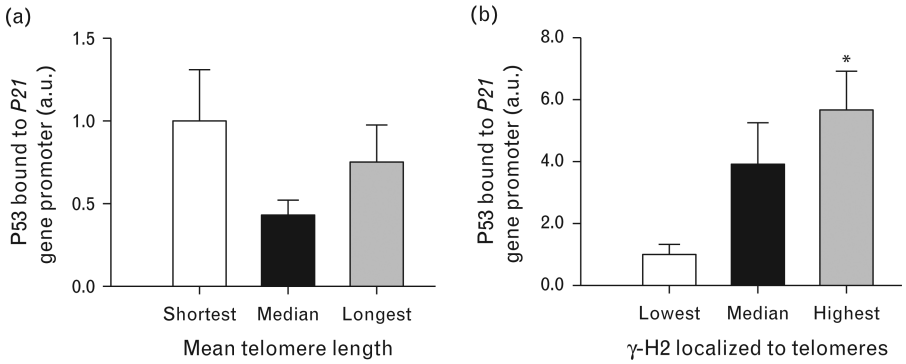

FIGURE 2.

Influence of telomere dysfunction on P53/P21-induced senescence. (a) P53 bound to P21 gene promoter across tertiles of mean telomere length and (b) P53 bound to *P21* gene promoter across tertiles of γ-H2 localized to telomeres ($P < 0.01$ compared with lowest tertile). Data presented are mean ± SEM normalized to control group. γ-H2, p-histone γ-H2A.X (ser139); P53, tumor suppressor protein p53; *P21*, cyclin-dependent kinase inhibitor 1A.

TABLE 1

Age-matched nonhypertensive and hypertensive participant characteristics

Characteristic	Nonhypertensive (*n* = 26)	Hypertensive (*n* = 29)	*P*
Age (years)	63.7 ± 1.5	63.8 ± 2.4	0.48
Sex (M/F)	16/10	18/11	0.48
BMI (kg/m^2)	27.2 ± 0.9	30.1 ± 1.1	0.01*
SBP (mmHg)	135.8 ± 3.8	140.8 ± 3.5	0.17
DBP (mmHg)	78.4 ± 2.4	78.5 ± 2.4	0.48
PP (mmHg)	57.5 ± 2.5	62.3 ± 2.5	0.09
Prescription medications			
Calcium channel blockers	0.0% (0)	24.1% (7)	N/A
β-Blockers	0.0% (0)	17.2% (5)	N/A
ACE inhibitors	0.0% (0)	37.9% (11)	N/A
Angiotensin blockers	0.0% (0)	17.2% (5)	N/A
Diuretics	0.0% (0)	34.5% (10)	N/A

ACE, angiotensin-converting enzyme; PP, pulse pressure.

*Data presented are mean ± SEM, % (*n*), and *n* across groups ($P < 0.05$).

TABLE 2

Logistic regression results

Backward likelihood ratio		
Step 1	Hosmer–Lemeshow ($P = 0.47$)	Pseudo $R^2 = 0.26$
Predictor	Δ in –2 log likelihood	P
γ-H2	4.77	0.03*
mTL	0.18	0.68
BMI	6.49	0.01*
Step 2	Hosmer–Lemeshow ($P = 0.78$)	Pseudo $R^2 = 0.25$
Predictor	Δ in –2 log likelihood	P
γ-H2	4.76	0.03*
BMI	6.83	0.01*
Forward likelihood ratio		
Step 1	Hosmer–Lemeshow ($P = 0.51$)	Pseudo $R^2 = 0.14$
Predictor	Δ in –2 log likelihood	P
BMI	5.48	0.02*
Step 2	Hosmer–Lemeshow ($P = 0.78$)	Pseudo $R^2 = 0.25$
Predictor	Δ in –2 log likelihood	P
BMI	6.83	0.01*
γ-H2	4.76	0.03*

γ-H2, p-histone γ-H2A.X (ser139) localized to telomeres; mTL, mean telomere length

*$P < 0.05$.