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## Rise in Insulin Resistance Is Associated With Escalated Telomere Attrition

Jeffrey P. Gardner, PhD; Shengxu Li, MD; Sathanur R. Srinivasan, PhD; Wei Chen, MD, PhD; Masayuki Kimura, MD, PhD; Xiaobin Lu, MD; Gerald S. Berenson, MD; Abraham Aviv, MD

**Background**—Insulin resistance predisposes to cardiovascular disease and shortens human lifespan. We therefore tested the hypothesis that a rise in insulin resistance in concert with gain in body mass is associated with accelerated white blood cell telomere attrition.

**Methods and Results**—We measured white blood cell telomere dynamics and age-related changes in insulin resistance and body mass index in young adults of the Bogalusa Heart Study. Over 10.1 to 12.8 years, the relative changes in telomere length were correlated with the homeostasis model assessment of insulin resistance ( $r = -0.531$ ,  $P < 0.001$ ) and changes in the body mass index ( $r = -0.423$ ,  $P < 0.001$ ).

**Conclusions**—These findings provide the first tangible nexus of telomere biology with insulin resistance and adiposity in humans. (*Circulation*. 2005;111:2171-2177.)

**Key Words:** telomere ■ insulin ■ obesity ■ aging ■ stress

Obesity shortens the human lifespan because it frequently causes metabolic derangements, including insulin resistance, that lower the threshold for a host of aging-related diseases, particularly cardiovascular disease.<sup>1-4</sup> In contrast, caloric restriction stalls aging, as evidenced by its ability to extend both the median and maximal lifespan in many organisms, including mammals.<sup>5</sup> A battery of metabolic indicators in rhesus monkeys describes the protection afforded by caloric restriction against risk factors, including insulin resistance, that predispose to diseases of aging.<sup>6</sup> Because experimental models of lifelong caloric restriction in people are impractical, other venues may be explored to assess the link between caloric intake and aging in humans.

It has been proposed that (excluding pregnancy), “not gaining weight after early adulthood” may serve as an epidemiological paradigm of caloric restriction in humans.<sup>7</sup> Accordingly, gain in body mass (primarily due to adiposity) may hasten biological aging. Because body fat reflects not only caloric consumption but also energy expenditure, the “not-gaining-weight” model is not strictly caloric restriction. Still, elucidation of the impact of weight gain or loss during adult life on a host of biological processes is likely to broaden our understanding of human aging.

A number of specific markers of energy metabolism, glucose regulation, inflammation, and oxidative stress have been used to gauge biological aging in the caloric restriction model.<sup>5,6</sup> However, single measurements of some of these

indicators may not reflect the pace of aging over time but display the metabolic status at the time of examination. Serial measurements of such markers over a long period of time may be necessary to chronicle the effect of caloric restriction on aging.

An important question is whether variables other than hormones or metabolic indicators may distinguish biological from chronological age in models of human aging. A number of studies have suggested that telomeres may be one of these indicators. Telomeres, the TTAGGG tandem repeats at the ends of chromosomes, become progressively shortened with each replication of cultured human somatic cells (reviewed in Wong and Collins<sup>8</sup>), and their loss is accelerated by oxidative stress.<sup>9</sup> Critically short telomeres may trigger replicative senescence, albeit other processes, including the capped/uncapped telomeric status and telomerase activity, are major determinants in this phenomenon. Thus, telomere length largely registers the replicative history, the cumulative oxidative burden, and, in part, the proliferative potential of somatic cells in culture.

Cross-sectional analyses have demonstrated that telomere length of white blood cells (WBCs) is inversely correlated with age.<sup>10-12</sup> In addition, individuals with short telomeres in WBCs are more likely to manifest accelerated vascular aging,<sup>11</sup> atherosclerosis,<sup>13,14</sup> and premature death.<sup>15</sup> These observations suggest that by recording the cumulative impact of somatic cell replication (due to growth and turnover) and

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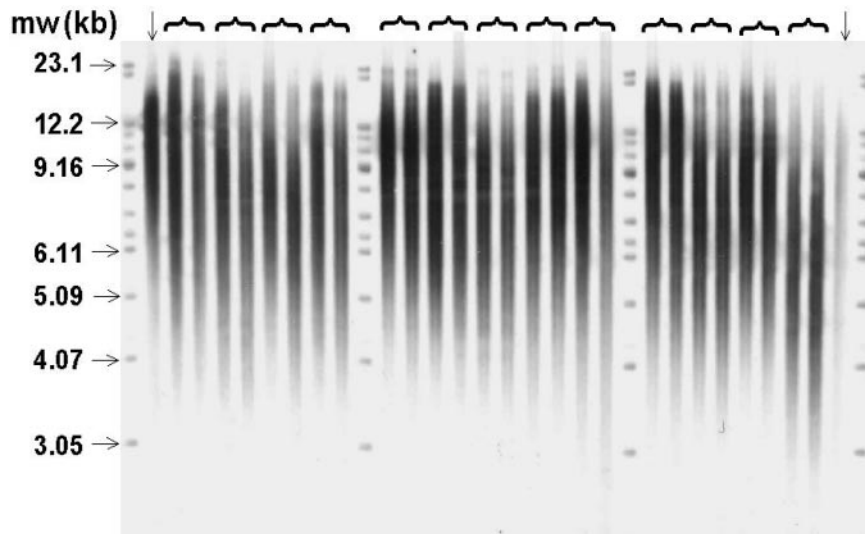
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**Figure 1.** Illustration of Southern blot for TRF length. Four molecular-weight ladders are positioned at intervals on gel. Vertical arrows denote TRF references obtained from human kidney (left) and human placenta (right) DNA. Baseline (left) and follow-up (right) samples from each individual are linked by brackets.

the accruing burden of oxidative stress, telomere dynamics may provide an account, above and beyond chronological age, of the biology of human aging and age-related cardiovascular disease. A recent review article<sup>16</sup> underscores this concept and suggests that more epidemiological data are necessary to establish the links between cardiovascular disease and telomere dynamics.

After age adjustment, variation in WBC telomere length among humans may arise from variability in telomere length at birth, levels of telomerase activity in progenitor compartments, and patterns of cell divisions after birth. Telomere length is highly variable in newborns<sup>17</sup> and adults.<sup>10–12</sup> It is unknown whether the variability in telomere length during adulthood simply reflects the variability observed at birth or whether the rate of telomere erosion is different among adult subjects. In addition, no information is available about WBC telomere attrition rate and its relationship with cardiovascular risk factors, including obesity and insulin resistance, among adults.

To test the hypothesis that a rise in insulin resistance accelerates telomere erosion and to explore individual variation in telomere attrition rate among humans, we measured the terminal restriction fragment (TRF) length of WBC DNA from males and females (ages 21.0 to 43.5 years) from the Bogalusa Heart Study<sup>18</sup> who donated 2 sequential blood samples at a time interval of at least 10 years.

## Methods

### General Consideration and Biochemical Analyses

The study subjects included 22 white males, 28 white females, 8 black males, and 12 black females who participated in the 1988 to 1991 and 2000 to 2001 cross-sectional cardiovascular screenings of young adults of the Bogalusa Heart Study. They were selected on the basis of the availability of stored blood samples from both examinations. Among those selected, 9 developed hypertension (blood pressure >140/90 mm Hg or taking antihypertensive medication), 1 had been hypertensive since baseline, and 1 developed diabetes (fasting glucose >125 mg/dL), which was untreated with medication. All participants gave informed consent to participate in the study. The Institutional Review Boards of Tulane University and the University of Medicine & Dentistry of New Jersey, New Jersey Medical School, approved the research.

We examined 2 variables that describe the relationships between telomere dynamics and other parameters, namely, change over time and yearly change. Because TRF length was not the same among individuals at baseline visit, we also examined the relationship between the relative yearly change in TRF length (ie, the rate of change in TRF length/TRF length at baseline) and parameters of interest. In addition to measuring fasting glucose and insulin, we determined the homeostasis model assessment of insulin resistance (HOMA-IR), a highly reliable surrogate indicator of insulin resistance.<sup>19</sup> Only 49 of the 70 subjects had available fasting glucose and insulin measurements both at baseline and at follow-up visits. For this reason, correlations between TRF length change versus fasting glucose, insulin, and HOMA-IR were performed for only 49 subjects. A commercial radioimmunoassay kit was used to measure plasma immunoreactive insulin (Padebas Pharmacia). Plasma glucose was measured by an enzymatic method with the Beckman Instant Glucose Analyzer (Beckman Instruments).

### Measurements of TRF Length

Briefly, DNA isolated from frozen blood (Gentra Systems) was digested overnight with the restriction enzymes *Hinf* I (10 U) and *Rsa* I (10 U; Roche Diagnostics Corp). A total of 28 DNA samples ( $\approx 3 \mu\text{g}$  each) and 4 DNA ladders (1-kb DNA ladder plus  $\lambda\text{DNA}/\text{HindIII}$  fragments; GIBCO BRL) were resolved on a 0.5% agarose gel (20 $\times$ 20 cm) at 50 V. After 16 hours, the DNA was depurinated for 30 minutes in 0.25 N HCl, denatured 30 minutes in 0.5 mol/L NaOH/1.5 mol/L NaCl, and neutralized for 30 minutes in 0.5 mol/L Tris (pH 8)/1.5 mol/L NaCl. The DNA was transferred for 1 hour to a positively charged nylon membrane (Roche Diagnostics) with a vacuum blotter. Membranes were hybridized at 65°C with the telomeric probe [digoxigenin 3'-end labeled 5'-(CCCTAA)<sub>3</sub>] overnight in 5 $\times$  SSC, 0.1% Sarkosyl, 0.02% SDS, and 1% blocking reagent (Roche Diagnostics). Membranes were washed 3 times at room temperature in 2 $\times$  SSC, 0.1% SDS each for 15 minutes and once in 2 $\times$  SSC for 15 minutes. The digoxigenin-labeled probe was detected by the digoxigenin luminescent detection procedure (Roche Diagnostics) and exposed on Hyperfilm (Amersham Biosciences). Each DNA sample was resolved in duplicate on different gels. Baseline and follow-up samples were run in adjacent lanes (Figure 1). If the difference between the duplicate results was >5%, a third measurement was performed, and the mean of the 2 results with <5% difference was taken. Fewer than 5% of the samples required a third measurement. The coefficient of variation of samples resolved on different gels on different occasions was <1.5%.

### Statistical Analysis

Statistical analyses were performed with SAS (version 8.0). General linear model was used to examine the effect of race, sex, age, and

**TABLE 1. Mean±SD Values of Age, BMI, TRF Length, Glucose, Insulin, and HOMA-IR at Baseline and Follow-Up by Race and Sex**

Parameters*	White		Black		P†	
	Male	Female	Male	Female	Race	Sex
Age, y						
Baseline	26.4±3.1	26.1±3.2	28.2±2.9	24.5±3.2	NS	<0.05‡
Follow-up	37.9±3.3	37.6±3.2	40.0±3.0	35.9±3.0	NS	<0.01‡
BMI, kg/m <sup>2</sup>						
Baseline	25.7±4.4	23.3±3.4	24.9±4.1	25.0±6.6	NS	NS
Follow-up	29.4±5.2	26.7±4.4	30.7±5.9	28.6±6.8	NS	NS
P for change§	<0.001	<0.001	<0.01	<0.05		
TRF length, kb						
Baseline	7.45±0.718	7.46±0.82	7.67±0.73	8.02±0.80	<0.05	NS
Follow-up	7.13±0.80	7.11±0.77	7.29±0.64	7.58±0.94	NS	NS
P for change§	<0.001	<0.001	<0.01	<0.01		
Insulin, μU/mL						
Baseline (n=61)	8.8±3.7	8.0±3.0	9.9±4.9	10.4±4.5	NS	NS
Follow-up	15.6±13.2	10.8±8.9	12.8±6.9	10.8±5.3	NS	NS
P for change§	0.01	NS	NS	NS		
Glucose, mg/dL						
Baseline (n=49)	83.6±8.3	76.0±6.9	81.1±10.9	78.8±6.1	NS	NS
Follow-up	89.5±14.5	81.3±10.7	83.3±12.4	83.1±12.5	NS	NS
P for change§	NS	NS	NS	NS		
HOMA-IR						
Baseline (n=49)	1.9±1.0	1.3±0.4	2.0±1.0	2.0±1.0	NS	NS
Follow-up	3.7±3.7	2.3±2.3	2.8±1.8	2.3±1.2	NS	NS
P for change§	<0.05	NS	NS	NS		

\*n=70 for those not specified in parentheses.

†P values were adjusted for covariates where appropriate.

‡Only in blacks.

§Pairwise *t* test was used.

time of screening and potential interactions on TRF length. Insulin and HOMA-IR values were log transformed. Pairwise *t* test was used to examine the difference between baseline and follow-up measurements. Pearson correlation and multiple regression analysis were used to examine the relation between change (or rate of change) in variables of interest and TRF dynamics, with adjustment for covariates where appropriate.

## Results

### General Characteristics of the Cohort

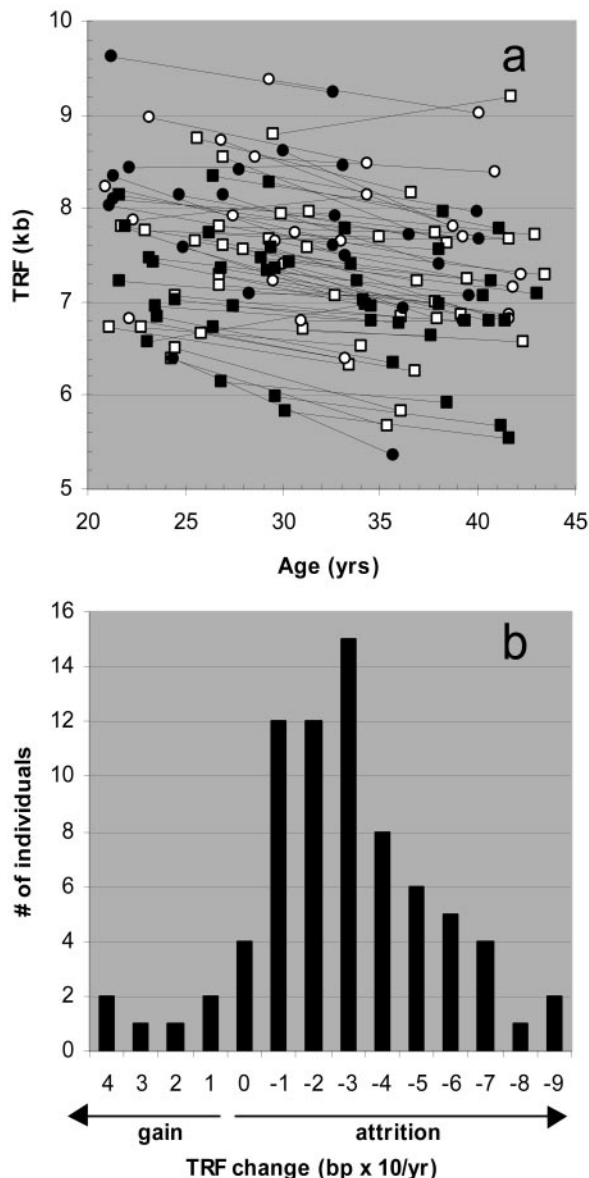
Physical and metabolic characteristics of the cohort at baseline and follow-up visits are summarized in Table 1. Blacks had longer age-adjusted TRF length than whites at baseline and follow-up visits, but the difference was significant only at the baseline visit ( $P<0.05$ ). White males showed higher insulin and HOMA-IR levels at the follow-up visit than at the baseline visit. The lack of change in fasting insulin, glucose, and HOMA-IR with age for other members of the cohort is due to the fact that although some individuals showed increased levels of fasting insulin and glucose, others showed a decline in these parameters at the follow-up compared with the baseline visits.

### Individual Yearly Changes in Telomere Length Are Highly Variable

Yearly TRF change was normally distributed, with 64 subjects showing telomere attrition, 5 exhibiting telomere gain, and 1 unchanged (Figure 2, a and b). Of those exhibiting telomere gain, 2 subjects showed an increase in TRF length of only 0.8 and 2.3 bp/y. Thus, for all practical purposes, their TRF length was unchanged in the interim between baseline and follow-up DNA analysis. Based on TRF attrition (or gain) for each subject between the baseline and follow-up DNA samples, the mean individual TRF attrition was 31.3 bp/y (0.0313 kb/y). This value is in agreement with reported WBC telomere attrition rates ( $\approx 20$  to 40 bp/y) obtained from cross-sectional analyses in larger adult cohorts.<sup>10,11,13</sup>

### Yearly Changes in Telomere Length Highly Correlate With Changes in Body Mass Index and Insulin Resistance

Table 2 summarizes the main correlation coefficients of association between changes in parameters of interest (gain and loss in telomere length and rise and fall in body mass index [BMI], fasting insulin, glucose, and HOMA-IR) over



**Figure 2.** a, Change in individual TRF lengths in young adults. Lines link TRF measurements of baseline and follow-up DNA samples for periods ranging from 10.1 to 12.8 years. Open symbols indicates whites; closed symbols, blacks; squares, males; and circles, females. b, Distribution of yearly change in TRF length in study cohort. Value of abscissa refers to attrition (–) or gain (+) in TRF length  $\times 10/\text{year}$ .  $n=70$ .

the time of follow-up. Changes in TRF length highly correlated with changes in indices of body mass and glucose regulation.

Figure 3 depicts the relationship of the relative change in telomere length (loss or gain) per year and yearly changes in BMI ( $n=70$ ; Figure 3a) and HOMA-IR ( $n=49$ ; Figure 3b) and the yearly changes in BMI versus HOMA-IR (Figure 3c). The relative yearly change in telomere length was significantly correlated with yearly changes in BMI and HOMA-IR. Moreover, the yearly change in HOMA-IR was highly correlated with the yearly change in BMI. We therefore used

**TABLE 2. Correlation Coefficients Between TRF Length Dynamic Measurements and Change in Variables of Interest, Adjusted for Age, Follow-Up Period, Race, and Sex**

Change Over Time	TRF Length	
	Change Over Time, kb	Relative Change, $\text{bp} \cdot \text{y}^{-1} \cdot \text{kb}^{-1}$
BMI, $\text{kg}/\text{m}^2$	–0.379*	–0.410†
Systolic blood pressure, mm Hg	–0.131	–0.131
Diastolic blood pressure, mm Hg	–0.103	–0.110
Pulse pressure, mm Hg	–0.067	–0.058
Insulin, $\mu\text{U}/\text{mL}$	–0.436*	–0.479†
Glucose, $\text{mg}/\text{dL}$	–0.415*	–0.484†
HOMA-IR	–0.470*	–0.522†
HDL cholesterol, $\text{mg}/\text{dL}$	0.201	0.179
LDL cholesterol, $\text{mg}/\text{dL}$	–0.105	–0.098
Triglycerides, $\text{mg}/\text{dL}$	–0.084	–0.111

\* $P<0.01$ ; † $P<0.001$ .

Relative change is expressed as change over time indexed to baseline TRF length.

multiple regression analysis to determine which of the 2 variables (relative change in HOMA-IR or relative change in BMI) independently accounted for the TRF length change.

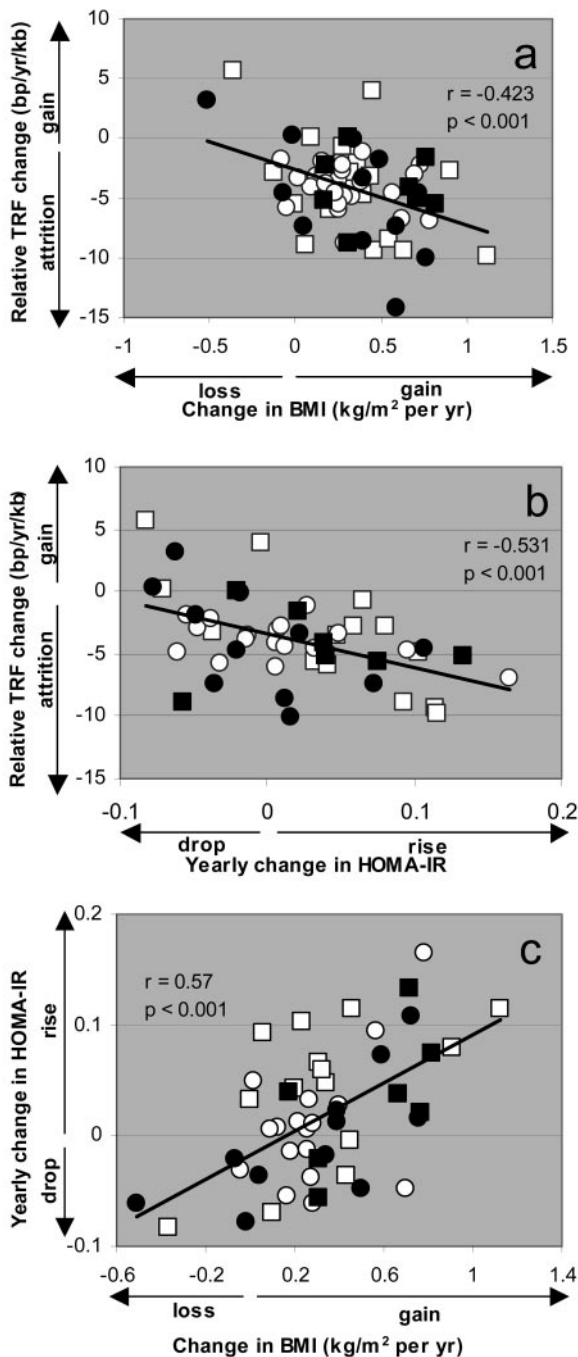
Table 3 shows that after adjustment for covariates, change in HOMA-IR but not change in BMI independently correlated with the relative yearly change in TRF length. This suggests that the relationship between yearly change in BMI and telomere dynamics is largely explained by the yearly change in HOMA-IR. Moreover, of the 3 individuals with substantial gains in telomere length between baseline and follow-up visits, 2 demonstrated BMI loss and a drop in HOMA-IR.

## Discussion

The central findings of this work are as follows: (1) yearly telomere length change in WBCs is highly variable among young adults, and (2) weight gain is associated with accelerated telomere attrition. Moreover, a rise in HOMA-IR largely accounts for the observed relationship between BMI change and telomere dynamics.

If telomere length strictly tracked chronological age with little interindividual variation in age-dependent telomere attrition, telomere dynamics would offer little information that explains disparity among humans in aging and age-related diseases. We suggest that regardless of whether telomeres are surrogate indicators of or determinants in processes that shape human aging, the discordance among individuals in age-dependent telomere attrition (or gain) may be the key to gaining a better understanding of aging and age-related diseases in humans. Because both telomere length<sup>10,12</sup> and BMI<sup>20</sup> are highly heritable and change with age, it is worth determining the concordance in habitability of these entities at different ages.

It is unlikely that the higher rate of telomere attrition is a cause rather than a consequence of the rise in HOMA-IR and



**Figure 3.** Yearly changes in BMI (a;  $n=70$ ) and HOMA-IR (b; log transformed;  $n=49$ ) vs relative yearly change in TRF length, and yearly change in HOMA-IR vs yearly change in BMI (c;  $n=49$ ). Open symbols indicate whites; closed symbols, blacks; squares, males; and circles, females.  $r$  Values for A and B reflect adjustment for follow-up period, age, race, and sex.

gain in adiposity, albeit causality cannot be inferred from association. Insulin resistance and obesity are conditions marked by a heightened oxidative stress.<sup>21–23</sup> Obesity is also associated with increased inflammation, because fat tissue is a major source of inflammatory cytokines.<sup>24,25</sup> Inflammation promotes an increase in WBC turnover, which would enhance

**TABLE 3.** Regression of Relative TRF Length Rates ( $\text{bp} \cdot \text{y}^{-1} \cdot \text{kb}^{-1}$ ) on Yearly Changes in BMI and HOMA-IR

Independent Variables	Regression Coefficient	$P$
Age at follow-up, y	0.2	0.267
Follow-up period, y	-0.1	0.932
Race	-0.6	0.526
Sex	-0.9	0.377
Yearly change in BMI, $\text{kg} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$	-1.7	0.309
Yearly change in HOMA-IR	-25.6	0.008

Race: 1=white, 2=black; sex: 1=male, 2=female.  
 $n=49$ .

telomere attrition. In addition, increased adiposity is accompanied by angiogenesis to accommodate the growing metabolic needs of the expanding fat mass,<sup>26</sup> which explains the increased central and total blood volume of obese subjects.<sup>27</sup> To maintain the peripheral WBC pool, obese persons may hence need to increase the total number of circulating WBCs.

The present study cannot decipher whether oxidative stress, inflammation, expanded blood volume, or their combination contributes to accelerated telomere attrition that accompanies weight gain. We note, nonetheless, that both oxidative stress<sup>23,28,29</sup> and inflammation<sup>30</sup> are considered pivotal factors in the biology of aging. We therefore speculate that oxidative stress, by enhancing telomere erosion per replication,<sup>9,31</sup> and inflammation, through increasing WBC turnover, are responsible for the increase in telomere erosion with a rise in insulin resistance in the present study cohort. In contrast, given the reported values of total blood volume in obese and lean subjects,<sup>27</sup> it is doubtful that a small increase in blood volume in the Bogalusa Heart Study subjects who gained weight would contribute considerably to overall telomere erosion within the observation period. Further support for this idea is derived from a study in a subset of the offspring cohort of the Framingham Heart Study that showed that WBC telomere length was inversely correlated not only with HOMA-IR but also with systemic oxidative stress (unpublished data). We note, however, that because telomere length at any age is the product of telomere length at birth and telomere attrition rate thereafter, cross-sectional analysis of telomere length, based on single measurements, requires large cohorts to capture links of biological variables with telomere attrition. This may be the reason that Jeanclous et al<sup>32</sup> did not observe significantly lower TRF length in patients with non-insulin-dependent diabetes mellitus than in their nondiabetic peers.

A central question is how much of weight gain or insulin resistance-related telomere erosion is a reflection of peripheral WBC homeostasis. Little is known about the biological half-life and turnover of different types of WBCs in humans. Studies in rodents suggest that self-replication of peripheral lymphocytes is a major contributor to the size of the peripheral WBC pool.<sup>33</sup> For instance, the rates of thymocyte production and the thymus output of naive T cells are not influenced by the size of the peripheral T-cell pool. It follows that maintenance of the peripheral pool depends on the

self-replication of peripheral cells. An unanswered question is whether increased oxidative stress and chronic demand for higher WBC production due to inflammation in adults, with a rise in insulin resistance and increased adiposity, accelerate telomere erosion in hematopoietic stem cells.

Telomerase activity is expressed in hematopoietic stem cells and lymphocytes.<sup>34,35</sup> It is theoretically possible that under certain circumstances, telomerase activity in normal stem cells may not only slow the rate of telomere erosion but also produce considerable telomere elongation, as observed in 3 subjects in the present study cohort. Another possibility is that a change in peripheral WBC homeostasis (due to reduced oxidative stress, inflammation, or, less likely, blood volume) may contribute to this process. At present, however, given the small number of subjects demonstrating age-dependent telomeric gain, we do not ascribe much significance to this finding.

Although stem cells express telomerase, they still exhibit telomere erosion with aging. Could acceleration in telomere attrition with insulin resistance be a factor in increased morbidity and premature death? It has been proposed that insulin resistance may serve as a predictor of age-related diseases.<sup>36</sup> In principle, insulin resistance–associated enhancement of telomere erosion, particularly if it accelerates telomere erosion in hematopoietic stem cells, ultimately may lead to premature replicative senescence of subsets of WBCs (eg, CD8 T cells). Such a scenario is based on the assumption that these cells may reach replicative senescence within the human lifespan.<sup>37,38</sup> Without the ability to undergo clonal expansion in response to antigenic stimulation, immunity would be compromised, as suggested by Cawthon et al.<sup>15</sup> This would mean that variations among individuals in the ability to mount clonal expansion later in life might depend on both telomere length at birth and telomere attrition rate throughout extrauterine life. We suspect that if it takes place, the tipping point in telomere length beyond which clonal expansion is severely compromised would occur only in the elderly.

In conclusion, aging is the progressive derangement of the individual's internal environment, which leads to an increased mortality risk with advancing age. This metabolic and structural breakdown appears to result from diverse causes that include the cumulative burden of oxidative stress<sup>23,28,29</sup> and inflammation.<sup>30</sup> Insulin resistance and obesity accelerate aging because they are states of increased oxidative stress and inflammation, which bring about a shorter lifespan. In biological terms, an accelerated WBC telomere attrition rate in insulin resistance and obese states is an affirmation of this concept. Our findings underscore the value of investigating telomere dynamics in vivo through tracking changes over time in telomere length in the same individuals.

### Acknowledgments

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