Insulin-Like Growth Factors and Leukocyte Telomere Length: The Cardiovascular Health Study

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The insulin-like growth factor (IGF) axis may affect immune cell replicative potential and telomere dynamics. Among 551 adults 65 years and older, leukocyte telomere length (LTL), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor–binding proteins 1 and 3 (IGFBP-1, IGFBP-3) were measured. Multivariate linear regression was used to model the association of LTL with IGF-1 and IGFBPs, while controlling for confounding and increasing precision by adjusting for covariates. We observed a significant association between higher IGF-1 and longer LTL after adjustment for age, sex, race, smoking status, body mass index, hypertension, diabetes, and serum lipids. The results suggested an increase of .08 kb in LTL for each standard deviation increase of IGF-1 ($p = .04$). IGFBP-1 and IGFBP-3 were not significantly associated with LTL. High IGF-1 may be an independent predictor of longer LTL, consistent with prior evidence suggesting a role for IGF-1 in mechanisms relating to telomere maintenance.

Key Words: Insulin-like growth factor—Telomere length—Epidemiology.

AGING is associated with progressive immune function dysregulation and impaired immune cell replication, leading to reduced capacity to respond to vaccines and greater susceptibility to infection among elderly individuals. In addition, short telomere length in peripheral leukocytes appears to be associated with increased risk of developing vascular diseases, metabolic disorders, and other age-associated phenotypes (1–4). Insulin-like growth factor-1 (IGF-1) is known to be an important promoter of cell growth, proliferation, and survival. Reduced IGF-1 with advancing age has been proposed to be a biological marker of aging and has been related to increased risk of diseases of old age (5,6). IGF-1 may relieve oxidative stress (7) and inflammation (8,9), which are believed to be important mechanisms contributing to increased immune cell turnover and telomere attrition (10). A recent prospective study showed accelerated immune decline in HIV-infected women with low IGF-1 levels (11), which is further evidence of a link between IGF-1 and immune cell replicative senescence.

We hypothesized that the IGF axis affects immune cell replicative potential by influencing telomere dynamics in elderly individuals. To address this hypothesis, we conducted a cross-sectional study of leukocyte telomere length (LTL) and circulating levels of IGF-1 and insulin-like growth factor–binding proteins (IGFBP-1, IGFBP-3) among 65+ year-old adults.

Methods

Participants

The present analyses were conducted among participants in the Cardiovascular Health Study (CHS), a population-based cohort of 5,888 men and women 65 years and older. Participants were 551 CHS enrollees who were randomly selected for inclusion in both an ongoing CHS ancillary study on IGFs (12–14) and an ongoing CHS ancillary study on telomere length (1). Participants were eligible for the CHS IGF ancillary study if they were free of prior myocardial infarction, stroke, and heart failure at study enrollment. Participants were eligible for the CHS telomere ancillary study if they consented to use of their DNA, had at least 12 μg of DNA available, and had stored leukocytes for additional DNA preparation.

Enrollment and Study Visits

CHS participants were enrolled in two phases, with 5,201 individuals enrolled in 1989–1990 and another 687 African American individuals enrolled in 1992–1993. Individuals

from each enrollment phase were included in the present study. CHS participants completed study visits at enrollment and annually during follow-up, which included clinical measurements (such as height, weight, and blood pressures), questionnaires about medical diagnoses and other health-related variables, and collection of overnight fasting peripheral blood specimens for extraction of plasma, serum, and DNA.

Measurement of IGFs

For selected participants, fasting plasma specimens collected at the initial study visit were obtained from the blood repository and analyzed for IGF-1, IGFBP-1, and IGFBP-3. Samples were assayed in duplicate by enzyme-linked immunosorbent assay using reagents from Diagnostic Systems Laboratory (Webster, TX). Within-batch and between-batch coefficients of variation were 6.9% and 6.0% for IGF-1, 3.5% and 3.1% for IGFBP-1, and 6.0% and 3.6% for IGFBP-3 (13). In a subset of participants, we found that IGF-1 and IGFBP levels were correlated over time within individuals when measured repeatedly at the initial study visit and subsequent visits; within-person correlations over more than 2–3 years were $r = .83$ for IGF-1, $r = .74$ for IGFBP-1, and $r = .83$ for IGFBP-3 (13).

Measurement of LTL

Using stored DNA from the 1992–1993 study visit, LTL was measured as the mean length of the terminal restriction fragments in peripheral leukocytes using the Southern blot method (1,15,16). All measures were performed in duplicate, with a Pearson correlation coefficient for the duplicates of $r = .97$ and an average coefficient of variation for pair sets of 1.5%.

Variable Definition

Information from the initial study visit (1989–1990 for original cohort members and 1992–1993 for the African American cohort) was used to define variables including age, smoking status, plasma lipids, body mass index (defined by measured height and weight), hypertension (defined as measured systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or history of treated hypertension), and diabetes (defined as measured fasting glucose >126 mg/dL or history of treated diabetes). Use of prescription medications was assessed using an inventory of recently used medications, and regular use of aspirin was determined through a direct question about aspirin use more than 2 days in the prior 2 weeks.

Statistical Analyses

The primary data analytic goal was to assess the association of IGF-1, IGFBP-1, and IGFBP-3 levels with LTL, independent of other variables that are known determinants of IGFs and LTL. In preliminary analyses, we examined each variable of interest to detect outliers and to confirm that distributions and between-variable correlations were as expected. Multivariate linear regression was used to model the association of LTL (defined as the dependent variable) with the independent variables IGF-1, IGFBP-1, and IGFBP-3, while controlling for confounding and increasing precision by adjusting for known correlates of IGFs and LTL. Initial models were adjusted for age, sex, race, and laboratory batch, and we then added adjustment variables for smoking status, body mass index, hypertension, diabetes, and lipids (high-density and low-density lipoprotein cholesterol) (1,8). We also present analyses after exclusion of treated diabetics and stratified on gender because of known differences in LTL and IGF levels by sex. Finally, several sensitivity analyses were conducted in order to strengthen the conclusion that observed correlations of IGF levels with LTL did not reflect correlations of these variables with age. To accomplish this, we added additional adjustment terms for age including age^2, age^3, and ln(age) to the model. We also repeated analyses after eliminating the upper and lower 15% of the age distribution.

Results

Subject characteristics included were 551 CHS participants with the following characteristics ($M \pm SD$ or %): age in years, 72.1 ± 5.0; non-White race, 13.3% ($n = 73$); ever smoker, 55.4% ($n = 305$); diabetic, 15.6% ($n = 86$); hypertensive, 65.9% ($n = 363$); and body mass index in kilogram per square meter, 27.3 ± 4.9.

Pearson correlations with age were $r = -.11$ ($p = .01$) for IGF-1, $r = .13$ ($p < .01$) for IGFBP-1, $r = -.16$ ($p < .01$) for IGFBP-3, and $r = -.15$ ($p < .01$) for LTL. Compared with women, men had significantly higher levels of IGF-1, lower levels of IGFBP-3, and a shorter LTL (Table 1).

The association between IGF-1 levels and LTL approached, but did not achieve, statistical significance after adjustment for age, race, sex, and laboratory batch ($p = .09$; Table 2, Model 1). A significant association between lower IGF-1 and shorter LTL was present after additional adjustment for correlates of IGF and LTL levels including smoking status, body mass index, hypertension, diabetes, and serum lipids (Table 2, Model 2). The association between IGF-1 and LTL and the association between age and LTL were of similar magnitude but in opposite directions, with a difference in LTL (β) per *SD* of IGF-1 = .08 kb ($p = .04$) and a difference in LTL (β) per *SD* of age = −.09 kb ($p < .01$). Results were similar after exclusion of treated diabetics (difference in LTL per *SD* of IGF-1 = .09 kb, $p = .03$). After stratifying analyses by sex, we found that LTL was significantly associated with IGF-1 level only among men ($\beta = .12$, $p = .03$ in age-, sex-, and batch-adjusted analyses; $\beta = .15$, $p < .01$ in fully adjusted analyses). This association was not present among women ($\beta = .02$, $p = .63$

Table 1. Demographic, Clinical, and Biomarker Data Among 194 Men and 357 Women 65 Years and Older

	All (N = 551)	Men (N = 194)	Women (N = 357)
Age (years)	72.1 ± 5.0	72.5 ± 5.4	71.8 ± 4.8
Non-White race	13.3 (73)	12.9 (25)	13.4 (48)
Income			
<$25,000	59.5 (328)	51.0 (99)	64.1 (229)
$25,000–$50,000	23.8 (131)	28.9 (56)	21.0 (75)
>$50,000	12.7 (70)	18.0 (35)	9.8 (35)
Education			
<High school	27.4 (151)	26.3 (51)	28.0 (100)
High school or equivalent	39.4 (217)	31.4 (61)	43.8 (156)
>High school	33.2 (183)	42.3 (82)	28.3 (101)
Ever smoker	55.4 (305)	66.5 (129)	49.3 (176)
Hypertensive	65.9 (363)	61.9 (120)	68.1 (243)
Diabetic	15.6 (86)	16.1 (31)	15.4 (55)
Estrogen use (women only)	—	—	9.8 (54)
Cholesterol-lowering medication use	5.5 (30)	7.2 (14)	4.5 (16)
Any hypertensive medication use	47.2 (259)	40.2 (78)	51.0 (181)
Oral hypoglycemic agent use	5.8 (32)	9.3 (18)	3.9 (14)
Insulin use	3.1 (17)	4.1 (8)	2.5 (9)
Aspirin use	31.7 (174)	32.0 (62)	31.6 (112)
BMI (kg/m^2)	27.27 ± 4.92	26.69 ± 3.81	27.59 ± 5.40
High-density lipoprotein cholesterol (mg/dL)	54.08 ± 14.72	47.35 ± 11.34	57.74 ± 15.06
Low-density lipoprotein cholesterol (mg/dL)	131.53 ± 36.20	126.16 ± 35.67	134.42 ± 36.20
Systolic blood pressure (mmHg)	138.71 ± 21.52	139.18 ± 21.36	138.46 ± 21.64
Diastolic blood pressure (mmHg)	71.64 ± 12.21	73.96 ± 12.04	70.39 ± 12.13
IGF-1 (μg/L)	148.11 ± 56.92	161.07 ± 59.50	141.06 ± 54.27
IGFBP-1 (μg/L)	30.36 ± 19.11	28.30 ± 17.73	31.49 ± 19.75
IGFBP-3 (μg/L)	4019.70 ± 909.11	3697.45 ± 853.94	4194.82 ± 891.18
Leukocyte telomere length (kb)	6.32 ± 0.62	6.17 ± 0.56	6.41 ± 0.64

Note: Continuous variables reported as $M \pm SD$; categorical variables reported as % (n). BMI = body mass index; IGF-1 = insulin-like growth factor-1; IGFBP = insulin-like growth factor–binding protein.

in age-, sex-, and batch-adjusted analyses; β = .03, p = .54 in fully adjusted analyses). We found that the results were qualitatively similar, but with wider confidence intervals, when we included more detailed adjustment for age and when we excluded individuals in the upper and lower 15% of observed ages.

Discussion

We found evidence of an association between low circulating IGF-1 and shortened LTL. This provides further support for a link between the IGF axis, a major regulator of cell proliferation and survival, with replicative capacity and telomere dynamics in immune cells. This preliminary report needs to be replicated in additional studies with larger sample size. Limitations include a lack of data on leukocyte telomere shortening measured prospectively, changes in IGF-1 levels over time, and telomerase activity.

Aging is associated with an increased fraction of T cells that have reached end-stage maturation, characterized by reduced proliferative capacity and short telomeres (17). Telomeres are repeat TTAGGG sequences at the end of chromosomes that shorten with each cell division. Telomeres appear to function as a "mitotic clock" because telomere length is a record of the replicative history and an index of the replicative potential of somatic cells in culture (18). The present findings suggest a correlation between shortened LTL and reduced IGF-1 levels with aging. This is plausible given prior evidence that IGF-1 may reduce oxidative stress (7) and inflammation (8,9). IGF-1 has also been shown to inhibit the apoptosis of hematopoietic progenitor cells (19),

Table 2. Association of IGF-1, IGFBP-1, IGFBP-3, and Age With Leukocyte Telomere Length Among 551 Men and Women 65 Years and Older

	β	*SE* (β)	*p* Value
Model 1			
IGF-1 (per *SD*)	.06	.04	.09
IGFBP-1 (per *SD*)	.03	.03	.22
IGFBP-3 (per *SD*)	−.02	.04	.54
Age (per *SD*)	−.09	.03	<.01
Model 2			
IGF-1 (per *SD*)	.08	.04	.04
IGFBP-1 (per *SD*)	.04	.03	.23
IGFBP-3 (per *SD*)	−.04	.04	.30
Age (per *SD*)	−.09	.03	<.01

Note: β represents change in leukocyte telomere length associated with 1 *SD* change in IGF-1, IGFBP-1, or IGFBP-3. Model 1: Adjusted for IGF-1, IGFBP-1, IGFBP-3, age, race, sex, and laboratory batch. Model 2: Adjusted for covariates in Model 1 plus smoking status (ever vs never), body mass index, hypertension, diabetes, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. IGF-1 = insulin-like growth factor-1; IGFBP = insulin-like growth factor–binding protein.

increase lymphocyte numbers by increasing or maintaining thymic mass (20), and increase proliferative response in activated mononuclear cells (21). It is possible that the effects of IGF-1 on telomere length may represent a balance between direct or indirect IGF-1 effects that upregulate telomerase activity and competing mechanisms whereby increased cellular turnover associated with IGF-1 leads to accelerated telomere attrition. An unexpected observation was the finding that IGF-1 and LTL were more strongly associated in men than in women. This may have been due to chance, although the finding is consistent with other data suggesting gender differences in the regulation and effects of telomere loss, as well as in IGF activity (1,22).

In summary, low IGF-1 may be an important independent predictor of shortened LTL. This extends prior evidence suggesting a role for IGF-1 in mechanisms relating to telomere maintenance in immune cells (21). We found possible gender differences in the association between telomere length and IGF axis proteins, and concurrently with the work described here, we investigated this association in other populations with results that were only partially concordant with those presented here (A. Aviv, MD, personal communication). Identifying reasons for the discrepant findings between populations will require further investigation. If confirmed, these data will provide the basis for future investigations of how the interplay between the IGF system and telomere regulation affect immune aging and risk of age-associated diseases.

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