Higher circulating levels of IGF-1 are associated with longer leukocyte telomere length in healthy subjects

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A B S T R A C T

Mutations that inhibit the insulin-like growth factor-1 (IGF-1) extend the lifespan of worms, flies and mice. However, it appears that relatively low circulating levels of IGF-1 in humans are associated with aging-related diseases and diminished longevity. As leukocyte telomere length (LTL) is ostensibly a biomarker of human aging, we examined the relationship between LTL and blood IGF-1 in a healthy cohort. Our sample comprised 476 healthy, unrelated Caucasians (208 men and 268 women), aged 16–104 years, living in the West Coast of Southern Italy. We measured LTL by Southern blots and IGF-1 by enzyme-linked immunoassay. Both IGF-1 and LTL diminished with age (IGF-1, $r = -0.601$, $P < 0.001$; LTL, $r = -0.706$, $P < 0.001$). Age-adjusted LTL was positively associated with IGF-1 level throughout the age range of the cohort ($r = 0.270$, $P < 0.001$). IGF-1 accounted for about 10% of the inter-individual variation in LTL over and above the effect of age. Our findings suggest that both circulating IGF-1 and LTL are indices of healthy aging in humans. Further research will be necessary to establish whether LTL will ultimately be used in clinical settings as an index of healthy aging.

1. Introduction

The signaling of insulin and insulin-like growth factor-1 (IGF-1) is evolutionarily conserved among eukaryotes. Worms and flies evidently possess a single insulin/IGF-1 pathway, but in mammals the pathway has split into two: the insulin pathway and the growth hormone (GH)/IGF-1 pathway (Kenyon, 2005). Though the two pathways overlap, insulin regulates first and foremost glucose homeostasis, while the GH/IGF-1 axis primarily functions in growth, development and somatic maintenance.

Mutations in key genes of the insulin/IGF-1 pathway prolong the lifespan of *Caenorhabditis elegans* (Kenyon et al., 1993; Paradis et al., 1999; Berdichevsky et al., 2006), *Drosophila Melanogaster* (Tatar et al., 2001; Clancy et al., 2001) and the mouse (Brown-Borg et al., 1996; Holzenberger et al., 2003; Kurosu et al., 2005). However, humans who display low levels of IGF-1 due to hypopituitarism, resistance to GH, or deficiency of GH do not live longer than their peers (Besson et al., 2003; Bartke, 2005). In fact, they might experience diminished longevity. What is more, epidemiological/clinical studies show that a lower IGF-1 is associated with atherosclerotic cardiovascular disease (Kaplan et al., 2005).

These conflicting trends between model organisms and humans suggest that in humans, the link between the IGF-1 and longevity might not fit neatly into a simple paradigm. We examined, therefore, the associations of leukocyte telomere length (LTL), ostensibly a biomarker of human aging (Aviv, 2006) with circulating levels of IGF-1 and with insulin resistance, given that the age-related trajectories of these indices of growth and metabolism are often diametrically opposite. As not only low levels of IGF-1 but also short LTL is associated with aging-related diseases, principally atherosclerosis (Samani and van der Harst, 2008) and diminished human longevity (Bakaysa et al., 2007; Kimura et al., 2008), we have elected to study individuals free of any major aging-related disease. In this way we were able to track

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the relationships among the three major variables (IGF-1, insulin resistance and LTL) across a wide age range without the confounding effects of aging-related diseases and their treatments.

2. Materials and methods

2.1. The cohort and study setting

All participants were living in the five districts of the Campania region located on the West Coast of Southern Italy. We included in this study only subjects born in and whose parents and grandparents were from Campania. At enrolment, we obtained medical history, anthropometric measurements and data regarding present cigarette smoking and the use of medications. For the enrollment of successfully aged individuals, i.e., those healthy subjects older than 85 years, we obtained a list of living subjects born between 1900 and 1913. The subjects were contacted at home or in their institution and examined by physicians previously trained to obtain relevant parameters for this study. Since our study called for studying only healthy individuals, we excluded subjects with cancer, type 2 diabetes (T2D), hypertension and major cardiovascular and endocrine diseases. We also excluded subjects using drugs affecting insulin/glucose and/or plasma lipid levels (e.g., oral hypoglycemic agents and statins) and those with abnormal laboratory results for liver, kidney, and thyroid functions.

Subjects (and whenever necessary, relatives) gave informed consent to participate in the study, which had been approved by the Ethical Committee of the appropriate Institutions.

2.2. Measurement of LTL by the mean length of the terminal restriction fragments (TRF)

The mean length of the TRF was measured as previously described (Kimura et al., 2007).

2.3. Assays of IGF-1, glucose and insulin and determination of insulin resistance by the homeostatic model of insulin resistance (HOMA-IR)

Blood samples were collected in the morning after the participants had been fasting for at least 8 h. IGF-1 was measured by enzyme-linked immunoassay (ELISA) (R&D System, Inc., Minneapolis, MN). Plasma fasting glucose was determined by the glucose oxidase method (Beckman Glucose Autoanalyzer, Fullerton, CA) and fasting plasma insulin by enzyme-linked immunoassay (Mercodia AB, Uppsala, Sweden). HOMA-IR was computed as previously described (Matthews et al., 1985). HOMA-IR has been validated to be a good index of insulin resistance in subjects with broad range of insulin sensitivity (Matthews et al., 1985) and has a good correlation with the insulin mediated glucose uptake, as determined by euglycemic hyperinsulinemic glucose clamp (Bonora et al., 2000).

2.4. Statistical analysis

We used ANOVA to analyze differences between women and men and smokers vs. non-smokers, analysis of covariance (ANCOVA), Pearson product-moment correlations to test associations among variables and multivariate linear regression analyses to test the independent association of covariates with LTL. We used linear model analysis to test the independent contribution of covariates as well as the effect of their interaction on LTL. Statistical analyses were performed using SPSS software package. Data in Table 1 and the narrative are presented as mean ± SD.

3. Results

3.1. General characteristics of the cohort

The cohort consisted of 476 unrelated Caucasians (208 men and 268 women) aged 16–104 years (55 ± 22 years). General characteristics of this group are displayed in Table 1. Women were older, had a lower BMI and smoked less than men.

3.2. Relations of LTL, IGF-1 and age

Fig. 1 displays the relationship of LTL (panel A) and IGF-1 (panel B) with age. Both variables diminished with age throughout the age range of the cohort. There was a strong correlation between LTL and IGF-1 (panel C). Since both variables declined with age, panel D displays the relationship between age-adjusted LTL and IGF-1. After age-adjustment, LTL was positively and significantly correlated with IGF-1.

3.3. Relation of the homeostatic model of insulin resistance with age and with LTL

HOMA-IR was used as an index of insulin resistance. It clearly increased with age for individuals younger than 85 years, but its level was relatively low in the successfully aged individuals, i.e., those older than 85 years (Fig. 2). HOMA-IR was therefore adjusted for age, but only for individuals younger than 85 years (Table 1). HOMA-IR values for individual older than 85 years were 1.66 ± 0.74 for men and 1.73 ± 0.84 for women ($P = 0.725$).

LTL was correlated negatively with HOMA-IR ($r = -0.123$, $P = 0.023$) and BMI ($r = -0.118$, $P = 0.032$) in individuals younger than 85 years. However, after age-adjustment, these correlations were not statistically significant.

3.4. The individual and joint contributions of the IGF-1 and HOMA-IR to variation in LTL

Both IGF-1 and HOMA-IR changed with age, though the successfully aged individuals deviated from the age-dependent increase in HOMA-IR, observed in the younger subset. Accordingly, we analyzed whether IGF-1 and HOMA-IR along with other variables might account for some of the inter-individual variation in LTL. First, we examined the extent that IGF-1 explained inter-individual variation in LTL in the entire cohort. Second, we examined how much HOMA-IR explained for inter-individual variation in LTL for the younger individuals. Third, we examined the joint influence of IGF-1 and HOMA-IR on the inter-individual variation of LTL in the younger individuals. Finally, we examined the same for the successfully aged individuals.

Table 2 shows that age alone accounted for 49.8% of the variation in LTL. A regression model with age and IGF-1 accounted for 55.2% of these variations. Further addition of BMI, gender and smoking status into the model provided minimal information over and above age and IGF-1 about variation in LTL.

Table 3 shows that for younger individuals, age is the main factor explaining variation in LTL with other variables, including BMI, HOMA-IR, smoking and gender playing no substantial role in LTL inter-individual variation. Age alone accounted for 19.1%,

Table 1
Characteristics.

Characteristic	Men ($N = 208$)	Women ($N = 268$)	Both ($N = 476$)	P for sex
Age (years)	51 (20)	59 (25)	55 (22)	<0.001
Body mass index (kg/m^2)	25 (1.9)	23 (3.2)	24 (2.8)	<0.001
Smokers (%)[a]	29.3 ($n = 61$)	8.6 ($n = 23$)	17.6 ($n = 84$)	0.001*
LTL (kb)	6.07 (0.6)	6.08 (0.6)	6.07 (0.6)	0.823
IGF-1 (ng/mL)	215 (51)	211 (49)	213 (50)	0.446
HOMA-IR[b]	2.52 (1.1)	2.43 (1.1)	2.48 (1.1)	0.42

Data are presented as means (standard deviation). LTL, IGF-1 and HOMA-IR were age-adjusted, but see text regarding age-adjustment for HOMA-IR.

[a] Current vs. former or never.

[b] Applies only to individuals younger than 85 years.

* Chi Square: 34.6.

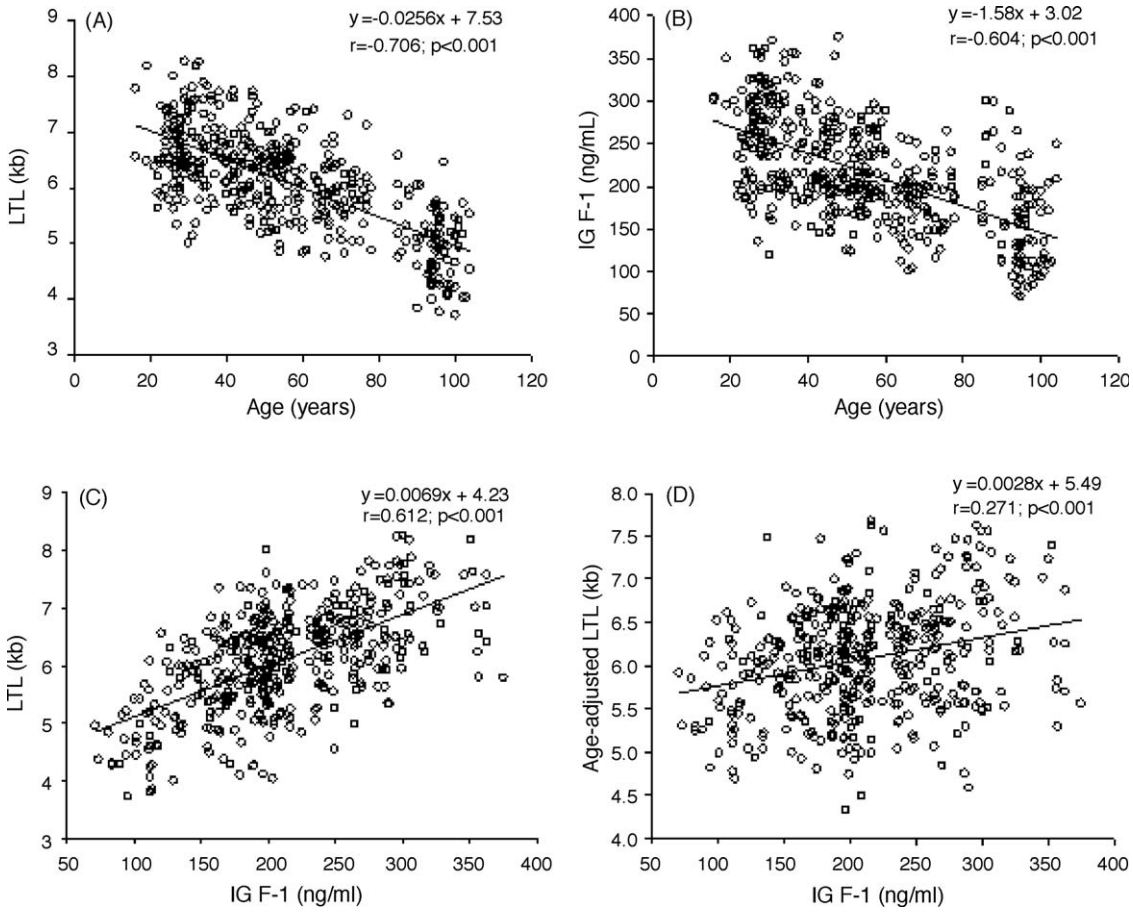

Fig. 1. Relations between LTL and age (A), IGF-1 and age (B), LTL vs. IGF-1 (C) and age-adjusted LTL vs. IGF-1 (D).

while the joint effect of age and IGF-1 accounted for 28.7% of the variation. BMI, HOMA-IR, smoking and gender accounted only marginally for the variations.

Finally, Table 4 displays the joint effect of IGF-1, HOMA-IR and other variables on LTL in the successfully aged individuals. In these subjects, age accounted for only 7.2% of the inter-individual variation, while age and IGF-1 jointly explained 18.1% of this variation. Again, HOMA-IR did not account for much of the inter-individual variation in LTL. While LTL was longer, though not significantly, in women than men (5.97 ± 0.58 kb vs. 5.82 ± 0.63 kb, respectively), the addition of gender into the model only modestly and non-significantly modified the results.

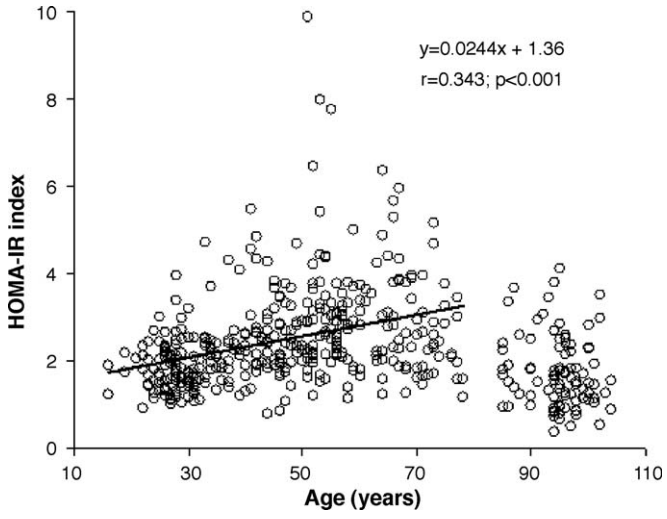

Fig. 2. Relation between HOMA-IR and age. The regression line equation, *P* and *r* values pertain to individuals younger than 85 years.

4. Discussion

The central findings of this work are that LTL and the level of IGF-1 declined with age throughout the entire age range of our cohort. In contrast, while HOMA-IR increased with age, this upward trajectory applied only to individuals younger than 85 years. For the successfully aged individuals, the mean HOMA-IR value was equivalent to that observed at the age of 28 ± 3 years. Such findings confirmed previous observations of progressive shortening of LTL (Valdes et al., 2005; Demissie et al., 2006; Bekaert et al., 2007; Kimura et al., 2007) and reduced levels of IGF-1 (Paolisso et al.,

Table 2
Regression of LTL on age, IGF-1 and other variables in all individuals.

Independent variable	Estimate	*P*	Model R^2
Intercept	7.535 ± 0.076	<0.001	
Age	−0.026 ± 0.001	<0.001	49.8
Intercept	6.277 ± 0.188	<0.001	
Age	−0.019 ± 0.001	<0.001	
IGF-1	0.004 ± 0.001	<0.001	55.2
Intercept	6.291 ± 0.338	<0.001	
Age	−0.020 ± 0.002	<0.001	
IGF-1	0.004 ± 0.001	<0.001	
BMI	0.002 ± 0.010	0.870	
Smoking	−0.130 ± 0.084	0.120	
Gender	0.041 ± 0.064	0.519	56.5

Table 3
Regression of LTL on age, BMI, IGF-1, HOMA-IR and other variables in younger individuals.

Independent variable	Estimate	*P*	Model R^2
Intercept	7.274 ± 0.104	<0.001	
Age	−0.019 ± 0.002	<0.001	19.1
Intercept	7.461 ± 0.346	<0.001	
Age	−0.021 ± 0.002	<0.001	
BMI	−0.005 ± 0.013	0.695	
HOMA	0.016 ± 0.032	0.628	
Smoking	−0.127 ± 0.090	0.159	
Gender	0.015 ± 0.075	0.842	21.2
Intercept	5.908 ± 0.222	<0.001	
Age	−0.012 ± 0.002	<0.001	
IGF-1	0.004 ± 0.001	<0.001	28.7
Intercept	5.895 ± 0.240	<0.001	
Age	−0.013 ± 0.002	<0.001	
IGF-1	0.005 ± 0.001	<0.001	
HOMA	0.030 ± 0.030	0.316	
Smoking	−0.117 ± 0.080	0.143	
Gender	0.039 ± 0.066	0.554	29.4

1997; Benbassat et al., 1997; Sugimoto et al., 1998; Fischer et al., 2004) with age. They also concur with findings that insulin resistance of successfully aged persons diverges from the upward trajectory of insulin resistance with age (Barbieri et al., 2003, 2008). Importantly, age-adjusted LTL was positively correlated with IGF-1, meaning that individual with relatively higher levels of IGF-1 displayed a longer LTL. However, in this ostensibly healthy cohort, age-adjusted LTL was not significantly associated with HOMA-IR.

At the ranges of 16–104, 16–85 and 85–104 years, age respectively explained 49.8%le (Table 2), 19.1% (Table 3) and 7.2% (Table 4) of the inter-individual variation in IGF-1. These differences among the models related to the age range of the individuals in the respective models — age explaining less variation in LTL at a narrower age range.

Though some of the younger individuals in our cohort might develop aging-related diseases later in life, at the time of blood collection, our entire cohort comprised healthy individuals of a wide age range. Accordingly, a case can be made that in our subjects, the slopes of the downward trajectories of IGF-1 levels and LTL define biological aging, divorced from aging-related diseases. In contrast, insulin resistance is an index of predilection to a subset of aging-related diseases, principally atherosclerosis (Razani et al., 2008). This might explain the association of age-adjusted LTL with insulin resistance, as expressed in HOMA-IR, in a cohort in which participants displaying aging-related diseases were not excluded (Demissie et al., 2006). In addition, while we have not observed a significant gender effect on LTL in this cohort, other studies reported longer LTL in women than in men (Benetos et al., 2001; Bekaert et al., 2007; Vasan et al., 2008), though this was not found in all studies (Njajou et al., 2007). The discrepancies between the studies might also relate to the less susceptibility of women to atherosclerosis and the inclusion of individuals who might suffer from this disease in those studies.

Table 4
Regression of LTL on age, IGF-1, HOMA-IR and other variables in successfully aged individuals.

Independent variable	Estimate	*P*	Model R^2
Intercept	8.481 ± 1.347	<0.001	
Age	−0.037 ± 0.014	0.011	7.2
Intercept	6.827 ± 1.363	<0.001	
Age	−0.026 ± 0.014	0.062	
IGF-1	0.004 ± 0.001	0.001	18.1
Intercept	6.713 ± 1.370	<0.001	
Age	−0.026 ± 0.014	0.067	
IGF-1	0.004 ± 0.001	0.005	
HOMA	0.073 ± 0.080	0.366	18.9
Intercept	7.145 ± 1.425	<0.001	
Age	−0.031 ± 0.015	0.037	
IGF-1	0.004 ± 0.001	0.006	
HOMA	0.068 ± 0.080	0.398	
Gender	0.164 ± 0.151	0.280	20.1

Among the variables examined, IGF-1 level predominated in its ability to explain inter-individual variation in LTL after adjustment for age. It explained approximately 10% in the inter-individual variation in LTL above that provided by age both for individuals younger than 85 years (Table 3) and for the successfully aged participants (Table 4). Thus, throughout the wide age range of our cohort, a higher IGF-1 level denoted a longer LTL.

Shortened LTL has been consistently linked to aging-related diseases (Aviv, 2006; Samani and van der Harst, 2008). Moreover, though the association between LTL and mortality in the elderly had been controversial (Cawthon et al., 2003; Martin-Ruiz et al., 2005; Bischoff et al., 2006; Harris et al., 2006; Honig et al., 2006) more recent studies in the powerful model of same-sex twins found that the co-twins with the shorter LTL were prone to die first (Bakaysa et al., 2007; Kimura et al., 2008). Based on these observations it seems that LTL is a biomarker of human aging and an index of survival in the elderly, perhaps because LTL shortening registers the accruing systemic burden of oxidative stress and inflammation (Aviv, 2006). The positive association of age-adjusted LTL with IGF-1 thus suggests that both a relatively long LTL and a high IGF-1 level might be advantageous in that they connote successful aging and better survival.

How then can studies showing that lower IGF-1 levels are associated with increased susceptibility to atherosclerosis and other aging-related disorders be reconciled with observations in model organisms? Blocking the IGF-1/insulin pathway in the worm increased longevity (Kenyon, 2005), a phenomenon attributed to the up regulation of genes encoding a suite of proteins that augment resistance to stress, oxidative stress, in particular (Honda and Honda, 1999; Murphy et al., 2003; McElwee et al., 2004; Berryman et al., 2008; Honda et al., 2008). Similar links between longevity and stress resistance were implicated in the fruit fly (McCarroll et al., 2004) and Ames dwarf mouse (Brown-Borg et al., 1996; Brown-Borg and Rakoczy, 2000, 2005).

The down regulation of insulin/IGF-1 pathway in post-mitotic organisms would be beneficial because it augments their anti-oxidant ability without compromising tissue repair through proliferation. The mouse, like other mammals, has highly proliferative tissues, the repair of which might benefit from the mitogenic action of IGF-1.

Also humans must resort to mechanisms that counter IGF-1 mediated increase in oxidative stress. At the systemic level these mechanisms may include the action of IGF-1 on the vascular endothelium. The endothelium is a systemic organ that partitions the blood from the rest of the soma. In this way, the endothelium is both the target of and a contributor to systemic oxidative stress and inflammation, which are registered by the pace of LTL shortening.

In the vascular endothelium, IGF-1 up regulates nitric oxide synthase (eNOS), which would cause vasorelaxation — a highly beneficial phenomenon to the aging vasculature. This up regulation would also diminish oxidative stress, unless eNOS is 'uncoupled' (Seinosuke and Yokoyama, 2004; Cooper et al., 2007; Paulis and Simko, 2007). Importantly, the up regulation of eNOS antagonizes the vasoconstrictive, pro-oxidant and pro-inflammatory actions of angiotensin II (Paulis and Simko, 2007) and suppresses oxidized LDL-mediated generation of superoxide

(Sukhanov et al., 2007). Thus, the interaction between IGF-1 and the endothelium might diminish the systemic burden of oxidative stress/inflammation. In fact, circulating IGF-1 appears to be a powerful anti-atherosclerotic agent that diminishes the oxidative/inflammatory burden at the systemic level (Sukhanov et al., 2007). It is this systemic effect of IGF-1 that might ultimately explain the IGF-1 LTL link in humans.

It is noteworthy that parallel studies have examined the relationship between IGF-1 and LTL in Flemish and Danish cohorts and found no significant association between age-adjusted LTL and IGF-1 (unpublished data). In contrast, Kaplan et al. (2009) observe association between age-adjusted LTL and IGF-1 among 551 participants (65% women, 13% African Americans) in the US-based Cardiovascular Health Study, which is a community-dwelling 65+ year old population. The underlying reason for the differences among these studies is unclear, but they might relate to a host of factors, including demography, geography and health status.

A recent study proposed that elevated levels of IGF-1 in centenarian women arises from a rare mutation in the gene encoding an IGF-1 receptor, resulting in diminished IGF-1-mediated signaling (Suh et al., 2008). The authors suggested that as per model organisms, exceptional longevity in humans might relate, at least in part, to the down regulation of the IGF-1 pathway. However, their conclusion was based on findings in very few and highly selected individuals. In contrast, our observation of the link between IGF-1 and LTL is derived from a large cohort of a wide age distribution. As many factors contribute to the circulating levels of IGF-1, it is unlikely that in and of themselves, rare variants of the IGF-1 receptor would be the sole explanation for the findings in our sample. However, the recent finding that the *FOXO3a* genotype is associated with human longevity lends support for a role of the IGF-1 pathway in human aging (Willcox et al., 2008).

Finally, we would like to underscore the limitation of this study. The cohort was highly homogeneous and limited to a narrow geographic region. Moreover, the cross-sectional nature of the study cannot provide data whether a longer LTL in individuals who display higher levels of IGF-1 reflects diminished rate of age-dependent LTL shortening, longer birth LTL, or both.

In conclusion, we show that regardless of age, LTL is positively associated with the levels of IGF-1. Further studies need to be undertaken to explore the biological ramifications of this association to human health and longevity.

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