Associations of plasma IGF1, IGFBP3 and estradiol with leucocyte telomere length, a marker of biological age, in men

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Abstract

Objective: Effects of insulin-like growth factor 1 (IGF1) and its binding proteins (IGFBPs) on ageing, and their interaction with sex hormones, remain uncertain. We examined associations of plasma IGF1, IGFBP1, IGFBP3, estradiol and testosterone, with leucocyte telomere length (LTL), a marker of biological age, in 2999 community-dwelling men aged 70–84 years.

Methods: Plasma IGF1, IGFBP1 and IGFBP3 measured using immunoassay, sex hormones using mass spectrometry. LTL measured by PCR, expressed as ratio of telomeric to single-copy control gene DNA (T/S ratio). Linear regression models adjusted for age and cardio-metabolic risk factors, median splits defined low/high groups.

Results: Mean age was 76.7 ± 3.2 years. IGF1 and IGFBP3 showed age-adjusted correlations with LTL (coefficient 0.59, $P = 0.001$ and 0.45, $P = 0.013$ respectively), IGFBP1 did not. In multivariable-adjusted models IGF1 and IGFBP3 (but not IGFBP1) were associated with LTL (T/S ratio 0.015 higher per 1 s.d. increase in IGF1, $P = 0.007$, and 0.011 per 1 s.d. IGFBP3, $P = 0.049$). IGF1 and estradiol were independently associated with longer telomeres (T/S ratio 0.012 higher per 1 s.d. increase in estradiol, $P = 0.027$, when included in model with IGF1). Testosterone was not associated with LTL. Men with both high IGF1 (>133 $\mu\text{g/L}$) and high estradiol (>70 pmol/L) had longer LTL compared to men with lower values (multivariable-adjusted T/S ratio 1.20 vs 1.16, $P = 0.018$).

Conclusions: Higher IGF1 and IGFBP3 are independently associated with longer telomeres in older men. Additive associations of higher IGF1 and higher estradiol with telomere length are present. Further studies are needed to determine whether these hormonal exposures cooperate to slow biological aging.

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Introduction

Reduced activity of the growth hormone (GH)-insulin-like growth factor 1 (IGF1) axis has been associated with extended lifespan in experimental models of invertebrates and mice (1, 2). IGF1 is a major downstream effector of GH that is present in relatively stable concentrations in the blood, providing an integrated measure of pulsatile GH secretion (3). IGF1 in the circulation binds to at least six IGF-binding proteins (IGFBPs). Of these two are of particular interest: IGFBP1 which is inversely associated with metabolic syndrome, and IGFBP3 which is the most abundant IGF-binding protein present in the circulation (4, 5).

In humans lower IGF1 concentrations have been associated with higher mortality due to ischemic heart disease (6). Consistent with this, higher serum IGF1 bioactivity has been associated with extended survival and reduced cardiovascular risk (7). However, there are contrasting results as low (8) and high (9) IGF1 concentrations have been associated with all-cause mortality. Similarly, contrasting results for IGFBP1 have been reported, with one study finding an association of low IGFBP1 with mortality from ischemic heart disease (6), and others associating higher IGFBP1 or increases in IGFBP1 with all-cause mortality (10, 11, 12). Lower IGFBP3 concentrations were associated with increased mortality (8, 12). In addition to cardiovascular risk and longevity, the IGF1 axis has been associated with frailty in middle-aged and older men (13). The pathways by which different components of the IGF1 axis might influence a range of health outcomes during aging remain unclear.

Telomeres are essential DNA-protein complexes at the ends of chromosomes which protect them from fusion and degradation (14). Telomere shortening is associated with cellular senescence and is postulated to be both a contributory and interactive factor in diseases associated with ageing (14). Peripheral blood leucocyte telomere length (LTL) correlates with telomere length in vascular and other tissues (15, 16, 17); thus, shorter LTL represents a biomarker for advanced biological age. Associations of IGF1 with LTL have been reported, but the nature of associations between IGFBP1 and IGFBP3 with LTL are unclear (18, 19, 20). Furthermore, there is an interaction between the IGF1 axis and sex hormones, as estrogens mediate GH secretion in men (21). Higher estradiol concentrations are associated with longer LTL in men (22, 23), putatively via activation of the enzyme telomerase which lengthens telomeres (24, 25). However, studies examining whether the IGF1 axis and sex hormones might act together to influence telomere length are lacking.

The aim of this study was to clarify associations of plasma IGF1 and also IGFBP1 and IGFBP3 with LTL in a population-based cohort of older men, and to test the hypothesis that the IGF1 axis and sex hormones additively influence telomere length in men.

Subjects and methods

Study population

The Health In Men Study (HIMS) is a population-based cohort study of community-dwelling older men from Perth, Western Australia (26). In total, 12 203 men aged ≥ 65 years completed a questionnaire and attended for physical examination in Wave 1 (W1, 1996–1999). Subsequently, 4246 of these men then aged 70–89 years completed a second questionnaire, and attended for physical examination and venesection in Wave 2 (W2, 2001–2004). Men were predominantly of Caucasian ethnic origin. The University of Western Australia Human Research Ethics Committee approved the study, and all men gave written informed consent. After excluding 104 men taking androgen-related medications, 51 men with history of orchidectomy and then 559 men with history of prostate cancer, there were 3532 men in the dataset. We further excluded 128 men aged 85–89 years as the number of men in this age stratum was small compared to other ages, and these men may have been healthy survivors less representative of older men as a whole. Some men had one or more missing data on IGF1, IGFBP1 or IGFBP3 ($n=188$), LTL ($n=355$) or other key covariates ($n=16$) and were also excluded. After these exclusions there were 2999 community-dwelling men aged 70–84 years available for the analysis.

Definition of medical comorbidities and physical activity levels

Alcohol consumption and physical activity were determined by questionnaire at W1, and smoking status by questionnaire at W2. Physical activity was the sum of the number of hours in a usual week of non-vigorous and 2 $\times$ the number of hours of vigorous physical activities, reflecting the higher exercise intensities associated with vigorous activities. Blood pressure, height and weight recorded at W2 were analysed. BMI was defined as weight (kg) divided by height (m) squared. A history of cardiovascular disease (CVD) was defined as any self-reported history of heart attack or stroke, heart bypass

surgery or balloon angioplasty, aortic aneurysm, or surgery to the aorta, carotid or lower limb arteries at W2. Use of lipid-lowering and hypertensive medications was recorded from questionnaire responses at W2. Men diagnosed with diabetes, reporting use of glucose-lowering medication, or with fasting or non-fasting glucose at W2 of ≥ 7.0 mmol/L or ≥ 11.1 mmol/L respectively, were considered to have diabetes.

Laboratory assays for circulating IGF1, IGFBP1, IGFBP3 and sex hormones

Blood samples were collected between 08:00 and 10:30 h at W2. Plasma and serum aliquots were prepared immediately after phlebotomy and stored at -80°C until assayed. Plasma total IGF1, IGFBP1 and IGFBP3 were assayed using reagent kits of single lot numbers from Diagnostics Systems Laboratories Inc. (DSL, supplied by Beckman Coulter) as previously described (4). The non-extraction IGF1 ELISA, the total IGFBP1 ELISA and the active IGFBP3 ELISA kits were used. The assays were automated using a Grifols Triturus ELISA processor (Vital Diagnostics, Castle Hill, NSW, Australia). Between-run imprecision (coefficient of variation) were IGF1: 12.2% at 117 $\mu\text{g/L}$ and 8.6% at 216 $\mu\text{g/L}$; IGFBP1: 8.6% at 3.1 $\mu\text{g/L}$ and 5.2% at 49 $\mu\text{g/L}$; IGFBP3: 16.8% at 540 $\mu\text{g/L}$ and 4.4% at 4300 $\mu\text{g/L}$. Plasma total estradiol and testosterone concentrations were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described (27). Interassay coefficients of variation were estradiol: 8.6% at 103 pmol/L and 8.1% at 308 pmol/L; testosterone: 6.5% at 2.0 nmol/L, 6.8% at 5.9 nmol/L, and 3.9% at 29.8 nmol/L.

Measurement of LTL

From blood samples collected at W2 aliquots of leucocyte DNA were prepared and stored at -80°C until required. An optimized PCR-based methodology for accurate measurement of LTL utilizing the protocol described by Cawthon *et al.* (28) was employed. Briefly, telomere lengths of the leucocyte DNA samples were measured by a multiplex quantitative PCR method (23). Each sample was amplified for telomeric DNA and for beta-globin, a single-copy control gene, which was used as an internal control to normalize the starting amount of DNA. The K562 cell line was used as a standard (29). Periodic reproducibility experiments were performed to confirm adequate normalization. All samples, standards, and controls were run in triplicate, and the median value used for analyses.

A standard curve derived from K562 cell line was used to transform the cycle threshold into nanograms of DNA. The amount of telomeric DNA (T) was divided by the amount of single-copy control gene DNA (S), producing a relative measurement of the telomere length (T/S ratio). The coefficient of variation for the quantitative PCR across all batches was $<10\%$. Some men did not provide a leucocyte DNA sample at W2 (choosing to provide blood samples only for plasma and serum); thus, LTL data were unavailable for them and they were excluded from the analysis.

Statistical analysis

SAS version 9.4 was used to analyze the data. Characteristics of the study sample are presented as mean $\pm$ s.d. for continuous variables, and n (%) for categorical variables. The chi-square test was used to compare categorical variables and t -test for quantitative variables. In analyses of IGF1, IGFBP1 and IGFBP3 vs age and LTL vs age, data are expressed as mean and the s.e.m. Correlation coefficients and partial correlation coefficients were calculated for crude and age-adjusted associations of IGF1, IGFBP1 and IGFBP3 with T/S ratio, and for IGF variables with estradiol and testosterone. Linear regressions of T/S ratio on each IGF variable separately were performed with adjustment for age and cardio-metabolic risk factors. The adjustment variables were age, BMI, CVD history, alcohol, smoking, physical activity, cholesterol, HDL, lipid medication, diabetes, systolic BP, hypertension medication and if the blood sample was taken fasting. Results are presented as the estimated coefficient (P value) where the coefficient represents the change in T/S ratio for a 1 SD change in the IGF variable. To examine the joint associations of IGF variables and sex hormones on LTL, linear regressions of T/S ratio on each IGF variable with either estradiol or testosterone included in the model were performed with adjustment for age and cardio-metabolic risk factors. In addition, we analyzed the differences in T/S ratio according to low/high groups for IGF variables and sex hormones using median splits. Linear regression models were fitted that compared the mean T/S ratio across the four groups (low IGF variable/low sex hormone (L/L), low IGF variable/high sex hormone (L/H), high IGF variable/low sex hormone (H/L) and high IGF variable/high sex hormone (H/H)). Results are presented as the estimated (multivariable-adjusted) mean T/S ratio for each of the four groups together with P values for the comparison of each group to the low IGF variable/low sex hormone (L/L) group. A P value <0.05 was considered significant.

Results

Characteristics of the study population

Characteristics of the 2999 men comprising the final study sample and the 405 men excluded due to missing key data are shown in Table 1. The excluded men drank on average slightly less alcohol, and fewer were current smokers, otherwise they had similar baseline characteristics and cardiovascular profiles to the study sample. Mean age of the 2999 men in the study sample was 76.7 years, 14.2% had a history of diabetes and 33.5% had CVD. In the study sample 2362 men (78.8%) provided fasting blood samples, and fasting/non-fasting status was adjusted for in the analysis.

Table 1 Characteristics of the study sample aged 70–84 years ($n = 2999$) and those excluded due to missing relevant data ($n = 405$). The table shows mean $\pm$ S.D. or n (%). P values are from chi-square tests for categorical variables and from t-tests for quantitative variables.

Characteristic	Study sample ($n = 2999$)	Excluded subjects ($n = 405$)	P value*
Age (years)	76.7 $\pm$ 3.2	77.0 $\pm$ 3.3	0.053
BMI (kg/m ²)	26.6 $\pm$ 3.7	26.3 $\pm$ 3.3	0.152
Cardiovascular disease history	1006 (33.5)	123 (30.4)	0.203
Alcohol (g/day)	12.0 $\pm$ 15.6	10.1 $\pm$ 14.4	0.020
Smoking			
Never	985 (32.8)	128 (31.6)	0.040
Former	1830 (61.0)	264 (65.2)	
Current	184 (6.1)	13 (3.2)	
Physical activity (h/week)	7.00 $\pm$ 7.14	7.09 $\pm$ 7.90	0.814
Cholesterol (mmol/L)	4.89 $\pm$ 0.95	4.94 $\pm$ 0.99	0.288
HDL (mmol/L)	1.39 $\pm$ 0.36	1.41 $\pm$ 0.34	0.385
Lipids medication	1182 (39.4)	134 (33.1)	0.014
Diabetes	425 (14.2)	60 (14.9)	0.699
Systolic blood pressure (mmHg)	147 $\pm$ 20	149 $\pm$ 20	0.155
Hypertension medication	1656 (55.2)	206 (50.9)	0.098
Fasting blood sample	2362 (78.8)	314 (77.5)	0.571
IGF1 (μ g/L)	141.4 $\pm$ 58.8		
IGFBP1 (μ g/L)	26.6 $\pm$ 20.5		
IGFBP3 (μ g/L)	3778 $\pm$ 894		
Estradiol (pmol/L)	73.1 $\pm$ 29.0		
Testosterone (nmol/L)	13.1 $\pm$ 5.0		
LTL (T/S ratio)	1.18 $\pm$ 0.29		

*Comparison of full study sample with excluded group for those with data on this variable.

Associations of IGF1, IGFBP1, IGFBP3 and LTL with age

The relationships of IGF variables and LTL with age are shown in Table 2. IGF1 declined by 18.4 μ g/L per decade of age ($P < 0.0001$), IGFBP1 increased by 12.1 μ g/L per decade of age ($P < 0.0001$) and IGFBP3 declined by 467 μ g/L per decade of age ($P < 0.0001$). T/S ratio showed an overall estimated decline of 0.063 per decade of age ($P = 0.0002$).

Correlations of IGF1, IGFBP1 and IGFBP3 with LTL

The correlations and age-adjusted correlations between each IGF variable and LTL are shown in Table 3. IGF1 and IGFBP3 showed significant positive correlations and age-adjusted correlations with LTL. IGFBP1 was not significantly correlated with LTL. Correlations of estradiol but not testosterone with LTL were reported previously (22, 23).

Associations of IGF variables with estradiol and testosterone

Of the 2999 men in the study sample, 27 did not have recorded values for estradiol and testosterone, leaving 2972 men for further analysis. A matrix of age-adjusted correlations between IGF variables, estradiol and testosterone is shown in Table 4. Estradiol correlated with IGFBP1 ($r = 0.09$) and was inversely correlated with IGF1 ($r = -0.08$) and IGFBP3 ($r = -0.10$, all $P < 0.0001$). Similar results were seen for testosterone.

Regression analyses of IGF1, IGFBP1, IGFBP3 and LTL

Linear regressions of T/S ratio on IGF variables after adjustment for age, cardio-metabolic risk factors and CVD history are shown in Table 5. IGF1 and IGFBP3 (but not IGFBP1) showed a significant multivariable adjusted association with LTL. The estimated difference in T/S ratio was 0.015 per 1 s.d. increase in IGF1 ($P = 0.007$) and 0.011 per 1 s.d. increase in IGFBP3 ($P = 0.049$).

Independent associations of IGF1 and IGFBP3, and estradiol, analyzed as continuous variables, with LTL

Linear regressions of T/S ratio on IGF variables in models also including either estradiol or testosterone, after adjustment for age, cardio-metabolic risk factors and

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Table 2 Relationships of IGF1, IGFBP1, IGFBP3 and leucocyte telomere length (LTL, expressed as the T/S ratio) with age in 1999 community-dwelling men aged 70–84 years. Table shows mean $\pm$ S.E.

Age (years)	n	IGF1 (μ g/L)	IGFBP1 (μ g/L)	IGFBP3 (μ g/L)	LTL (T/S ratio)
70–74	1162	148.8 $\pm$ 1.77	23.3 $\pm$ 0.52	3921 $\pm$ 26.2	1.20 $\pm$ 0.009
75–79	1330	138.7 $\pm$ 1.60	27.5 $\pm$ 0.58	3755 $\pm$ 23.8	1.17 $\pm$ 0.008
80–84	507	131.8 $\pm$ 2.40	32.2 $\pm$ 1.02	3512 $\pm$ 39.9	1.14 $\pm$ 0.012
All	2999	141.4 $\pm$ 1.07	26.6 $\pm$ 0.37	3778 $\pm$ 16.3	1.18 $\pm$ 0.005
Average change per decade of age*	2999	–18.4 $\pm$ 3.34	12.1 $\pm$ 1.15	–467 $\pm$ 50.3	–0.063 $\pm$ 0.017

*Estimated from linear regression on age.

CVD history, are shown in Table 6. There was no evidence of any IGF*estradiol interaction when IGF1, IGFBP1, IGFBP3 and estradiol were modeled as continuous variables (all $P > 0.05$, data not shown). IGF1 and estradiol were independently associated with longer telomeres, with an estimated increase in T/S ratio of 0.015 per 1 s.d. increase in IGF1 ($P = 0.006$) and 0.012 per 1 s.d. increase in estradiol ($P = 0.027$), when both estradiol and IGF1 were included in the same model (Table 6A). When IGFBP1 and estradiol were included in the same model, only estradiol was associated with LTL (Table 6B). IGFBP3 and estradiol were independently associated with longer telomeres, with an estimated increase in T/S ratio of 0.013 per 1 s.d. increase in IGFBP3 ($P = 0.024$) and 0.012 per 1 s.d. increase in estradiol ($P = 0.026$), when both estradiol and IGFBP3 were included in the same model (Table 6C). Testosterone was not associated with LTL in any of the models.

Additive associations of IGF1 and estradiol, analyzed using median splits, with LTL

Results using median splits are shown in Table 7. For IGF1/estradiol in the fully adjusted model, men in the H/H group had the highest mean LTL, men in the H/L and L/H groups had intermediate LTL, and men in the L/L group the shortest LTL. Only men in the H/H group had significantly different mean LTL compared to L/L (mean T/S ratio 1.20 vs 1.16, $P = 0.018$) in the fully adjusted model. In the age-adjusted model for IGFBP3/estradiol, men in the H/H group had the highest mean LTL (mean T/S ratio 1.21 vs 1.17, $P = 0.030$ vs L/L), but this association was attenuated in the fully adjusted model (T/S ratio mean 1.20 vs 1.17, $P = 0.061$). There was no difference between groups for IGFBP1/estradiol.

Consideration of IGF1/IGFBP3 ratio

The mean for IGF1/IGFBP3 ratio was 0.037 (s.d. 0.013). The age-adjusted correlation of IGF1/IGFBP3 with LTL

(T/S ratio) was 0.05 ($P = 0.006$) which was similar to the result for IGF1 (as shown in Table 3). The age-adjusted correlation between IGF1 and IGF1/IGFBP3 was very high at 0.78 ($P < 0.0001$); thus, results for IGF1/IGFBP3 ratio would be very similar to results for IGF1.

Discussion

In this study of community-dwelling older men, higher plasma IGF1 and IGFBP3 (but not IGFBP1) were associated with longer telomeres as assessed by LTL, independently of age and cardiometabolic risk factors. Furthermore, there were independent associations of circulating IGF1 and estradiol, and IGFBP3 and estradiol, with longer telomeres. Men with both plasma IGF1 and estradiol concentrations above the median value, had the longest telomeres, consistent with an additive association of these hormones with telomere length.

Previous studies assessing the relationship between IGF1 and LTL had not shown associations with IGFBPs and had not examined whether sex hormones and IGF1 might additively influence LTL (18, 19, 20). Moverare-Skrtic *et al.* studied 2744 men aged 69–81 years from the Osteoporotic Fractures in Men-Sweden study, finding that men with LTL in the lowest tertile had lower serum IGF1 compared to men in the two highest tertile groups (18). In a multivariable regression analysis a 1 s.d. higher IGF1 concentration was associated with 12% lower risk

Table 3 Correlations and age-adjusted correlations of IGF1, IGFBP1 and IGFBP3 with leucocyte telomere length (LTL, T/S ratio) in 1999 men aged 70–84 years. Data are shown as correlation coefficient (P value).

	Correlation with T/S ratio (P value)	Age-adjusted correlation with T/S ratio (P value)
IGF1	0.065 (<0.001)	0.059 (0.001)
IGFBP1	–0.011 (0.559)	0.002 (0.902)
IGFBP3	0.056 (0.002)	0.045 (0.013)

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Table 4 Correlations of IGF1, IGFBP1, IGFBP3, estradiol and testosterone in 2972 older men aged 70–84 years. Age-adjusted correlation coefficients and their corresponding P values (in brackets) are shown.

	IGF1	IGFBP1	IGFBP3	Estradiol	Testosterone
IGF1	1.000	−0.142 (<0.001)	0.593 (<0.001)	−0.079 (<0.001)	−0.112 (<0.001)
IGFBP1		1.000	−0.095 (<0.001)	0.092 (<0.001)	0.247 (<0.001)
IGFBP3			1.000	−0.098 (<0.001)	−0.119 (<0.001)
Estradiol				1.000	0.475 (<0.001)
Testosterone					1.000

of having LTL in the lowest tertile of values, with no data shown for IGFBP1 or IGFBP3 (18). Kaplan *et al.* studied 551 adults aged ≥ 65 years comprising 194 men and 357 women, and found that higher IGF1 was independently associated with longer LTL, but IGFBP1 and IGFBP3 were not (19). It is possible that their negative result with respect to IGFBP3, may relate to the smaller size of their study sample. Barbieri *et al.* reported an analysis of 476 adults aged 16–104 years with 208 men and 268 women, finding an age-adjusted association of IGF1 with LTL, and

no data shown for IGFBP1 or IGFBP3 (20). None of these studies examined sex hormones in conjunction with IGF1 and IGFBPs.

In our study, higher plasma IGF1 analyzed as a continuous variable was independently associated with longer LTL, with a substantive effect size. Thus a 1 s.d. increase in plasma IGF1 was associated with an increase in T/S ratio of 0.015, compared with estimated differences

Table 5 Multivariable linear regression showing associations of variables with LTL in 2999 community-dwelling men aged 70–84 years. Data shown are estimated coefficients (P values) where the coefficient represents the change in T/S ratio for a 1 S.D. change, or between categories, for each of the variables of interest.

	Coefficient (P value)
A: Multivariable model*	
Age (s.d. = 3.2 years)	−0.0215 (<0.001)
BMI (s.d. = 3.7 kg/m ²)	−0.0128 (0.029)
Cardiovascular disease history (yes vs no)	0.0008 (0.949)
Alcohol (s.d. = 15.6 g/day)	0.0033 (0.568)
Smoking	
(Current vs never)	0.0039 (0.869)
(Former vs never)	0.0062 (0.601)
Physical activity (s.d. = 7.1 h/week)	−0.0010 (0.847)
Cholesterol (s.d. = 0.95 mmol/L)	0.0154 (0.013)
HDL (s.d. = 0.36 mmol/L)	−0.0234 (<0.001)
Lipids medication (yes vs no)	0.0174 (0.171)
Diabetes (yes vs no)	0.0023 (0.883)
Systolic blood pressure (s.d. = 20 mmHg)	0.0059 (0.287)
Hypertension medication (yes vs no)	0.0255 (0.029)
Fasting blood sample (yes vs no)	0.0090 (0.498)
B: Model with IGF variables†	
IGF1 (s.d. = 58.8 µg/L)	0.0147 (0.007)
IGFBP1 (s.d. = 20.5 µg/L)	0.0003 (0.967)
IGFBP3 (s.d. = 894 µg/L)	0.0110 (0.049)

*Model includes all the variables shown. †Model includes age, BMI, CVD history, alcohol, smoking, physical activity, cholesterol, HDL, lipid medication, diabetes, systolic BP, hypertension medication and if the blood sample was taken fasting, and each of the IGF variables individually in three separate models (one shown in each row).

Table 6 Multivariable linear regression showing associations of IGF variables and sex hormones with LTL in 2972 community-dwelling men aged 70–84 years. Data shown are estimated coefficients (P values) where the coefficient represents the change in T/S ratio for a 1 S.D. change, for each of the variables of interest.

	Coefficient (P value)
A: Model with IGF1 and estradiol*	
IGF1 (s.d. = 58.8 µg/L)	0.0149 (0.006)
Estradiol (s.d. = 29.0 pmol/L)	0.0121 (0.027)
Model with IGF1 and testosterone*	
IGF1	0.0138 (0.012)
Testosterone (s.d. = 5.0 nmol/L)	−0.0015 (0.801)
B: Model with IGFBP1 and estradiol†	
IGFBP1 (s.d. = 20.5 µg/L)	−0.0001 (0.981)
Estradiol	0.0110 (0.045)
Model with IGFBP1 and testosterone†	
IGFBP1	0.0011 (0.856)
Testosterone	−0.0033 (0.579)
C: Model with IGFBP3 and estradiol‡	
IGFBP3 (s.d. = 894 µg/L)	0.0127 (0.024)
Estradiol	0.0123 (0.026)
Model with IGFBP3 and testosterone‡	
IGFBP3	0.0113 (0.048)
Testosterone	−0.0013 (0.828)

*Model includes age, BMI, CVD history, alcohol, smoking, physical activity, cholesterol, HDL, lipid medication, diabetes, systolic BP, hypertension medication and if the blood sample was taken fasting, and IGF1 and sex hormone in the same model. †Model includes age, BMI, CVD history, alcohol, smoking, physical activity, cholesterol, HDL, lipid medication, diabetes, systolic BP, hypertension medication and if the blood sample was taken fasting, and IGFBP1 and sex hormone in the same model.

‡Model includes age, BMI, CVD history, alcohol, smoking, physical activity, cholesterol, HDL, lipid medication, diabetes, systolic BP, hypertension medication and if the blood sample was taken fasting, and IGFBP3 and sex hormone in the same model.

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Table 7 T/S ratio in older men according to concentrations of IGF variables and estradiol stratified using median splits.* Data are shown as adjusted mean (P value) from linear regression for T/S ratio according to IGF variable/estradiol groups.

IGF variable (median)	Low IGF and low estradiol	Low IGF and high estradiol [†]	High IGF and low estradiol [†]	High IGF and high estradiol [†]
Group sizes				
IGF1	729	755	755	733
IGFBP1	796	693	688	795
IGFBP3	714	769	770	719
Age-adjusted T/S ratio means				
IGF1 (133)	1.16	1.16 (0.877)	1.18 (0.147)	1.20 (0.007)
IGFBP1 (21.7)	1.18	1.18 (0.623)	1.17 (0.786)	1.18 (0.545)
IGFBP3 (3770)	1.17	1.16 (0.629)	1.18 (0.779)	1.21 (0.030)
Multivariable-adjusted T/S ratio means [‡]				
IGF1 (133)	1.16	1.17 (0.870)	1.18 (0.257)	1.20 (0.018)
IGFBP1 (21.7)	1.18	1.18 (0.674)	1.17 (0.713)	1.18 (0.584)
IGFBP3 (3770)	1.17	1.17 (0.646)	1.17 (0.983)	1.20 (0.061)

*Estradiol median is 70 pmol/L. Medians for IGF variables are shown in brackets in the table in units of $\mu\text{g/L}$. [†]P value for comparison with low IGF variable and low estradiol group. [‡]Adjusted for age, BMI, CVD history, alcohol, smoking, physical activity, cholesterol, high-density lipoprotein cholesterol (HDL), lipid medication, diabetes, systolic BP, hypertension medication and if the blood sample was taken fasting.

of 0.021 for being 3.2 years (1 s.d.) younger, and 0.013 for having a BMI 3.7 kg/m^2 (1 s.d.) lower. These results are supportive of the concept that IGF1 might favorably influence ageing trajectories. Higher IGF1 concentrations have been associated with lower risk of mortality in some epidemiological studies (7, 8), but other such studies have reported neutral (10, 11) or even adverse associations of IGF1 with mortality (9). IGF1 has multiple biological actions including modulation of cell growth and proliferation, including in the vasculature (30). Thus, the association of higher IGF1 with longer telomeres, a marker for younger biological age, is consistent with findings from studies which have associated lower IGF1 concentrations with cardiovascular risk factors such as hypertension and diabetes (31), presence of arterial plaque (32), deaths from ischemic heart disease (6) and worsening frailty phenotype (13). Contrary to studies in laboratory animals which associate reduced activity of the GH/IGF1 axis with longevity (1, 2) or studies in patients with acromegaly who have pathologically excessive GH secretion and shorter telomeres (33), our results are consistent with higher IGF1 concentrations within the physiological range being associated with slower biological aging in older men.

We found that higher IGFBP3 concentrations were independently associated with longer LTL, with a 1 s.d. increase in IGFBP3 associated with an increase in T/S ratio of 0.011, approximately half the difference seen with a 1 s.d. change in age. IGF1 and IGFBP3 concentrations are correlated, and in some studies the associations of lower IGFBP3 with specific outcomes in men mirror the associations of lower IGF1, for example with metabolic syndrome (4), all-cause mortality (8) and worsening

frailty (13). However, analyses of other outcomes show divergent associations, for example, in the HIMS cohort men with higher plasma IGFBP3 had a lower incidence of dementia, while plasma IGF1 was not a predictor of dementia risk (34). In another analysis, higher IGFBP3 (but not IGF1) was associated with colorectal cancer risk (35). IGFBP3 has been shown to bind endothelial cells (36), be actively internalized via an endocytic pathway (37), and is involved in regulation of apoptosis (38) and adipocyte differentiation via nuclear localization (39). Therefore, while it is possible that the association of higher IGFBP3 with longer LTL reflects the parallel association of IGF1 with this endpoint, an alternative explanation might be that IGFBP3 may modulate LTL via mechanisms distinct from IGF1. Notably, all trans retinoic acid and activated retinoid X receptor α (RXR α) have recently been found to regulate LTL (40) as have polymorphic variants of PPAR gamma (41); that IGFBP3 heterodimerizes with RXR α (42) and interacts with PPAR gamma (39), could link IGFBP3 activity to LTL through nuclear receptor binding and regulation, independent of IGF1.

In our study there was no association of IGFBP1 with LTL. There is a strong inverse association of IGFBP1 with risk of metabolic syndrome (4). In one study lower IGFBP1 concentrations were associated with higher ischemic heart disease but not all-cause mortality (6), in other studies higher IGFBP1 concentrations were associated with greater all-cause mortality (10, 12). Therefore the overall impact of IGFBP1 on aging remains to be elucidated, and it does not appear to be associated with LTL.

Testosterone stimulates GH secretion in hypogonadal men via aromatization to estradiol (43), and blockade

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of aromatase with letrozole reduces circulating estradiol and peak GH response to arginine in men (21). However, while administration of GH increases circulating IGF1 in healthy men, there was no additive effect of GH combined with testosterone in this setting (44). We found that estradiol and testosterone were inversely associated with both IGF1 and IGFBP3, after adjusting for age. However, estradiol and IGF1 concentrations were independently and positively associated with LTL, as were estradiol and IGFBP3. A 1 s.d. increase in plasma estradiol was associated with an increase in T/S ratio of 0.012, similar in magnitude to that seen with a 1 s.d. increase in IGF1, when both were included in the same regression model. A similar result was seen with estradiol and IGFBP3. Men with both estradiol and IGF1 concentrations above the median value, had longer telomeres compared to men with both of estradiol and IGF1 below the median. The difference between groups (mean T/S ratio 1.20 vs 1.16 in multivariable-adjusted model) is comparable to the difference seen between 5-year age strata (e.g. mean T/S ratio in men 70–74 years 1.20 vs men 75–79 years 1.17). Therefore, our results are consistent with independent and additive associations of higher estradiol and higher IGF1 with LTL in older men.

Of note, GH treatment increases, and GH receptor blockade decreases, both IGF1 and estradiol concentrations (45). GH increases the estradiol/testosterone ratio, consistent with an effect on aromatase activity (45), while IGF1 enhances aromatase activity *in vitro* (46). However, we found an inverse association of estradiol with IGF1, which makes it less likely that high concentrations of IGF1 might have contributed to increased conversion of testosterone to estradiol in our study cohort.

We previously reported associations of higher estradiol with longer telomere length in younger, middle-aged and older men (22, 23). The underlying mechanism may relate to increased expression and activity of telomerase, the enzyme which lengthens telomeres by synthesis of new telomeric repeats (TTAGGG)_n, as shown in cell line and animal models (14, 24, 25, 47). Of note, IGF1 has been shown to reduce telomere length in fibroblasts (33) but enhances the effect of phytohaemagglutinin to increase telomerase activity in cord blood mononuclear cells (48), suggesting that IGF1 exerts effects on telomere length *in vitro* which may be cell-type specific. Other studies, albeit in prostate cancer cells, have reported that IGF1 induced telomerase activity, mediated via the phosphoinositol 3-kinase-Akt kinase pathway, and increased mRNA expression and protein levels of the catalytic reverse transcriptase subunit of telomerase, hTERT (49). While

other studies have shown in prostate cancer cells that IGFBP2 stimulates telomerase activity (50), when tested no effect of IGFBP3 was observed *in vitro* (49). The novel observation of additive associations of higher estradiol and higher IGF1 with LTL may be due to contributory actions of each hormone on telomerase activity and regulation of telomere length. However, it is also possible that higher IGF1 may reflect higher protein intake which may favor longevity in older, but not younger, adults (51). Therefore an alternative explanation for our findings is that they reflect a direct contribution of higher estradiol to telomerase activity, and an indirect contribution of higher IGF1 as a marker for biological factors relevant to aging in older men.

Other studies have linked the IGF1 axis to longevity in a contrasting manner, noting that centenarians and their offspring have lower IGF1 concentrations and IGF1 bioactivity compared to controls matched to the offspring (52, 53). In the Leiden Longevity Study, nonagenarians with IGF1/IGFBP3 ratios in the lowest quartile had greater longevity and better functional status compared with those in the highest quartile (54). It is possible that those findings reflect a selected population of the very oldest survivors or contrasting influences of the IGF1 axis on different aspects of ageing. Further work is needed to clarify the role of both sex hormones and the IGF1 axis to influence biological ageing in relation to nutritional status, health and longevity (55).

Limitations of this study are the single measurement of plasma IGF1, IGFBP1 and IGFBP3 and LTL at one time-point. We did not have serial blood samples for repeated measurements of these variables. As this is a cross-sectional analysis, causation cannot be inferred therefore it is not possible to establish a clear cause-and-effect relationship between variables. We measured total IGF1 and did not measure either free IGF1 or IGF1 bioactivity (7), nor did we measure circulating IGFBP1 phosphorylation status which may affect IGF1 bioactivity (56). Nevertheless, assays of total IGF1, IGFBP1 and IGFBP3 at a single time-point have been informative in other studies (4, 6, 8, 9, 10, 12). As blood samples were collected from surviving men at Wave 2, it is possible that these men as a group might have had higher IGF1 concentrations and longer telomeres than may have been the case had sampling been undertaken at Wave 1 and involved men who subsequently died prior to Wave 2. However, this should not have affected our results as associations between IGF1 and LTL were analysed as continuous variables and using median splits, rather than relying on absolute threshold values.

Another limitation of the study was that of 4246 men included at Wave 2, only 2999 were available for the analysis. However, the major reasons for exclusion were medications or comorbidities likely to affect sex hormone status which could confound the analysis, and missing relevant data. As the missing relevant data were largely accounted for by men who did not provide DNA samples for assay of LTL, there is no reason to suspect this could have biased the findings. Nevertheless, the possibility of bias cannot be dismissed. The HIMS cohort comprises men who had previously attended an earlier wave of the study, who returned for re-assessment and blood sampling. Therefore, our results may be more applicable to generally healthier older men. Associations between the IGF1 axis, sex hormones and LTL may be different in populations of younger or middle-aged men. The HIMS population is predominantly of Caucasian ethnicity, so additional studies would be needed to confirm our findings in men from other ethnic groups, and we cannot extrapolate our results to women.

Strengths of the study are the large cohort of community-dwelling older men, in whom not only IGF1 but also IGFBP1 and IGFBP3, and also estradiol and testosterone results were available. Sex hormones were assayed using LC-MS/MS. We assayed LTL using a method that correlates with the gold standard of Southern blot (28), which is feasible and practical for epidemiological studies. The cohort of men aged 70–84 years identifies an important and expanding demographic vulnerable to ill health. The multivariable models included age, BMI, smoking, alcohol, physical activity, and history of diabetes and cardiovascular disease, making it less likely that the results were confounded by age, underlying ill-health or physical factors.

In conclusion, higher IGF1 and IGFBP3 in the physiological range are independently associated with longer telomeres, consistent with lower biological age, in older men. Additive influences of higher IGF1 and higher estradiol on telomere length are present, suggesting that the IGF1 axis and sex hormones jointly influence telomere length *in vivo*. Further work is needed to clarify the mechanisms underlying these associations, and thus, to explore whether hormonal exposures might cooperatively modulate biological ageing.

Declaration of interest

K K Y H is on the editorial board of *EJE*. K K Y H was not involved in the review or editorial process for this paper, on which he/she is listed as an author. The other authors have nothing to disclose.

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