Low Circulating Insulin-Like Growth Factor I Bioactivity in Elderly Men Is Associated with Increased Mortality

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Context: Low IGF-I signaling activity prolongs lifespan in certain animal models, but the precise role of IGF-I in human survival remains controversial. The IGF-I kinase receptor activation assay is a novel method for measuring IGF-I bioactivity in human serum. We speculated that determination of circulating IGF-I bioactivity is more informative than levels of immunoreactive IGF-I.

Objective: Our objective was to study IGF-I bioactivity in relation to human survival.

Design, Setting, and Study Participants: We conducted a prospective observational study at a clinical research center at a university hospital of 376 healthy elderly men (aged 73–94 yr).

Main Outcome Measures: IGF-I bioactivity was determined by the IGF-I kinase receptor activation assay. Total and free IGF-I were determined by IGF-I immunoassays. Mortality was registered during follow-up (mean 82 months).

Results: During the follow-up period of 8.6 yr, 170 men (45%) died. Survival of subjects in the highest quartile of IGF-I bioactivity was significantly better than in the lowest quartile, both in the total study group [hazard ratio (HR) = 1.8; 95% confidence interval (95% CI) = 1.2–2.8; $P = 0.01$] as well as in subgroups having a medical history of cardiovascular disease (HR = 2.4; 95% CI = 1.3–4.3; $P = 0.003$) or a high inflammatory risk profile (HR = 2.3; 95% CI = 1.2–4.5; $P = 0.01$). Significant relationships were not observed for total or free IGF-I.

Conclusion: Our study suggests that a relatively high circulating IGF-I bioactivity in elderly men is associated with extended survival and with reduced cardiovascular risk. **(*J Clin Endocrinol Metab* 93: 2515–2522, 2008)**

IGF-I has important anabolic and mitogenic effects and is involved in mechanisms of function, maintenance, and repair of many tissues (1). Involvement of IGF-I in the process of aging has been studied extensively in the last two decades. Studies in invertebrates have suggested that disruption of signaling pathways similar to the IGF-I pathway extends lifespan (2–5). GH/IGF-I-deficient mice and rats have increased longevity compared with controls (3, 6, 7). Also, decreased IGF-I receptor (IGF-IR) signaling activity in female heterozygous knockout mice was shown to retard the process of aging (8). However, the precise role played by IGF-I in human survival remains controversial (9).

IGF-I is part of a complex system consisting of two growth factors (IGF-I and IGF-II), at least six high-affinity IGF-binding proteins (IGFBP-1 to -6) and several IGFBP proteases (1, 10). It is believed that only 0.5–1% of total IGF-I levels circulates in a free form, whereas about 99% of circulating IGF-I is bound by IGFBPs. Only free IGF-I is considered to interact with the IGF-IR (11).

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; CV, coefficients of variation; CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; HR, hazard ratio; hs, highly sensitive; IGFBP, IGF-binding protein; IGF-I KIRA, IGF-I-specific kinase receptor activation assay; IGF-IR, IGF-I receptor; IRP, inflammatory risk profile; LDL, low-density lipoprotein; SBP, systolic blood pressure.

Most of what is known about regulation of IGF-I in the circulation is based on measurements using specific IGF-I immunoassays. Because IGFBPs interfere with the accurate determination of IGF-I by immunoassay, various techniques have been developed to remove IGFBPs from samples or neutralize their influence on IGF-I immunoreactivity (11, 12). However, it is important to note that IGFBPs are important modulators of IGF-I bioactivity (1). IGFBPs are able to alter IGF-I bioactivity without changing the extractable concentrations of total IGF-I (11).

Recently, a highly sensitive and IGF-I-specific kinase receptor activation assay (IGF-I KIRA) was developed to determine IGF bioactivity in human serum (13, 14). This bioassay determines the ability of circulating IGF-I to activate the IGF-IR by quantification of intracellular receptor autophosphorylation upon IGF-I binding. Unlike an immunoassay, the IGF-I KIRA does not disregard modifying effects of IGFBPs and IGFBP proteases on the interaction between IGF-I and the IGF-IR (15, 16). Therefore, the IGF-I KIRA method is a new tool that could help broaden our understanding of the IGF-I system in humans, in both normal and pathological conditions.

The objective of the present study was to investigate whether IGF-I bioactivity was related to survival in a cohort of healthy elderly men. In addition, we studied the relationship between IGF-I bioactivity and cardiovascular disease (CVD) in this cohort.

Subjects and Methods

Study population

The design of the Zoetermeer study, a prospective cohort study conducted in clinically healthy independently living Caucasian elderly men, has been reported previously (17). Individuals were drawn from the municipal register of Zoetermeer, The Netherlands. Inclusion criteria were male sex, age at least 70 yr, and a sufficient physical and mental status to visit the study center independently. The Medical Ethics Committee of the Erasmus Medical Center approved the study. Of 1567 invited men, 403 participated and gave written informed consent. At baseline, medical histories were obtained from all participants, and serum samples were collected from 376 individuals. General practitioners were contacted about the status of participants in subsequent years. Cause of death was derived from death certificates and could only be verified in a limited number of subjects. The maximum follow-up time was 8.6 yr. Information on nonfatal events was not registered.

Anthropometric measurements

Height and weight were measured. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in duplicate. Hypertension was defined as SBP higher than 160 mm Hg and/or DBP higher than 90 mm Hg or antihypertensive treatment. Lean body mass and fat mass were measured using dual-energy x-ray absorptiometry (Lunar Corp., Madison, WI) (18).

Assays

At baseline, a venous blood sample was collected after an overnight fast. Serum and plasma aliquots were stored immediately after processing at −40 C. Total IGF-I levels were measured by RIA (Mediagnost GmbH, Tübingen, Germany) (19). Intraassay and interassay coefficients of variation (CV) were 1.6 and 6.4%, respectively. Free IGF-I levels were measured by a noncompetitive, two-site immunoradiometric assay (Beckman Coulter, Inc., Webster, TX). Intraassay and interassay CV were 10.3 and 10.7%, respectively. IGFBP-1 and IGFBP-3 levels were determined by specific RIA as previously described (19). For IGFBP-1 and IGFBP-3, intraassay CV were 3.4 and 1.9%, respectively, and interassay CV were 8.1 and 9.2%, respectively. Insulin was measured by a commercially available RIA (Medgenix Diagnostics, Brussels, Belgium). Intraassay and interassay CV were 8.0 and 13.7%, respectively. Immunoassays were performed soon after sample collection (in 1998) to reduce the possibility of analyte degradation.

Insulin sensitivity was calculated according to the homeostasis model assessment (HOMA) model 2 (HOMA Calculator version 2.2; Oxford Centre for Diabetes, Endocrinology and Metabolism). Total and high-density lipoprotein (HDL) cholesterol and triglycerides were measured using a commercially available kit (17). Low-density lipoprotein (LDL) cholesterol levels were calculated. C-reactive protein (CRP) levels were determined with a highly sensitive (hs) method (hs-CRP) using a latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Liederbach, Germany).

IGF-I KIRA

IGF-I bioactivity was measured using an in-house IGF-I KIRA as previously described (13). Human embryonic renal cells (293 EBNA; Invitrogen, Karlsruhe, Germany) stably transfected with copy DNA of the human IGF-IR gene were used as readout after stimulation with either recombinant IGF-I standards (Austral Biologicals, San Ramon, CA) or 1/10 diluted serum samples. IGF-I standards, two control samples, and serum samples from study participants were measured in duplicate on each culture plate. Intraassay and interassay CV were 6.0 and 10.9%, respectively. IGF-I KIRA measurements were performed in 2005. There are no data on the effects of long-term storage on sample IGF-I activity in the KIRA. However, because all samples were analyzed after an equal period of storage, any variable effect of storage time is likely removed.

Statistical analysis

Data were analyzed using SPSS for Windows, release 12.0 (Chicago, IL). Only data from the 376 participants for whom serum samples were available were included in the analyses. The Shapiro-Wilk test was used to test normality of variables (data were considered to be normally distributed when $P > 0.05$). For data that did not meet the criteria for normality, logarithmic transformations were applied. Baseline characteristics are presented as means ± SD, medians with 25th and 75th percentiles (P_{25}–P_{75}), or numbers. Correlation coefficients (r_s) between IGF-I parameters were calculated using nonparametric tests (Spearman's correlation coefficient). Univariate general linear models were used to calculate adjusted differences in means of variables between groups (F tests were used to evaluate significance). Linear regression was used to calculate the relation between IGF-I bioactivity and age. A multiple linear regression model was used to test equality of slopes (IGF-I bioactivity = $\beta_0 + \beta_1 \times \text{age} + \beta_2 \times Z + \beta_3 \times \text{age} \times Z + E$), where $Z = 1$ for nonsurvivors, $Z = 0$ for survivors, and E denotes independent, identically normally distributed error terms. IGF-I bioactivity and free IGF-I were calculated as percentage of total IGF-I according to the formula: X (picomoles/liter)/total IGF-I (picomoles/liter) × 100% (X = IGF-I bioactivity or free IGF-I level, respectively).

Cox proportional hazard models were used to analyze survival (Wald tests were used to evaluate significance of variables in the hazard model). The time-to-event variable was specified as time from baseline examination to death denoted in months. Individuals who did not have an event during the time that the subject was part of the study (n = 206) were censored. Continuous IGF-I risk factors were grouped (group 1, ≤25th; group 2–3, 25–75th; and group 4, ≥75th percentiles) and used as categorical covariates in survival analyses. For all IGF-I parameters, risk was calculated relative to the highest quartile. Within the final model, age, body mass index (BMI), smoking, SBP, diabetes, LDL, and HDL were considered possible confounders and were used as baseline time-constant covariates. Both crude and adjusted hazard ratios (HR) were estimated. Assumptions of proportionality of hazards were verified by graphical inspection.

TABLE 1. Baseline characteristics of survivors and nonsurvivors in the study cohort (n = 376)

Variable	Survivors (n = 206)	Nonsurvivors (n = 170)	*P* value
Mean ± SD			
Age (yr)	77.1 ± 3.0	78.5 ± 3.9	<0.001
BMI (kg/m^2)	25.8 ± 2.9	25.2 ± 3.0	0.29
Lean mass (kg)	52.3 ± 5.5	51.4 ± 5.4	0.21[a]
Fat mass (kg)	21.3 ± 5.7	21.0 ± 5.7	0.84[a]
SBP (mm Hg)	157 ± 25	156 ± 24	0.85[b]
DBP (mm Hg)	84 ± 11	83 ± 11	0.49[b]
Total cholesterol (mmol/liter)	5.8 ± 1.0	5.7 ± 1.1	0.53[b]
Glucose (mmol/liter)[c]	5.5 ± 1.2	5.4 ± 1.0	0.82[b]
Median (P_{25}–P_{75})			
Insulin (IU/liter)[c]	8.0 (6.0–10.0)	8.3 (6.2–10.6)	0.27[b]
HOMA (%S)	96 (71–124)	94 (75–124)	0.73[b]
Number (%)			
Smoking	37 (18%)	33 (19%)	0.89
Hypertension	114 (55%)	116 (68%)	0.01[b]
Myocardial infarction	24 (12%)	39 (23%)	0.002
Malignancy	18 (9%)	14 (8%)	0.91
Diabetes mellitus	14 (6.8%)	17 (10%)	0.30

All values are unadjusted. *P* values were adjusted for age. Insulin sensitivity in percentage (%S) was calculated according to the HOMA model 2 (HOMA Calculator version 2.2; Oxford Centre for Diabetes, Endocrinology and Metabolism). P_{25}–P_{75}, range between the 25th and 75th percentiles.

[a] *P* values for lean mass and fat mass were additionally adjusted for height.

[b] *P* values for blood pressures, lipids, glucose, insulin, and HOMA (%S), were additionally adjusted for BMI.

[c] Diabetics were excluded in the analysis of means for glucose, and insulin.

To control for confounding by CVD or inflammatory risk status, crude data were stratified and survival analysis was repeated as described previously. To correct for CVD, crude data were stratified into two groups split by the presence (n = 133) or absence (n = 242) of prevalent CVD. Presence of CVD was defined as having a medical history of myocardial infarction or cerebrovascular disease and/or being treated or having symptoms of angina pectoris, congestive heart failure, or claudicatio intermittens (17). To correct for inflammatory risk status, continuous baseline hs-CRP levels were split at 3 mg/liter, resulting in subjects with a low to medium inflammatory risk profile (IRP) (hs-CRP ≤ 3 mg/liter; n = 238) and subjects with a high IRP (hs-CRP > 3 mg/liter; n = 138) (20, 21). Survival analysis using IGF-I risk groups as categorical covariates was performed in IRP and CVD strata as described previously.

In general, cause of death was based on death certificates that could not be verified in the majority of subjects. Therefore, cause of death was not controlled for in all Cox proportional hazard models. Trends across IGF-I risk groups were based on models with linear effect of the risk factors (Armitage trend test). Two-sided *P* values are reported, and *P* values < 0.05 were considered statistically significant.

Results

Baseline characteristics of participants, categorized by survivors (n = 206, 55%) and nonsurvivors (n = 170, 45%), are shown in Table 1. Mean age at baseline was 77.7 ± 3.5 (SD) (range, 73–94) years. Mean time to death was 81.9 (range, 3–103) months.

Baseline levels of IGF system parameters are shown in Table 2. Overall, IGF-I bioactivity and free IGF-I levels accounted for 2.6% (range, 0.2–5.9%) and 0.6% (range, 0.2–2.9%) of circulating total IGF-I levels, respectively ($P < 0.001$). These fractions were not significantly correlated with each other ($r_s = 0.04$; $P = 0.48$). At baseline, only mean IGFBP-1 levels differed significantly between survivors and nonsurvivors (Table 2). In addition, the mean calculated bioactive IGF-I fraction was significantly higher in survivors compared with nonsurvivors (mean ± SEM, 2.7 ± 0.06 *vs.* 2.4 ± 0.07%, adjusted for age $P = 0.04$), whereas the calculated mean free IGF-I fraction did not differ between these groups ($P = 0.62$). Also the mean total IGF-I/IGFBP-3 ratio did not differ between survivors and nonsurvivors (adjusted for age $P = 0.21$).

IGF-I bioactivity was significantly correlated with all studied parameters of the IGF system (Table 3) and to the total IGF-I/IGFBP-3 ratio ($r_s = 0.26$; $P < 0.001$). Furthermore, mean baseline IGF-I bioactivity was negatively related to age [slope (β) = −4.5 pmol/liter·yr; $P = 0.01$, data not shown]. However, the rate of decline in IGF-I bioactivity with age did not differ between survivors (β = −3.5 pmol/liter·yr) and nonsurvivors (β = −4.1 pmol/liter·yr; $P = 0.77$). We found no significant correlations between IGF-I bioactivity and BMI, waist to hip ratio, lean mass, fat mass, fasting glucose, or insulin (data not shown).

Survival analyses for risk factor groups (1, 2–3, and 4) of total IGF-I, free IGF-I, and IGF-I bioactivity were performed. HR for mortality rate between groups are shown in Table 4.

Cox proportional hazard plots are shown in Fig. 1, A–C. A significant relationship was found between groups of IGF-I bioactivity and mortality (Table 4 and Fig. 1C). Subjects within the highest quartile of IGF-I bioactivity (group 4) had a lower mortality rate than subjects in groups with lower IGF-I

TABLE 2. Means at baseline of parameters of the IGF-I system in the total study population, in survivors and in nonsurvivors after follow-up

Variable	Mean or geometric mean (95% CI)			*P* value[a]
	All subjects (n = 376)	Survivors (n = 206)	Nonsurvivors (n = 170)	
IGF-I bioactivity (pmol/liter)	333 (320–346)	344 (328–361)	317 (296–337)	0.09
Total IGF-I (nmol/liter)	13.3 (12.9–13.6)	13.3 (12.7–13.7)	13.2 (12.6–13.9)	0.72
Free IGF-I (pmol/liter)	76.2 (72.2–80.3)	77.0 (72.1–82.2)	75.4 (69.1–82.2)	0.58
IGFBP-1 (nmol/liter)	1.1 (1.1–1.2)	1.1 (1.0–1.1)	1.2 (1.1–1.3)	0.03
IGFBP-3 (nmol/liter)	90.5 (88.0–93.0)	93.4 (90.1–96.9)	86.7 (83.1–90.2)	0.20

For Free IGF-I and IGFBP-1, geometric means are shown and 95% CI were calculated by forward-backward log-transformation.

[a] Age-adjusted *P* values were calculated for survivors *vs.* nonsurvivors.

TABLE 3. Correlation coefficients between parameters of the IGF-I system in the study population (n = 376)

	IGF-I bioactivity	Total IGF-I	Free IGF-I	IGFBP-1
Total IGF-I	0.49[b]			
Free IGF-I	0.19[b]	0.37[b]		
IGFBP-1	−0.25[b]	−0.15[b]	−0.10[a]	
IGFBP-3	0.34[b]	0.52[b]	0.17[b]	−0.23[b]

Correlations are presented as Spearman coefficients.

[a] $P < 0.05$.

[b] $P < 0.01$.

bioactivity (groups 1 and 2–3). This remained significant after adjustment for age, BMI, smoking, SBP, diabetes, LDL, and HDL (Table 4). For total IGF-I and free IGF-I, no significant relationships were found (Table 4 and Fig. 1, A and B, respectively). In addition, when survival was analyzed using quartiles of total IGF-I/IGFBP-3 ratios, no significant relationship was found (adjusted for age *P* for trend = 0.41, data not shown).

The data were then stratified into subgroups with either positive (n = 133) or negative medical history of CVD (n = 242). Mean baseline levels of IGF-I bioactivity and all other measured parameters of the IGF system did not differ between these two CVD groups (data not shown).

There was a significant relationship between groups of IGF-I bioactivity and mortality in subjects with prevalent CVD (Table 4 and Fig. 2A).

Subjects in the lowest quartile (group 1) of IGF-I bioactivity had a significantly higher mortality rate than subjects in the highest quartile (group 4) (unadjusted *P* for trend = 0.003; adjusted *P* for trend = 0.004).

For group 1 *vs.* 2–3, the HR for mortality rate was significant [unadjusted HR = 2.3 (95% CI = 1.4–3.8; *P* = 0.001); adjusted HR = 2.1 (95% CI = 1.3–3.6; *P* = 0.005)].

IGF-I bioactivity was significantly inversely related to hs-CRP levels ($r_s = -0.17$; $P < 0.001$, adjusted for age and BMI). Of all other measured parameters of the IGF-I system, only IGFBP-1 levels were significantly positively correlated with hs-CRP levels (r = 0.15; *P* = 0.04, adjusted for age and BMI).

Using hs-CRP as a marker of inflammation and mortality risk, we stratified data into a subgroup with a low to medium (n = 238) and with a high (n = 138) IRP. At baseline, mean IGF-I bioactivity was lower in subjects with a high IRP than in subjects with a low to medium IRP (mean ± SEM, 312 ± 11.6 *vs.* 344 ± 7.8 pmol/liter; *P* = 0.01, adjusted for age and BMI). Means of other parameters of the IGF-I system did not differ between IRP subgroups.

The relationship between IGF-I bioactivity groups and mor-

TABLE 4. Estimated HR between groups of IGF-I bioactivity, total IGF-I, and free IGF-I in all subjects and after stratification for CVD+ and CVD− and $IRP_{low/med}$ and IRP_{high}, respectively, during a follow-up period of 8.6 yr

	All subjects			CVD−			CVD+			$IRP_{low/med}$			IRP_{high}		
Group	HR	95% CI	*P* for trend	HR	95% CI	*P* for trend	HR	95% CI	*P* for trend	HR	95% CI	*P* for trend	HR	95% CI	*P* for trend
Unadjusted															
IGF-I bioactivity															
1	1.8	1.2–2.8		1.6	0.9–2.9		2.4	1.3–4.3		1.4	0.8–2.5		2.3	1.2–4.5	
2–3	1.2	0.8–1.8		1.4	0.8–2.5		1.1	0.6–1.9		1.0	0.6–1.6		1.9	1.0–3.6	
4	Ref.		0.01	Ref.		0.12	Ref.		0.01	Ref.		0.32	Ref.		0.01
Total IGF-I															
1	0.9	0.6–1.4		0.8	0.4–1.4		1.2	0.7–2.1		0.8	0.5–1.8		1.0	0.5–1.8	
2–3	0.6	0.4–0.9		0.7	0.4–1.2		0.6	0.4–1.1		0.6	0.3–0.9		0.9	0.5–1.6	
4	Ref.		0.36	Ref.		0.29	Ref.		0.92	Ref.		0.12	Ref.		0.25
Free IGF-I															
1	1.1	0.8–1.7		1.2	0.7–2.1		1.2	0.7–2.2		0.8	0.5–1.3		1.8	1.0–3.4	
2–3	0.7	0.5–1.1		0.8	0.5–1.3		0.8	0.5–1.3		0.7	0.4–0.9		1.1	0.6–2.1	
4	Ref.		0.93	Ref.		0.75	Ref.		0.76	Ref.		0.15	Ref.		0.25
Adjusted															
IGF-I bioactivity															
1	1.6	1.0–2.5		1.3	0.7–2.5		2.5	1.4–4.8		1.3	0.7–2.3		2.1	1.1–4.1	
2–3	1.4	0.8–1.8		1.4	0.8–2.5		1.2	0.7–2.1		1.0	0.6–1.7		2.0	1.0–3.8	
4	Ref.		0.04	Ref.		0.31	Ref.		0.01	Ref.		0.47	Ref.		0.03
Total IGF-I															
1	0.9	0.6–1.3		0.8	0.4–1.4		1.1	0.6–1.9		0.7	0.4–1.3		1.1	0.6–2.1	
2–3	0.7	0.5–1.0		0.8	0.5–1.4		0.6	0.3–1.0		0.6	0.4–0.9		1.0	0.5–1.9	
4	Ref.		0.25	Ref.		0.37	Ref.		0.77	Ref.		0.10	Ref.		0.87
Free IGF-I															
1	1.0	0.7–1.6		1.3	0.7–2.3		1.1	0.6–2.0		0.9	0.5–1.6		1.7	0.9–3.4	
2–3	0.7	0.5–1.1		0.9	0.5–1.5		0.7	0.4–1.2		0.6	0.4–1.0		1.0	0.6–1.9	
4	Ref.		0.81	Ref.		0.94	Ref.		0.98	Ref.		0.39	Ref.		0.15

Individuals were grouped according to their baseline levels of three different IGF-I parameters (IGF-I bioactivity, total IGF-I, and free IGF-I): group 1, IGF-I ≤ 25th percentile; group 2–3, IGF-I between 25th and 75th percentile; group 4, IGF-I ≥ 75th percentile. In all models, group 4 is the reference (Ref.) group (HR = 1.0). For adjusted data, models were adjusted for age, BMI, smoking, SBP, diabetes, LDL, and HDL. CVD−, Absence of cardiovascular disease; CVD+, presence of cardiovascular disease; IRP_{high}, high IRP; $IRP_{low/med}$, low to medium IRP.

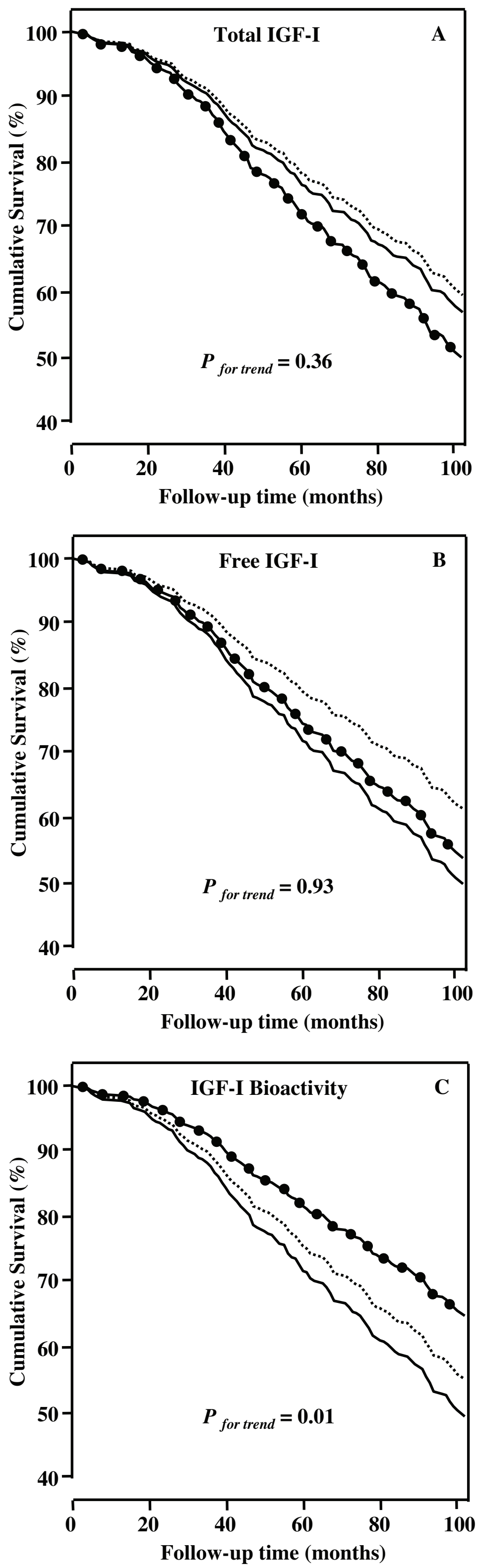

FIG. 1. Cox proportional hazard plots (percent) for groups of circulating total IGF-I levels (A), circulating free IGF-I levels (B) and IGF-I bioactivity levels (C). *P* for trend reached statistical significance only in the IGF-I bioactivity groups. Groups of IGF-I bioactivity are shown as follows: group 1 (—), ≤25th percentile; group 2–3 (– – –), between 25th and 75th percentile; and group 4 (—●—), ≥75th percentile. Trends across IGF-I bioactivity risk groups were based on Cox proportional hazard models with linear effect of the risk factor (Armitage trend test). Maximum time of follow-up was 103 months.

tality was significant, but only in subjects with a high IRP (Table 4 and Fig. 2B). Subjects in the highest quartile (group 4) had significantly better survival than subjects in the lowest quartile (group 1) ($P = 0.01$). This relationship remained significant after adjustment for age, BMI, smoking, SBP, diabetes, LDL, and HDL ($P = 0.03$). Exclusion of the first year of follow-up did not affect this relationship because the difference in mortality rate remained significant for group 1 *vs.* 4 [unadjusted HR = 2.0 (95% CI = 1.1–4.3; $P = 0.02$); adjusted HR = 2.0 (95% CI = 1.0–4.0; $P < 0.05$)]. For group 1 *vs.* 2–3, the HR for mortality rate was not significant [unadjusted HR = 1.2 (95% CI = 0.8–2.0; $P = 0.38$); adjusted HR = 1.0 (95% CI = 0.6–1.8; $P = 0.88$)].

Lastly, IRP and CVD data were combined. At baseline, mean IGFBP-1 levels were significantly higher in subjects with a positive medical history of CVD and/or a high IRP (n = 211) compared with individuals without prevalent CVD and a low/medium IRP ($P = 0.004$, data not shown).

A significant trend for mortality rates was found across IGF-I bioactivity groups in subjects with a high IRP and/or a positive medical history of CVD (unadjusted *P* for trend = 0.003; adjusted *P* for trend = 0.005; Fig. 2C). Mortality rate was highest in group 1, the quartile with the lowest IGF-I bioactivity (for estimated HR, see Fig. 2C legend).

Neither total nor free IGF-I showed any significant relationships with mortality rate in CVD and IRP subgroups (Table 4).

Discussion

This 8-yr prospective study in elderly men showed that higher circulating IGF-I bioactivity is associated with better overall survival. Individuals in the lowest quartile of IGF-I bioactivity had a 1.8-fold increased mortality risk compared with individuals in the highest quartile. Interestingly, for total and free IGF-I measurements as well as for the total IGF-I/IGFBP-3 ratios, we could not find such relationships.

Although men with lower IGF-I bioactivity might have died earlier or might have been excluded from the study because their physical condition (illness, frailty, or other causes) prevented a visit to the research center (22), the strength of our study is its prospective design, which is likely to reduce this form of selection bias.

The IGF-I KIRA was used to measure IGF-I bioactivity (13, 14), which was significantly associated with other IGF-I system parameters measured by immunoassay. However, none of these associations had correlation coefficients greater than 0.5. This suggests that in comparison with immunoassays, the KIRA produces different information about circulating IGF-I.

In most IGF-I immunoassays, various techniques are used to remove IGFBPs (15). However, one of the major functions of IGFBPs is to modulate IGF-I bioavailability. IGFBP-1 is thought to be an important direct modulator of IGF-I bioactivity (10). In our study, IGF bioactivity correlated better with circulating IGFBP-1 levels than with either total or free IGF-I levels, suggesting that IGFBP-1 indeed influences IGF-I bioactivity.

Of interest is the observed discrepancy between IGF-I bioactivity and free IGF-I in our study. Both parameters are be-

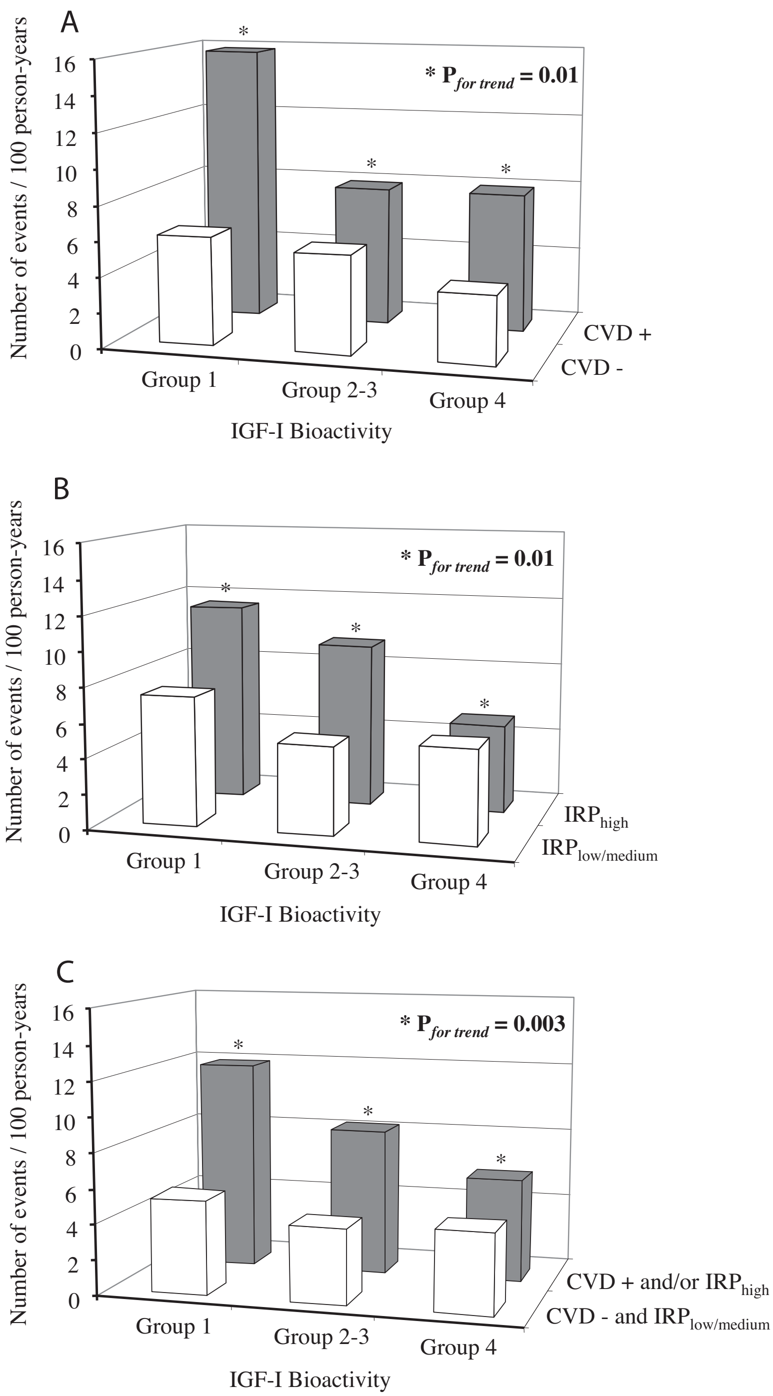

FIG. 2. Crude incidence rates [number of events (deaths) per 100 person-years] are shown for groups of circulating IGF-I bioactivity levels according to strata of absence or presence of a medical history of CVD (CVD− *vs.* CVD+, respectively) (A), low/medium or high IRP ($IRP_{low/medium}$ *vs.* IRP_{high}, respectively) (B), and combined subgroups of CVD and IRP (CVD+ and/or IRP_{high} *vs.* CVD− and $IRP_{low/medium}$) (C). A significant linear trend (*) between groups of IGF-I bioactivity was found only in subjects with prevalent CVD (A), a high IRP (B), or prevalent CVD and/or a high IRP (C). C, For group 1 *vs.* 2–3, unadjusted HR = 1.5 (95% CI = 1.0- 2.3; $P < 0.05$); for group 1 *vs.* 4, HR = 2.3 (95% CI = 1.3–3.8; $P = 0.002$); and for group 2 *vs.* 3, HR = 1.5 (95% CI = 0.9–2.5; $P = 0.11$). HR adjusted for age, BMI, smoking, SBP, diabetes, LDL, and HDL were 1.4 (95% CI = 0.9–2.4; $P = 0.15$), 2.2 (95% CI = 1.3–3.8; $P = 0.004$), and 1.6 (95% CI = 0.9–2.6; $P = 0.08$), respectively. Maximum time of follow-up was 103 months. Trends across IGF-I bioactivity risk groups were based on Cox proportional hazard models with linear effect of the risk factor (Armitage trend test). Groups of IGF-I bioactivity are as follows: group 1, IGF-I bioactivity ≤ 25th percentile; group 2–3, IGF-I bioactivity between 25th and 75th percentile; group 4, IGF-I bioactivity ≥ 75th percentile. IRP subgroups were based on hs-CRP levels: $IRP_{low/medium}$ = CRP ≤ 3 mg/liter; IRP_{high} = CRP > 3 mg/liter.

lieved to be informative about the fraction of circulating total IGF-I that interacts with the IGF-IR. Mean IGF-I bioactivity was significantly greater than mean free IGF-I level, and correlation between these parameters was poor. An explanation could be that the IGF-I KIRA is more sensitive than free IGF-I levels in estimating the concentration of circulating IGF-I that interacts with the IGF-IR, because the KIRA is probably better at detecting the modulatory effects of IGFBPs and IGFBP proteases. In addition, because the IGF-I KIRA measures the overall ability of serum to activate the IGF-IR *in vitro*, our data do not allow us to discriminate between the relative contributions of IGF-I and IGF-II to the IGF-I KIRA signal. Therefore, IGF-II-mediated effects could also have contributed to the discrepancy between the IGF-I KIRA and free IGF-I levels. From previous experiments, it is known that IGF-II has a cross-reactivity of about 12% to the IGF-IR (13). However, as has been suggested previously, from a biological point of view, it is not important whether IGF-IR activation is caused mostly by IGF-I or IGF-II (23).

In this study, a relatively high circulating IGF-I bioactivity was associated with a lower mortality risk. This is in contrast to results reported in animal studies, where low circulating IGF-I levels were associated with increased survival. An explanation could be that in these animal studies, effects of IGF-I on the rate of aging were studied during lifelong exposure, whereas our study provides insight into IGF-I activity only toward the end of life. In addition, insulin and IGF-I have very different functions and signaling pathways in mammals compared with their homologs in lower species (*e.g. Caenorhabditis elegans* and *Drosophila*). Furthermore, lifespan in humans is measured in decades as opposed to months or days in rodents, flies, and nematodes (24). Another explanation could be that catabolism and/or a systemic inflammatory response as a consequence of subclinical (undiagnosed) diseases may have induced resistance to IGF-I production. It could be that in our study, low IGF bioactivity may not be a cause but rather an effect, serving as a reporter for disease or catabolism.

Humans have the potential to live for over 100 yr, and the precise interactions of all factors that influence aging is complex. In the Western world, CVD and cancer are the most important determinants of human survival. Although cancer is also a common cause of death in animal models used to study longevity, CVD is not and is more specific to humans. When we stratified for the presence or absence of CVD, we found that low IGF-I bioactivity was an important independent risk factor for mortality rate in individuals with CVD. Interestingly, comparative results were found when data were stratified according to IRP (based on CRP levels), and the observed trend became even stronger when both groups were combined.

Of note, CRP is a nonspecific marker of systemic inflammatory responses and has emerged as the most powerful predictor of mortality due to CVD events among 11 other biomarkers (21). In our study, CRP levels were significantly inversely related to IGF-I bioactivity and positively to IGFBP-1. Although a decline in IGF-I bioactivity could be a component of the hormonal alterations that occur in any illness or catabolic state, the difference in mortality rate between the highest and the lowest quartile of IGF-I bioactivity in our study remained significant when all mortalities in the first year of follow-up were excluded from the analyses. Thus, undiagnosed illness or catabolic states at baseline probably did not cause the relationship between IGF-I bioactivity and survival.

Interestingly, there are different clinical models that support the negative regulation of CRP and other inflammatory markers by GH/IGF-I administration (25, 26) For example, Sesmilo *et al.* (27) found low levels of CRP in patients with active acromegaly, which rose when IGF-I levels normalized after administration of a GH receptor antagonist. Verma *et al.* (28) found that CRP negatively influences proliferation, differentiation, and survival of isolated endothelial progenitor cells *in vitro* and their ability to produce nitric oxide (NO), whereas for IGF-I, opposite effects have been reported (29). Altogether these findings point to the possibility of a relationship between IGF-I bioactivity, CRP, and CVD. However, it remains to be seen whether reduced IGF-I bioactivity is an endocrine contributor to mortality risk or simply an epiphenomenon related to overall health/resistance to inflammation.

In conclusion, this prospective study provides evidence that low circulating IGF-I bioactivity in elderly men is associated with increased mortality, especially in those individuals in which an age-related proinflammatory state exists, with its attendant higher risk of mortality from CVD. Remarkably, for total and free IGF-I measurements, we could not find such relationships. Compared with IGF-I immunoassays, the IGF-I KIRA may offer the unique possibility of measuring the net modulating effects of IGFBPs and IGFBP proteases on IGF-IR activation by bioactive IGF-I available in human serum. In this respect, our study suggests that determination of IGF-I bioactivity may help to clarify the controversies that exist about the precise role of IGF-I in human survival.

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