Insulinlike Growth Factor Binding Protein-1 and Ghrelin Predict Health Outcomes Among Older Adults: Cardiovascular Health Study Cohort

Robert C. Kaplan,[1] Garrett Strizich,[1] Chino Aneke-Nash,[1] Clara Dominguez-Islas,[3] Petra Bůžková,[3] Howard Strickler,[1] Thomas Rohan,[1] Michael Pollak,[5] Lewis Kuller,[6] Jorge R. Kizer,[2] Anne Cappola,[7] Christopher I. Li,[4,8] Bruce M. Psaty,[9] and Anne Newman[6]

[1]Department of Epidemiology and Population Health and [2]Division of Cardiology, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461; [3]Department of Biostatistics and [4]Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington 98195; [5]Department of Medicine and Oncology, McGill University, Montreal, Quebec, Canada H4A 3J1; [6]Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261; [7]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania 19104; [8]Epidemiology Program, Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109; and [9]Cardiovascular Health Research Unit, Departments of Epidemiology and Health Services, University of Washington & Group Health Research Institute, Group Health Cooperative, Seattle, Washington 98101

Context: Multiple diseases may explain the association of the growth hormone/insulinlike growth factor-I (GH/IGF-I) axis with longevity.

Objective: To relate circulating GH/IGF-I system protein levels with major health events

Design and Setting: This is a cohort study set in 4 US communities.

Participants: Adults (N = 2268) 65 years and older free of diabetes and cardiovascular disease

Measurements: We assessed insulinlike growth factor binding protein-1 (IGFBP-1) and ghrelin in fasting and 2-hour oral glucose tolerance test (OGTT) blood samples, as well as fasting IGF-I and IGFBP-3. Hazard ratios for mortality and a composite outcome for first incident myocardial infarction, stroke, heart failure, hip fracture, or death were adjusted for sociodemographic, behavioral, and physiological covariates.

Results: During 13,930 person-years of follow-up, 48.1% of individuals sustained one or more components of the composite outcome and 31.8% died. Versus the lowest quartiles, the highest quartiles of fasting and 2-hour ghrelin were associated with 27% higher (95% confidence interval [CI]: 6%, 53%) and 39% higher (95% CI: 14%, 71%) risks of the composite outcome, respectively. The highest quartile of 2-hour IGFBP-1 was associated with 35% higher (95% CI: 1%, 52%) risk of the composite end point. Similarly, higher mortality was significantly associated with higher fasting and 2-hour ghrelin levels and with 2-hour IGFBP-1 level. When examined together, 2-hour post-OGTT levels of IGFBP-1 and ghrelin tended to predict outcomes better than fasting levels.

Conclusions: Circulating IGFBP-1 and ghrelin measured during an OGTT predicted major health events and death in older adults, which may explain the influence of the GH/IGF-I axis on lifespan and health.

Abbreviations: BMI, body mass index; BP, blood pressure; CHS, Cardiovascular Health Study; CI, confidence interval; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; GH, growth hormone; HF, heart failure; HR, hazard ratio; hs-CRP, high-sensitivity C-reactive protein; IGF, insulinlike growth factor; IGF-I, insulinlike growth factor-I; IGFBP, insulinlike growth factor binding protein; LLD, lower limit of detection; MI, myocardial infarction; OGTT, oral glucose tolerance test; SD, standard deviation.

Insulinlike growth factor (IGF)–axis proteins have anabolic and metabolic actions that affect development, growth, and survival. Extensive evidence suggests that insulinlike growth factor-I (IGF-I) may influence pathways that determine longevity in humans and other organisms (1, 2). Understanding this phenomenon has been challenging because a variety of mediators regulate the effects of IGF-I, which itself mediates the effects of growth hormone (GH) and reciprocally inhibits pituitary GH secretion. For example, IGF-I binding proteins (IGFBP-1 through IGFBP-6) influence IGF-I bioavailability and activity (3), and ghrelin stimulates the production of GH from the hypothalamus (4).

Circulating GH/IGF–related proteins have been shown to predict multiple disease end points (5–8) that in combination may account for the association of IGF-I with longevity. Prior epidemiological studies typically examined end points individually, rather than in combination, vs fasting measures of GH/IGF–related proteins. Some GH/IGF–axis proteins are physiologically modulated after food intake. Ghrelin, a gastric hormone with GH-stimulating and orexigenic effects (9), is proportionally decreased in the postprandial state compared with the fasting state (10). Insulin in the portal circulation acutely regulates circulating levels of IGFBP-1 by inhibiting the transcription of *IGFBP1* in the liver (11), such that circulating IGFBP-1 measurements taken after a glucose load are fivefold to 10-fold lower than measurements taken in the fasting state (12).

In the Cardiovascular Health Study (CHS) of older adults, we obtained measurements of GH/IGF–system proteins both during fasting and after glucose loading. The CHS also collected a variety of end points that were previously associated with IGF-I, including cardiovascular disease (CVD) (6–8), hip fracture (5), and death (1). This information was used in an 8-year follow-up study to link GH/IGF protein levels, disease, and survival.

Methods

Study population and setting

The CHS is a population-based, prospective, longitudinal study of community-dwelling adults aged ≥65 years recruited in Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania (13). In 1989–1990, 5201 participants were recruited into the CHS, and in 1992–1993, an additional 687 African American participants were enrolled. At annual study visits, data and biospecimens were obtained. Follow-up was through semiannual participant contacts and database matches (National Death Index, Medicare). The present analysis used as study baseline the 1996–1997 CHS visit, at which time 1180 CHS participants were deceased, 784 were contacted by phone or mail only, and 296 were not contacted. Among 3673 who were alive and completed an in-person visit, 3390 provided a blood sample. The study was approved by the institutional review board at each center, and all participants gave informed consent. The funding agency did not participate in the completion of this report.

Baseline variables

At the 1996–1997 clinic visit, CHS assessed medical history and medications, body mass index (BMI), blood pressure (BP), physical activity, alcohol consumption, smoking, estrogen and progestin use, and self-reported health status. Protein consumption was assessed with a food frequency questionnaire in 1995–1996.

Laboratory measurements were performed in 2012 using blood collected at the 1996–1997 study visit, after being processed and stored at −70°C according to the standardized, uniform procedures described previously (14, 15). Plasma IGF-I levels were measured in previously frozen archived specimens using enzyme-linked immunosorbent assay (ELISA) methods [1778 using materials from Immunodiagnostic Systems Ltd (IDS), Tyne & Wear, England, and 487 using materials from Diagnostics Systems Laboratories Inc., Webster, TX] (14). Other measurements included serum IGFBP-1 (ELISA, ALPCO, Salem, NH), IGFBP-3 (chemiluminescence method; IDS, Boldon, UK), and total ghrelin (both intact and des-octanoyl forms measured by sandwich ELISA; Millipore, Billerica, MA). Each biomarker was measured in fasting samples, and IGFBP-1 and ghrelin levels were additionally measured in blood that was collected 2 hours after a 75-g glucose load. Previous studies have shown high stability over specimen storage for up to 9 years and low variation within individuals and between laboratories for serum measurements of IGF-I analytes stored at −70C. (14, 16) IGFBP-1 levels below detectable levels were assigned to the lower limit of detection for the assay of 1.0 μg/L (4% of fasting specimens and 10% of postload specimens). For ghrelin, 11% of fasting specimens and 28% of postload specimens were below the lower limit of detection of the assay (<80 p/L). A subset of ghrelin levels (2.8% of fasting and 6.6% of 2-hour measures) was extrapolated to range from 10 to 80 pg/L using assay manufacturer calibration equations, whereas others falling below the extrapolation range were assigned a value of 10 pg/L. Interassay coefficients of variation were 11% to 14% for ghrelin, IGF-I, IGFBP-1, and IGFBP-3. Other serum laboratory values measured at the 1996–1997 study visit were creatinine, albumin, high-sensitivity C-reactive protein (hs-CRP), total cholesterol, glucose, and insulin (17).

Event follow-up

The CHS protocol captured incident health events through several means, including semiannual participant contacts and National Death Index matches. All incident major cardiovascular events and cerebrovascular events were adjudicated through review of medical records (18). Hospitalized hip fracture events were defined by searching the abstracts of all hospitalizations reported during follow-up for *International Classification of Diseases, Ninth Revision* codes of 820.xx (19). All deaths were reviewed to discern their cause and circumstances (20). We used up to 8 years of follow-up for event ascertainment to minimize misclassification due to changes in IGF protein levels over time.

Selection of subjects for the present analyses

To define survival free from major health events among the 3390 CHS participants who provided a blood sample at the 1996–1997 visit, we excluded all individuals who at the time of the baseline measurement of GH/IGF proteins had a history of myocardial infarction (MI; n = 227), stroke (n = 143), and heart failure (HF; n = 167) and an additional 170 who had had >1 of these CVD diagnoses. We also excluded diabetic participants (fasting plasma glucose level ≥126 mg/dL or taking antidiabetic agents) because of the effects of diabetes and its treatments on circulating protein levels (n = 342). Among 2341 remaining individuals, an additional 3% were excluded for incomplete data for smoking history (n = 41) and BMI (n = 32), yielding a sample of 819 men and 1449 women.

Outcomes

Using a time-to-event framework, we defined a composite primary outcome measure to incorporate the incidence of 5 conditions that have previously been associated with levels of circulating GH/IGF proteins, including (a) MI, (b) stroke, (c) HF, (d) hospitalized hip fracture, and (e) all-cause mortality. This outcome was defined as the first occurrence of any of the 5 event types during follow-up. A second primary outcome was all-cause mortality. Secondary outcomes included cause-specific mortality and individual incident events (MI, stroke, HF, and hip fracture).

Statistical analyses

Descriptive statistics of baseline variables were computed as stratified by sex and race. Spearman correlations examined relationships among variables. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of fasting IGF-I and IGFBP-3, as well as fasting and postprandial measures of IGFBP-1 and ghrelin, with outcomes. IGFBP-1 measures underwent log-transformation prior to analysis. Biomarkers other than IGFBP-1 were standardized, and all were included as linear predictors. Thus, we estimated the HR associated with a 1–standard deviation (SD) difference of IGF-I, IGFBP-3, and ghrelin or a twofold higher level of IGFBP-1. We used the continuous biomarker analyses as the primary means to gauge the statistical significance of the associations. We also examined HRs across quartiles.

Initial analyses adjusted for age, sex, race, and smoking status. We subsequently adjusted for potential confounders determined *a priori*, including alcohol consumption, self-reported health status, physical activity, protein consumption, hypertension, use of progestins or estrogens, serum albumin, serum cystatin C, serum hs-CRP, and BMI. Additional models adjusted for fasting insulin and IGF-I levels and 2-hour post–oral glucose tolerance test (OGTT) glucose level. To assess the incremental value of 2-hour measures over fasting measures of IGFBP-1 and ghrelin, we conducted analyses in which fasting and 2-hour measures were both included as independent variables. Lastly, those in the upper quartile for ghrelin, the upper quartile for IGFBP-1, or the upper quartile for both biomarkers were compared against those in the lower 3 quartiles for both.

Hotdeck methods were used to impute incomplete values for adjustment variables, including protein intake among 4% of participants, hs-CRP in 1%, and other covariates in <1% of participants (21). Schoenfeld residuals derived from age-, sex-, race/ethnicity-, and smoking history–adjusted and fully adjusted models were regressed against follow-up time for each predictor/outcome combination using both linear and loess regression; when we suspected possible violation of the proportional hazards assumption, sensitivity analyses were conducted in which we included an interaction term of the predictor variable, with time treated as both continuous and binary variables (follow-up years 1 to 4 vs years 5 to 8). Further sensitivity analyses involved exclusion of events that occurred during the first year of follow-up. We also assessed the sensitivity of results after (1) removal of HF, stroke, MI, or hip fracture from the composite outcome definition; (2) exclusion of individuals meeting plasma glucose thresholds for prediabetes or diabetes (2-hour glucose level ≥140 mg/dL, fasting glucose level 100 to 126 mg/dL); and (3) alternative imputation for ghrelin and IGFBP-1 values that fell below the lower limit of detection (LLD) at one-half the assay LLD. We examined first-order interactions between the IGF-related biomarkers and BMI dichotomized at the "overweight" threshold of 25 $kg \cdot m^{-2}$ (which also happened to be the approximate sample median). In a similar manner, we estimated sex-specific, race-specific, and age-specific associations. Two-tailed values of $P < 0.05$ were considered statistically significant. All statistical analyses were performed with Stata: Release 13 (StataCorp, College Station, TX) and SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Subject characteristics

Among 819 men and 1449 women included in the analyses, the mean age was 77.8 years (range, 68 to 102 years) and 14% were African American (Table 1). During a total of 13,930 person-years over 8 years of follow-up, 48.1% of these individuals sustained at least one of the health events that constituted the composite outcome (MI, stroke, CHF, hip fracture, or death).

Correlates of GH/IGF-axis biomarkers included anthropometric measures, diastolic BP, fasting and postprandial glucose and insulin measures, markers of kidney function, and hs-CRP (Table 2). IGF-I levels were correlated moderately with levels of IGF-binding proteins ($|r|$= 0.4 to 0.6). Ghrelin levels were modestly correlated with IGFBP-1 ($r < 0.3$) but not with other measures. We observed Spearman r correlations above 0.80 between fasting and 2-hour post-OGTT levels of IGFBP-1 and ghrelin.

Composite major health events outcome

Adjusted for age, sex, race/ethnicity, and smoking, risk of the composite disease outcome was associated with lower levels of IGFBP-3 and with higher fasting and 2-hour postload levels of both IGFBP-1 and ghrelin (Table 3). After further adjustment for alcohol consumption, self-reported health status, hypertension, physical activity, protein consumption, progestin and estrogen use, BMI, albumin, cystatin C, and hs-CRP protein, significant associations with the composite outcome remained for fasting and 2-hour levels of ghrelin and for 2-hour levels (but not

fasting levels) of IGFBP-1. For those biomarkers that were identified as statistically significant in continuous data analyses, quartile analyses suggested a stepwise increase in HRs across quartile values. Those who had fasting and 2-hour ghrelin in the upper quartile vs the lowest quartile had a 27% higher risk (95% CI, 6% to 53%) and 39% higher risk (95% CI, 14% to 71%) of the composite outcome, respectively. IGFBP-1 levels also were associated with the composite outcome; as for ghrelin levels, the adjusted hazard ratio was slightly higher for 2-hour levels compared with fasting levels, albeit CIs for the adjusted hazard ratios overlapped between the fasting and 2-hour measurements. Additional adjustment for levels of insulin or IGF-I did not appreciably alter the results. IGF-I level was not associated with the composite outcome.

Table 1. Characteristics of Cardiovascular Health Study Participants

	Men			Women		
	All (n = 819)	White[a] (n = 713)	Black (n = 106)	All (n = 1449)	White[a] (n = 1228)	Black (n = 221)
	Mean (SD)/Median (IQR)/%			Mean (SD)/Median (IQR)/%		
Age, y	78.0 (4.7)	78.4 (4.7)	75.3 (4.4)	77.7 (4.5)	77.9 (4.4)	76.5 (5.1)
Body mass index, kg/m^2	26.4 (3.7)	26.4 (3.7)	26.6 (3.7)	26.7 (4.8)	26.4 (4.6)	28.6 (5.6)
Waist circumference, cm	98.0 (9.8)	98.2 (9.7)	96.9 (10.5)	95.0 (14.2)	94.6 (14.1)	97.1 (14.7)
Systolic blood pressure, mm Hg	135.4 (20.0)	135.1 (19.6)	137.6 (22.6)	137.9 (20.5)	137.6 (20.5)	139.3 (20.6)
Diastolic blood pressure, mm Hg	72.1 (10.2)	71.8 (10.2)	74.4 (10.3)	69.9 (10.7)	69.3 (10.5)	73.2 (10.8)
Fasting glucose, mg/dL	94.4 (9.8)	94.6 (9.9)	93.1 (9.4)	91.7 (10.1)	91.3 (10.0)	93.8 (10.5)
2-hour glucose, mg/dL	129.9 (43.2)	129.3 (43.2)	133.7 (43.4)	138.7 (45.7)	138.7 (46.2)	138.7 (42.7)
Total cholesterol, mg/dL	188.5 (36.5)	188.1 (36.3)	191.4 (37.3)	212.9 (36.9)	212.5 (36.8)	214.9 (37.9)
Serum albumin, mg/dL	3.8 (0.3)	3.8 (0.3)	3.9 (0.3)	3.8 (0.3)	3.8 (0.3)	3.9 (0.3)
Fasting insulin IU/mL[b]	7.2 (4.8, 10.8)	7.1 (4.9, 10.9)	7.5 (4.3, 10.7)	7.2 (4.9, 10.9)	7.0 (4.8, 10.6)	8.1 (5.3, 12.3)
2-hour insulin IU/mL[b]	5.7 (5.1, 6.4)	5.7 (5.0, 6.3)	5.9 (5.2, 6.7)	6.0 (5.4, 6.6)	5.9 (5.3, 6.6)	6.1 (5.7, 6.8)
Cystatin C, mg/L[b]	1.1 (1.0, 1.3)	1.1 (1.0, 1.3)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)
hs-CRP, mg/L[b]	1.8 (0.9, 4.2)	1.8 (0.9, 4.1)	2.2 (1.0, 4.8)	2.4 (1.0, 4.9)	2.3 (1.0, 4.7)	2.8 (1.3, 5.9)
Physical activity, kcal/d[b]	1153 (460, 2404)	1188 (480, 2483)	810 (270, 2038)	675 (208, 1448)	713 (240, 1549)	510 (135, 1093)
Protein consumption, g/d[b]	77 (60, 97)	75 (59, 95)	84 (67, 107)	73 (57, 92)	73 (57, 91)	75 (55, 97)
Self-reported general health, %						
Excellent/very good	37.9	39.0	30.2	33.0	34.6	24.0
Good	46.4	46.4	46.2	47.8	48.2	45.7
Fair/poor	15.8	14.6	23.6	19.2	17.2	30.3
Hypertension, yes %	41.7	40.1	52.8	48.0	44.3	69.1
Smoking history, %						
Never	32.0	32.7	27.4	60.0	61.2	53.9
Former	59.8	61.3	50.0	32.5	31.6	37.6
Current	8.2	6.0	22.6	7.5	7.3	8.6
Alcohol consumption, %						
None	43.6	42.6	50.0	61.1	57.7	79.6
Moderate (1–7 drinks/wk)	37.9	38.0	37.7	31.6	34.2	17.7
High (≥7 drinks/wk)	18.5	19.4	12.3	7.3	8.2	2.7
Annual household income, %						
0 to $11,999	6.7	5.6	14.2	17.7	14.9	33.5
$12,000–$24,999	25.4	24.0	34.9	28.0	28.4	25.3
$25,000–$49,999	31.5	32.7	23.6	22.8	24.5	13.1
$50,000 and above	19.9	21.7	7.6	10.8	11.8	5.4
High education (≥12th grade), %	79.3	81.6	63.8	78.9	81.2	65.9
Estrogens, %	n/a	n/a	n/a	18.4	19.2	13.6
Progestins, %	n/a	n/a	n/a	4.3	4.9	0.9

Abbreviations: IQR, interquartile range; n/a, not applicable.

[a]Includes American Indian/Alaskan Native, Asian/Pacific Islander, and other.

[b]Values presented are median (IQR) because of nonnormal distribution.

Table 2. Univariate Statistics and Spearman Correlations for Predictors of Interest With Demographic and Clinical Covariates

	IGF-1 (μg/L)	IGFBP-3 (μg/L)	IGFBP-1 (μg/L)		Ghrelin (pg/mL)	
	Fasting	Fasting	Fasting	2-Hour	Fasting	2-Hour
Descriptive statistics						
n	2265	2265	2240[a]	1894[b]	2257[c]	1895[d]
n missing	3	3	28	374	11	373
Mean	101.2	3644.7	6.4[e]	3.7[e]	592.8	204.4
SD	34.1	843.1	2.4[e]	2.4[e]	462.3	181.4
Median	96.7	3649.0	6.6	3.6	502.1	169.6
25th percentile	78.0	3065.5	3.5	1.9	259.1	66.1
75th percentile	121.0	4162.0	11.8	7.0	825.0	291.4
Spearman correlations						
Fasting IGF-1	1	0.62[f]	−0.41[f]	−0.41[f]	−0.02	0.00
Fasting IGFBP-3	0.62[f]	1	−0.28[f]	−0.30[f]	0.08[f]	0.04
Fasting IGFBP-1	−0.41[f]	−0.28[f]	1	0.95[f]	0.26[f]	0.24[f]
2-Hour IGFBP-1	−0.4[f]	−0.30[f]	0.95[f]	1	0.25[f]	0.25[f]
Fasting ghrelin	−0.02	0.08[f]	0.26[f]	0.25[f]	1	0.87[f]
2-Hour ghrelin	0.00	0.04	0.24[f]	0.25[f]	0.87[f]	1
Age	−0.08[f]	−0.12[f]	0.25[f]	0.27[f]	0.06[f]	0.07[f]
Weight	0.17[f]	0.01	−0.45[f]	−0.42[f]	−0.33[f]	−0.31[f]
Height	0.19[f]	−0.08[f]	−0.15[f]	−0.13[f]	−0.13[f]	−0.12[f]
Body mass index	0.07[f]	0.07[f]	−0.44[f]	−0.42[f]	−0.29[f]	−0.29[f]
Waist circumference	0.09[f]	0.05[f]	−0.40[f]	−0.39[f]	−0.27[f]	−0.28[f]
Systolic blood pressure	−0.01	0.00	0.03	0.06[f]	0.00	0.00
Diastolic blood pressure	0.07[f]	0.03	−0.07[f]	−0.06[f]	−0.05[f]	−0.07[f]
Fasting insulin	0.17[f]	0.14[f]	−0.49[f]	−0.51[f]	−0.33[f]	−0.31[f]
2-Hour insulin	0.10[f]	0.16[f]	−0.40[f]	−0.41[f]	−0.22[f]	−0.25[f]
Fasting glucose	0.10[f]	0.09[f]	−0.25[f]	−0.28[f]	−0.13[f]	−0.15[f]
2-Hour glucose	0.04	0.12[f]	−0.14[f]	−0.16[f]	−0.04	−0.06[f]
Total cholesterol	0.04	0.24[f]	−0.10[f]	−0.09[f]	0.02	0.01
Cystatin C	0.03	−0.06[f]	0.17[f]	0.21[f]	−0.01	0.01
Serum albumin	0.14[f]	0.16[f]	−0.17[f]	−0.20[f]	0.01	−0.02
hs-CRP	−0.17[f]	−0.09[f]	−0.03	−0.03	−0.05[f]	−0.06[f]
Physical activity	0.07[f]	−0.01	−0.03	−0.03	−0.01	0.00
Protein consumption	0.04	−0.02	−0.05[f]	−0.03	−0.01	−0.01
Cigarette pack-year	0.06[f]	−0.02	−0.04	−0.04	0.05[f]	0.04

[a]Includes 2156 measurements of IGFBP-1 above the minimum detectable threshold of 1 μg/L, plus 84 observations below the detectable threshold to which we assigned the value of 1 μg/L.

[b]Includes 1705 measurements of IGFBP-1 above the minimum detectable threshold of 1 μg/L, plus 189 observations below the detectable threshold to which we assigned the value of 1 μg/L.

[c]Includes 2008 measurements of ghrelin, plus 63 extrapolated values in the range of 10 to 80 pg/mL, plus 186 observations below the detectable threshold of 10 pg/mL to which we assigned the value of 10 pg/mL.

[d]Includes 1373 measurements of ghrelin, plus 126 extrapolated values in the range of 10 to 80 pg/mL, plus 396 observations below the detectable threshold of 10 pg/mL to which we assigned the value of 10 pg/mL.

[e]Geometric means (SD) presented for IGFBP-1 measures.

[f]Spearman correlation coefficient significant at $P < 0.05$.

We next examined associations of GH/IGF–related predictors with individual components of the composite outcome. Higher risk of HF was significantly associated with lower IGF-I and IGFBP-3 levels and with higher IGFBP-1 and ghrelin levels (all $P < 0.05$; Table 4). Other associations between GH/IGF biomarkers and individual outcomes did not achieve statistical significance. When we removed HF from the composite outcome, risk of the composite outcome (minus HF) remained statistically significantly associated with ghrelin levels ($P < 0.003$) but not with IGFBP-1 levels (Table 3). Removing stroke, MI, or hip fracture from the composite had no effect on the nature of associations between GH/IGF biomarkers and the composite outcome (Supplemental Table 1), nor did further adjustment for fasting insulin and IGF-I levels (Supplemental Table 2) or 2-hour glucose level (data not shown).

Mortality

The upper quartiles of ghrelin levels in both fasting and 2-hour samples were associated with ~40% higher mortality risk compared with the lowest quartile ($P = 0.005$;

Table 3. Multivariable-Adjusted Hazard Ratios for Composite Outcome and All-Cause Mortality Across Quartiles of Growth Hormone/Insulinlike Growth Factor Function

	Composite Outcome[a]		Composite Outcome Excluding Heart Failure		All-Cause Mortality	
Quartile	Events	HR (95% CI)[b]	Events	HR (95% CI)[b]	Events	HR (95% CI)[b]
IGF-1						
1	279	1.00 (ref)	256	1.00 (ref)	191	1.00 (ref)
2	281	0.96 (0.81, 1.14)	255	0.94 (0.79, 1.13)	188	0.87 (0.71, 1.08)
3	256	0.97 (0.81, 1.16)	229	0.98 (0.81, 1.18)	151	0.80 (0.64, 1.00)
4	271	0.99 (0.83, 1.19)	247	1.03 (0.85, 1.24)	192	1.05 (0.85, 1.31)
1-SD increase		1.00 (0.93, 1.06)		1.02 (0.95, 1.09)		1.03 (0.96, 1.12)
P-continuous		0.8981		0.6436		0.3933
Fasting IGFBP-3						
1	322	1.00 (ref)	292	1.00 (ref)	225	1.00 (ref)
2	264	0.81 (0.68, 0.96)	237	0.82 (0.68, 0.98)	173	0.82 (0.67, 1.00)
3	263	0.92 (0.78, 1.10)	240	0.96 (0.80, 1.15)	171	0.93 (0.75, 1.15)
4	238	0.83 (0.69, 1.00)	218	0.90 (0.74, 1.09)	153	0.89 (0.71, 1.12)
1-SD increase		0.94 (0.88, 1.00)		0.96 (0.90, 1.03)		0.95 (0.88, 1.03)
P-continuous		0.0511		0.2560		0.2364
Fasting IGFBP-1						
1	221	1.00 (ref)	198	1.00 (ref)	128	1.00 (ref)
2	256	1.13 (0.94, 1.36)	231	1.08 (0.88, 1.31)	170	1.22 (0.96, 1.55)
3	276	1.06 (0.88, 1.29)	250	0.98 (0.80, 1.20)	173	0.98 (0.76, 1.25)
4	326	1.24 (1.01, 1.52)	300	1.11 (0.90, 1.39)	247	1.30 (1.00, 1.68)
Twofold increase[c]		1.06 (1.00, 1.12)		1.03 (0.96, 1.09)		1.07 (0.99, 1.16)
P-continuous		0.0722		0.3997		0.0674
2-Hour IGFBP-1						
1	167	1.00 (ref)	150	1.00 (ref)	92	1.00 (ref)
2	205	1.17 (0.95, 1.45)	185	1.15 (0.92, 1.43)	126	1.27 (0.96, 1.68)
3	229	1.24 (1.00, 1.54)	206	1.15 (0.91, 1.45)	143	1.25 (0.94, 1.66)
4	270	1.35 (1.07, 1.69)	246	1.23 (0.96, 1.57)	197	1.50 (1.12, 2.03)
Twofold increase[c]		1.09 (1.02, 1.17)		1.07 (1.00, 1.15)		1.13 (1.04, 1.23)
P-continuous		0.009		0.0598		0.0052
Fasting ghrelin						
1	252	1.00 (ref)	221	1.00 (ref)	159	1.00 (ref)
2	262	1.12 (0.94, 1.34)	241	1.19 (0.99, 1.44)	176	1.21 (0.97, 1.50)
3	281	1.18 (0.99, 1.40)	253	1.22 (1.02, 1.48)	179	1.15 (0.92, 1.43)
4	290	1.27 (1.06, 1.53)	270	1.36 (1.12, 1.65)	207	1.40 (1.12, 1.74)
1-SD increase		1.09 (1.03, 1.16)		1.10 (1.03, 1.18)		1.11 (1.03, 1.20)
P-continuous		0.0062		0.0028		0.0051
2-Hour ghrelin						
1	195	1.00 (ref)	176	1.00 (ref)	125	1.00 (ref)
2	213	1.17 (0.96, 1.43)	191	1.16 (0.94, 1.43)	122	1.08 (0.83, 1.39)
3	216	1.19 (0.97, 1.45)	192	1.18 (0.95, 1.46)	139	1.20 (0.93, 1.54)
4	248	1.39 (1.14, 1.71)	229	1.41 (1.14, 1.75)	173	1.39 (1.09, 1.79)
1-SD increase		1.11 (1.03, 1.18)		1.11 (1.04, 1.20)		1.15 (1.06, 1.24)
P-continuous		0.0039		0.0026		0.0009

[a]Including only the first of such events over up to 8 years' follow-up, the composite outcome encompasses 1088 total incident events, including 178 acute myocardial infarctions, 176 strokes, 233 first-onsets of congestive heart failure without prior or coincident myocardial infarction, 131 hospitalizations for hip fracture, and 376 deaths from causes not listed here.

[b]Models include adjustment for age at baseline, sex, race (indicator of black race), smoking status (indicator of former and current smokers), alcohol consumption, status of general health, hypertension, prehypertension, physical activity measured in kilocalories, protein consumption as caloric intake (measured at year 8), estrogen and progestin hormone consumption, serum albumin concentration, serum cystatin concentration, serum high-sensitivity C-reactive protein concentration, and body mass index.

[c]IGFBP-1 measures were modeled as log2 transformed.

Table 3). Higher IGFBP-1 measured 2 hours after OGTT, but not fasting, was associated with increased mortality ($P = 0.0052$). IGF-I and IGFBP-3 levels were not associated with mortality.

In analyses of cause-specific mortality (Table 5), associations with IGFBP-1 and ghrelin levels tended to be stronger for death due to causes other than cancer and CVD than for CVD-specific mortality or cancer-specific mortality. Higher IGF-I level was significantly associated with higher risk of cardiovascular mortality (HR per 1-SD increase, 1.20; 95% CI: 1.04, 1.37; $P = 0.0108$) but not with other mortality end points.

Joint analyses of IGFBP-1 and ghrelin

When fasting IGFBP-1 and 2-hour IGFBP-1 were both included as independent variables, the association with mortality was significant for 2-hour levels ($P = 0.0244$) but not for fasting levels ($P = 0.8833$; Table 6). Mutually adjusted analyses supported an association between 2-hour ghrelin and the composite outcome ($P = 0.0378$) and mortality ($P = 0.0165$) but no associations for fasting ghrelin ($P > 0.2$).

When we examined the risk associated with combinations of ghrelin and IGFBP-1 levels, highest risks of the primary outcomes were observed among individuals in the upper quartile of both measures and intermediate risk was observed among those in the upper quartile of only one of these measures (Supplemental Table 3).

Subgroup analyses

The associations of fasting ghrelin, fasting IGFBP-1, and 2-hour IGFBP-1 levels with the composite end point tended to be stronger among women than among men ($P_{\text{interaction}} < 0.05$ for ghrelin). We did not find heterogeneity in other associations across categories of sex, race, and BMI.

Analysis of time-varying effects

For fasting levels of IGF-I, IGFBP-1, and IGFBP-3, associations with the primary outcomes were found to vary over time ($P = 0.002$ to 0.029 for interaction with time). During the earliest period of follow-up, low IGF-I and IGFBP-3 levels and high fasting IGFBP-1 level tended to be associated with increased risk of the composite health outcome and death (Supplemental Table 4). Null associations were found during the longer term follow-up period. Also, interaction analyses suggested that the association between fasting IGFBP-3 and the composite outcome was modified by age at baseline (P value for interaction with baseline age, 0.039: among those <80 years old at baseline, HR = 0.89 per 1-SD increment of IGFBP-3, 95% CI: 0.82, 0.97; among those aged 80+ years, HR = 1.02, 95% CI: 0.92, 1.13). Associations of fasting IGFBP-1 tended to wane with time (for composite outcome, $P = 0.049$ for interaction with time; for death, $P = 0.027$). In contrast, associations of 2-hour IGFBP-1, fasting ghrelin, and 2-hour ghrelin with primary outcomes did not vary over time or with baseline age.

Sensitivity analyses

Additional sensitivity analyses excluding participants with impaired fasting glucose and/or impaired glucose tolerance as well as those in which all values below the LLD were assigned to one-half the LLD yielded results similar to those described previously. Analyses that did not consider events occurring in the first year of follow-up also did not appreciably alter the results.

Discussion

Although the GH/IGF signaling pathway has been one of the most highly scrutinized in the field of human longevity research (22), the search for components of the GH/IGF system that most strongly predict disease risks has been elusive. The current study suggests that among older adults, levels of circulating IGFBP-1 and ghrelin during a 2-hour OGTT may predict future risk

Table 4. Multivariable-Adjusted Hazard Ratios for Individual Incident Events Associated With Increased Levels of Growth Hormone/Insulinlike Growth Factor Function Measures

	Acute MI		Stroke		Congestive Heart Failure		Hip Fracture	
Predictor	**HR (95% CI)[a]**	***P***	**HR (95% CI)[a]**	***P***	**HR (95% CI)[a]**	***P***	**HR (95% CI)[a]**	***P***
Fasting IGF-1								
1-SD increase	1.06 (0.92, 1.21)	0.4340	1.06 (0.92, 1.22)	0.4203	0.88 (0.79, 0.99)	0.0393	1.06 (0.90, 1.25)	0.4879
Fasting IGFBP-3								
1-SD increase	0.97 (0.84, 1.12)	0.6746	1.00 (0.86, 1.16)	0.9943	0.84 (0.75, 0.95)	0.0044	1.04 (0.87, 1.24)	0.6849
Fasting IGFBP-1								
Twofold increase[b]	0.93 (0.81, 1.07)	0.3036	1.02 (0.89, 1.17)	0.7630	1.18 (1.06, 1.32)	0.0019	1.03 (0.88, 1.21)	0.6965
2-Hour IGFBP-1								
Twofold increase[b]	0.96 (0.83, 1.12)	0.6420	1.08 (0.93, 1.25)	0.2966	1.23 (1.10, 1.39)	0.0005	1.06 (0.88, 1.26)	0.5565
Fasting ghrelin								
1-SD increase	1.10 (0.96, 1.27)	0.1793	1.11 (0.97, 1.28)	0.1370	1.13 (1.01, 1.26)	0.0361	0.99 (0.84, 1.17)	0.8921
2-Hour ghrelin								
1-SD increase	1.14 (0.97, 1.32)	0.1032	1.12 (0.97, 1.30)	0.1204	1.14 (1.01, 1.28)	0.0324	0.99 (0.82, 1.19)	0.9226

[a]Effect estimates adjusted for age at baseline, sex, race (indicator of black race), smoking status (indicator of former and current smokers), alcohol consumption, status of general health, hypertension, prehypertension, physical activity measured in kilocalories, protein consumption as caloric intake (measured at year 8), estrogen and progestin hormone consumption, serum albumin concentration, serum cystatin concentration, serum high-sensitivity C-reactive protein concentration, and body mass index.

[b]IGFBP-1 measures were modeled as log2 transformed.

Table 5. Multivariable-Adjusted Hazard Ratios for Cause-Specific Mortality Associated With Increased Levels of Growth Hormone/Insulinlike Growth Factor Function Measures

	CV-Specific Mortality		Cancer-Specific Mortality		Noncancer, Non-CV Mortality	
Quartile	Events	HR (95% CI)[a]	Events	HR (95% CI)[a]	Events	HR (95% CI)[a]
IGF-I						
1	41	1.00 (ref)	63	1.00 (ref)	86	1.00 (ref)
2	56	1.24 (0.81, 1.89)	54	0.76 (0.52, 1.11)	78	0.80 (0.58, 1.10)
3	53	1.37 (0.89, 2.11)	32	0.50 (0.32, 0.77)	66	0.78 (0.56, 1.10)
4	63	1.69 (1.10, 2.59)	52	0.84 (0.57, 1.24)	77	0.94 (0.67, 1.32)
1-SD increase	213	1.20 (1.04, 1.37)	201	0.91 (0.78, 1.06)	307	1.02 (0.90, 1.15)
P-continuous		0.0108		0.2155		0.8069
Fasting IGFBP-3						
1	55	1.00 (ref)	66	1.00 (ref)	104	1.00 (ref)
2	53	1.08 (0.73, 1.59)	45	0.75 (0.51, 1.10)	74	0.71 (0.52, 0.97)
3	53	1.15 (0.77, 1.72)	47	0.84 (0.57, 1.25)	71	0.87 (0.63, 1.20)
4	52	1.21 (0.79, 1.84)	43	0.83 (0.54, 1.26)	58	0.74 (0.52, 1.05)
1-SD increase	213	1.10 (0.95, 1.28)	201	0.89 (0.76, 1.03)	307	0.89 (0.78, 1.01)
P-continuous		0.2041		0.1250		0.0724
Fasting IGFBP-1						
1	38	1.00 (ref)	41	1.00 (ref)	49	1.00 (ref)
2	62	1.40 (0.92, 2.12)	46	1.16 (0.75, 1.80)	62	1.13 (0.77, 1.66)
3	47	0.79 (0.50, 1.25)	52	1.17 (0.75, 1.83)	74	1.02 (0.69, 1.50)
4	63	0.91 (0.55, 1.50)	62	1.42 (0.88, 2.29)	121	1.50 (1.00, 2.25)
Twofold increase[b]	210	0.97 (0.85, 1.12)	201	1.09 (0.95, 1.25)	306	1.14 (1.01, 1.28)
P-continuous		0.7226		0.2324		0.0335
2-Hour IGFBP-1						
1	26	1.00 (ref)	31	1.00 (ref)	35	1.00 (ref)
2	45	1.54 (0.93, 2.56)	39	1.26 (0.78, 2.05)	42	1.09 (0.69, 1.73)
3	44	1.27 (0.75, 2.17)	46	1.47 (0.90, 2.40)	53	1.10 (0.69, 1.75)
4	57	1.24 (0.70, 2.21)	56	1.71 (1.02, 2.89)	83	1.55 (0.97, 2.49)
Twofold increase[b]	172	1.06 (0.90, 1.24)	172	1.13 (0.97, 1.31)	213	1.19 (1.04, 1.36)
P-continuous		0.4840		0.1137		0.0135
Fasting ghrelin						
1	38	1.00 (ref)	52	1.00 (ref)	69	1.00 (ref)
2	57	1.68 (1.10, 2.56)	49	0.96 (0.65, 1.43)	70	1.16 (0.83, 1.62)
3	60	1.64 (1.08, 2.51)	42	0.82 (0.54, 1.25)	77	1.16 (0.83, 1.63)
4	57	1.62 (1.04, 2.51)	58	1.17 (0.78, 1.76)	91	1.49 (1.07, 2.09)
1-SD increase	212	1.14 (0.99, 1.32)	201	1.01 (0.87, 1.17)	307	1.18 (1.06, 1.32)
P-continuous		0.0631		0.9057		0.0037
2-Hour ghrelin						
1	34	1.00 (ref)	40	1.00 (ref)	51	1.00 (ref)
2	34	1.19 (0.73, 1.95)	43	1.14 (0.73, 1.77)	45	0.95 (0.63, 1.44)
3	47	1.47 (0.92, 2.33)	40	1.10 (0.70, 1.72)	52	1.11 (0.74, 1.65)
4	57	1.63 (1.03, 2.60)	49	1.21 (0.78, 1.90)	66	1.40 (0.94, 2.08)
1-SD increase	172	1.17 (1.00, 1.35)	172	1.09 (0.94, 1.26)	214	1.18 (1.04, 1.35)
P-continuous		0.0428		0.2433		0.0129

Abbreviation: CV, cardiovascular.

[a]Effect estimates adjusted for age at baseline, sex, race (indicator of black race), smoking status (indicator of former and current smokers), alcohol consumption, status of general health, hypertension, physical activity measured in kilocalories, protein consumption as caloric intake (measured at year 8), estrogen and progestin hormone consumption, serum albumin concentration, serum cystatin concentration, serum high-sensitivity C-reactive protein concentration, and body mass index.

[b]IGFBP-1 measures were modeled as log2 transformed.

of mortality and major health events including MI, stroke, HF, and hip fracture. During follow-up, approximately half of the individuals studied, who were aged 78 years at baseline, experienced the composite outcome, which was designed to capture multiple conditions previously linked with the GH/IGF system. Significant associations of health outcomes and mortality with circulating IGFBP-1 and ghrelin levels persisted after adjustment for an extensive set of demographic, clinical, and biochemical variables. Although prior evidence has documented that low IGF-I and IGFBP-3 levels are also risk factors for mortality (1), in this study we found the ability of IGF-I and IGFBP-3 levels to predict mortality was modest and became weaker with advancing age.

Table 6. Associations of Ghrelin and IGFBP-1 With the Composite Outcome and All-Cause Mortality While Including Fasting and 2-Hour Measures in Models Simultaneously

	Composite Outcome		All-Cause Mortality	
Quartile	**Cases**	**HR (95% CI)[a]**	**Cases**	**HR (95% CI)[a]**
Fasting IGFBP-1				
1	181	1.00 (ref)	102	1.00 (ref)
2	199	0.94 (0.70, 1.27)	127	0.93 (0.64, 1.37)
3	221	0.83 (0.57, 1.22)	133	0.64 (0.39, 1.05)
4	262	1.01 (0.64, 1.59)	192	0.81 (0.45, 1.44)
Twofold increase[b]		0.97 (0.85, 1.10)		1.01 (0.86, 1.19)
P-continuous		0.6324		0.8833
2-Hour IGFBP-1				
1	165	1.00 (ref)	90	1.00 (ref)
2	204	1.23 (0.92, 1.65)	126	1.40 (0.95, 2.06)
3	228	1.39 (0.94, 2.03)	142	1.75 (1.06, 2.89)
4	266	1.33 (0.84, 2.10)	196	1.92 (1.07, 3.46)
Twofold increase[b]		1.08 (0.95, 1.23)		1.19 (1.02, 1.39)
P-continuous		0.2342		0.0244
Fasting ghrelin				
1	200	1.00 (ref)	120	1.00 (ref)
2	217	1.13 (0.90, 1.42)	139	1.26 (0.95, 1.69)
3	222	1.08 (0.82, 1.43)	139	1.15 (0.80, 1.63)
4	230	1.04 (0.76, 1.44)	159	1.22 (0.81, 1.82)
1-SD increase		1.03 (0.92, 1.16)		1.08 (0.95, 1.24)
P-continuous		0.5603		0.2422
2-Hour ghrelin				
1	194	1.00 (ref)	124	1.00 (ref)
2	212	1.17 (0.92, 1.47)	122	1.02 (0.75, 1.37)
3	215	1.12 (0.85, 1.48)	138	1.09 (0.77, 1.55)
4	248	1.42 (1.04, 1.95)	173	1.29 (0.87, 1.91)
1-SD increase		1.11 (1.01, 1.22)		1.15 (1.03, 1.30)
P-continuous		0.0378		0.0165

[a]Models include adjustment for age at baseline, sex, race (indicator of black race), smoking status (indicator of former and current smokers), alcohol consumption, status of general health, hypertension, prehypertension, physical activity measured in kilocalories, protein consumption as caloric intake (measured at year 8), estrogen and progestin hormone consumption, serum albumin concentration, serum cystatin concentration, serum high-sensitivity C-reactive protein concentration, and body mass index, as well as mutual adjustment for dummy variables for quartiles of fasting or 2-hour measure of each analyte, as appropriate.

[b]IGFBP-1 measures were modeled as log2 transformed.

IGFBP-1 is an inhibitor of IGF-I in humans that regulates IGF-I activity under varying conditions of food supply (23). Conditions of cachexia, malnutrition, inflammation, and trauma give rise to dramatic increases in circulating IGFBP-1 levels along with decreasing levels and bioactivity of IGF-I (24, 25). IGFBP-1 levels increase with short-term fasting and decrease rapidly after intake of a mixed meal or glucose load (12). Prior studies have implicated fasting IGFBP-1 level as a risk factor for CVD, disability, and mortality among older adults (15, 26, 27). We obtained a measurement of IGFBP-1 at 2 hours after ingestion of 75 g of glucose, which proved to be even more strongly associated with future adverse health events compared with fasting IGFBP-1 levels. In contrast to 2-hour measurements, fasting IGFBP-1 levels were not consistently associated with outcomes, especially after 2-hour levels were taken into account. Several potential mechanisms may explain the association of high IGFBP-1 levels with adverse health events. High IGFBP-1 level is associated with reduced activity of IGF-I, which in turn may reduce cellular proliferation and increase apoptosis, thereby producing loss of tissue mass and impairing tissue regeneration and repair (28). Lack of suppression of IGFBP-1 levels during the OGTT may reflect impaired hepatic function or degree of hepatic insulin resistance, and IGFBP-1 levels during OGTT correlate closely with fasting insulin and first-phase insulin secretion (29). Nevertheless, results were unaffected by considering glucose or insulin levels or diabetes status. It also may be important that postprandial levels of nutrition-sensitive biomarkers such as IGFBP-1 reflect typical circulating levels more accurately than levels measured under an imposed fast. This might explain the better predictive power of postload compared with fasting measurements.

Ghrelin, the endogenous ligand for the GH secretagogue receptor, is a key regulator of GH secretion, likely having both direct and indirect metabolic effects (30).

Primarily produced in the fundus of the stomach, circulating ghrelin responds markedly to food intake and appears to affect appetite and satiety. Several prior studies, mainly of cross-sectional design, have related ghrelin levels with risk of hypertension, hyperlipidemia, metabolic syndrome, vascular events, and bone health, albeit with inconsistent results (8, 31–34). We report one of the few studies to examine prospective associations of ghrelin with incident events in the older adult population. The response of ghrelin levels to feeding is diminished among older adults, who have a diminished suppression and recovery of ghrelin levels, and frail elderly have no ghrelin inhibition with food intake (35). This supports our findings attributing an adverse prognostic significance to high postload ghrelin level. A limitation of our approach was the large number of individuals with ghrelin levels below the lower assay limit of detection, whereas a more sensitive assay may provide greater ability to stratify health risks in the undetectable group.

Given our observational study design, which used measurements of GH/IGF in circulation rather than in other tissues, it remains a matter of speculation what mechanisms may link GH/IGF biomarkers with health events. We previously found that with advancing age, changes in circulating levels of IGF-system proteins (*e.g.*, decreasing IGF-I and rising IGFBP-1) were independent of concurrent changes in inflammatory, lipid, and sex hormone biomarkers (36). Dysregulation of the GH/IGF system in older persons may therefore be an aspect of aging that is distinct from changes in other physiological domains. Dampening of GH/IGF–mediated biological functions would potentially have broadly detrimental effects on tissue repair, maintenance of cellularity, nutrition, metabolism, and other fundamental processes that are mediated by this axis.

As expected, each GH/IGF biomarker measured in this study was correlated with multiple metabolic disease markers, including obesity and glucose, and diastolic BP level, although our main results persisted after adjustment for these covariates. HF risk was associated with all of the GH/IGF biomarkers examined, thus confirming the importance of GH/IGF in cardiac dysfunction (37). GH deficiency is common in patients with HF, who respond favorably to GH replacement (38). Mechanisms that have been proposed to explain the role of GH and IGF-I in the pathogenesis of HF, including endocrine system effects, nitric oxide production, abnormal vascular tone, and metabolic processes of cardiac cells, might also affect other organ systems and disease risks, thus explaining the associations in this study with non-HF end points (39). Moreover, it will be important to gain a better understanding of non-IGF-I–dependent cellular effects of IGFBP-1 to assess whether IGFBP-1 may itself be a disease mediator and therapeutic target (40).

Strengths of this study included the extensively characterized CHS cohort, which included multiple GH/IGF proteins; long-term disease outcomes; and other variables that might have confounded the associations of interest. Results should be generalized cautiously beyond the population studied, namely individuals who reached late life free of major CVD events and diabetes. Although we confirmed that low IGF-I and IGFBP-3 levels were associated with higher mortality, this association did not persist into the late period of follow-up, at which time the average age of participants exceeded 80 years. On the other hand, high IGFBP-1 and ghrelin levels continued to predict adverse outcomes over time.

In summary, since it became known that the GH/IGF axis affects survival and fitness in a range of simple and complex organisms, studies have sought to identify the potential relationship between the GH/IGF axis and health and disease in humans. We conclude that levels of IGFBP-1 and ghrelin, particularly after an OGTT, could serve as biomarkers for increased risk of diseases plausibly associated with the GH/IGF axis.

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A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

Address all correspondence and requests for reprints to: Robert Kaplan, PhD, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Belfer Building, Room 13015, Bronx, New York 10461. E-mail: robert.kaplan@einstein.yu.edu.

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