Title: Association of total IGF-I, IGFBP-1 and IGFBP-3 levels with incident coronary events and ischemic stroke

Authors: Robert C Kaplan PhD, Aileen P McGinn PhD, Michael N Pollak MD, Lewis H Kuller MD DrPH, Howard D Strickler MD, MPH, Tom E Rohan MD PhD, Anne R Cappola, MD, ScM, XiaoNan Xue PhD, Bruce M Psaty MD PHD

Institutional affiliations: Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY (RCK, APM, HDS, TER, XX); Cancer Prevention Research Unit, Departments of Medicine and Oncology, Lady Davis Research Institute of Jewish General Hospital and McGill University, Montreal, Quebec, Canada; (MNP); Department of Medicine and Epidemiology, University of Pittsburgh, Pittsburgh, PA (LHK); Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia (ARC); Departments of Epidemiology, Medicine and Health Services, Cardiovascular Health Research Unit, University of Washington, Seattle (BMP)

Short Title: IGF and incident CVD events

Keywords: insulin like growth factor, myocardial infarction, stroke, epidemiology

Revised Word count: 3,501

Revised abstract word count:250

Tables and Figures: 2

References: 37

Corresponding author: Robert C. Kaplan, PhD

Department of Epidemiology and Population Health

Albert Einstein College of Medicine

1300 Morris Park Avenue   Belfer 1306C

Bronx, NY 10461

Phone 718-430-4076 Fax 718-430-8780

Email: rkaplan@aecom.yu.edu

Abstract

Context: Prior observational studies have demonstrated that the growth hormone/insulin like-growth factor (GH/IGF) axis is associated with cardiovascular disease. However, this association has not been extensively studied among older adults.

Objective: To assess the association between levels of total IGF-I, IGF binding proteins (IGFBP-1, IGFBP-3) and risk of incident coronary events and ischemic stroke

Design and Participants: A case cohort analysis was conducted among adults >=65 years old in the Cardiovascular Health Study (CHS).

Main Outcome Measures: 534 coronary events (316 nonfatal MI's, 48 fatal MI's and 170 fatal CHD events), and 370 ischemic strokes were identified on follow-up. Comparison subjects were 1,122 randomly-selected participants from CHS.

Results: Mean follow-up time (years) was 6.7 for coronary events, 5.6 for strokes and 9.3 for comparison subjects. Hazard ratios (HRs) [95% confidence intervals] associated with baseline levels of total IGF-I and IGFBPs were estimated using multivariate adjusted Cox proportional hazards models. Neither IGF-I nor IGFBP-1 levels predicted risk of incident coronary events or stroke. IGFBP-3 had an inverse association with risk of coronary events (adjusted HR per standard deviation=0.88 [0.78-1.00], p=0.05), but was not associated with stroke. Exploratory analyses suggested that low IGF-I and low IGFBP-3 levels were significantly associated with higher risk of nonfatal MI (p<0.05), but not with risk of fatal MI or fatal CHD.

Conclusion: Circulating levels of total IGF-I or IGFBP-1 were not associated with risk of total coronary events or ischemic stroke among older adults, while low IGFBP-3 level was associated with increased risk of incident coronary events.

Key Words: myocardial infarction, stroke, epidemiology, insulin-like growth factor

Insulin-like growth factor-I (IGF-I), a central mediator of many of the effects of growth hormone (GH), is a cell survival and growth factor. IGF-I has substantial homology to proinsulin and has insulin-like metabolic effects [1]. IGF-I plays a critical role in the regulation of cell cycle, with mitogenic effects, and is an inhibitor of apoptosis and necrosis [2]. IGF-I may improve glucose metabolism through feedback inhibition of GH secretion, or via direct effects mediated through interactions of IGF-I with the IGF-I receptor (IGF-IR) or the hybrid IGF-I/insulin receptor. Several potential protective mechanisms of IGF-I on vascular disease processes have been described. Experimental infarction models suggest that IGF-I may promote survival of myocytes exposed to ischemic injury, in part by enhancing glucose uptake [3, 4]. Tumor necrosis factor alpha reduces IGF-I and increases IGF binding protein-3 (IGFBP-3) in vascular smooth muscle cells (VSMCs) [5]. This may in turn decrease VSMC survival in atherosclerotic plaques and promote plaque rupture. IGF-I has also been identified as a neuroprotectant agent [6], suggesting a possible protective association with risk of brain infarction.

In apparently healthy individuals, circulating levels of IGF-I and IGFBP-3 peak during puberty and thereafter decline with age, while IGFBP-1 levels increase with aging [7]. Several observations suggest that these age-related hormonal changes may influence the risk of cardiovascular disease (CVD) among older adults [8]. GH-deficient adults, who have low circulating IGF-I levels, are at high risk of CVD mortality [9] and have increased carotid artery wall thickness and endothelial dysfunction, which are partially reversed by GH replacement [10]. Several population-based prospective studies have suggested that low circulating levels of IGF-I within the normal range may predict increased risk of ischemic heart disease [11, 12] and ischemic stroke [13]. IGFBP-3 levels have been both directly and inversely associated with

prevalent and incident CVD [11, 13-17]. Low IGF-I levels may adversely affect the risk of

developing insulin resistance and related macrovascular complications [18].   Data on IGF levels

and CVD events among older adults are limited, however, as several of the prior studies have

been conducted among middle-aged populations [11, 13, 18]. Moreover, some previous studies

have had small sample size and have often identified CVD events through databases without

medical record review [12]. Because of the increasing off-label use of growth hormone for the

prevention of age-related conditions, it is critical to have an accurate assessment of the

association between low circulating IGF-I levels and cardiovascular disease risk among older

adults [19].

We assessed the association between baseline levels of total IGF-I, IGFBP-1, and IGFBP-3 and

risk of confirmed incident coronary events (including nonfatal myocardial infarction [MI], fatal

MI, and fatal CHD), ischemic stroke, and prevalent subclinical vascular disease, in a case-cohort

study among older adults (>=65 years) participating in the Cardiovascular Health Study (CHS).

METHODS

Study population   CHS is a prospective population-based cohort study of CVD in adults 65

years and older living in 4 US communities [20]. The original cohort of 5,201 participants was

recruited in 1989-1990. In 1992-1993, 687 additional participants were recruited, almost all of

whom were African-American, in order to enhance the racial/ethnic diversity of the cohort.

Potential subjects were identified from Medicare eligibility lists of the Health Care Financing

Administration (HCFA). Those eligible to participate included all persons living in the

household of each individual sampled, who: 1) were 65 or older at the time of examination; 2) expected to remain in the area for 3 years; and 3) were able to give informed consent.

Study visits CHS participants completed standardized clinic examinations and questionnaires at study baseline and at annual follow-up clinic visits. Retention through the last (ninth) visit was 95%. Data collection included an assessment of medical history, use of prescription medications, health-related behaviors, and demographic and socioeconomic factors. Physical exams included repeat right-arm blood pressures (BPs), ankle and brachial BPs obtained using a Doppler stethoscope [21], ultrasound of the carotid arteries [22], and twelve-lead resting electrocardiograms (ECGs). Fasting lipids, glucose, and other laboratory measurements were obtained on all subjects at the baseline visit and selected followup visits.

Information on CVD events Baseline history of MI, stroke, and congestive heart failure was elicited via interview. For MI and heart failure, medical records were then obtained and reviewed to confirm the accuracy of self-reported baseline history. In addition, if during the course of the study, information was obtained suggesting the occurrence of a incident CVD event prior to baseline, the study data on baseline history of CVD was updated to reflect this information. All incident CVD events and deaths during follow-up were identified through semi-annual participant contacts, notification of events by participants, and periodic searches of national administrative databases (e.g., HCFA Medicare Utilization database, National Death Index). Medical records for all deaths and CVD events were centrally reviewed and classified by events committees according to CHS criteria as described by Ives et al [23]. Incident coronary events included 1) non-fatal MIs (with symptoms, electrocardiographic findings, and enzymes

used to validate the presence of an MI), 2) fatal MIs, and 3) deaths that did not meet the predefined criteria for MI but were attributed to coronary heart disease after medical records review ("fatal CHD"). Strokes were validated by neurologist review. Neuroimaging studies were available for 86% of suspected transient ischemic attacks (TIAs) and strokes. Classification of major stroke types (ie, ischemic or hemorrhagic) and etiologic subtypes of ischemic stroke was performed using criteria adopted from the SHEP study[24]. A reliability study found kappa=0.86 for stroke versus no stroke, kappa=1.0 for ischemic versus hemorrhagic stroke, and kappa=0.77 for definition of ischemic stroke subtypes, suggesting substantial reliability for classification of stroke[25].

Selection of subjects for case cohort study   We selected the case groups for the present investigation of IGFs using CHS followup data through June 30, 2001. We identified all subjects who had a confirmed incident coronary event (including nonfatal MI, fatal MI, or fatal CHD) or incident ischemic stroke. In addition, a random sample of 1,122 CHS participants was selected from the baseline study population, without regard to subsequent case status, to serve as a shared comparison group for both coronary and stroke case groups (i.e., the random subcohort). Subjects who had a history of MI, stroke, or congestive heart failure at baseline were excluded from both case groups and the random subcohort.

Laboratory methods   Total IGF-I, IGFBP-1, and IGFBP-3 were measured using enzyme-linked immunosorbent assay methods (Diagnostics Systems Laboratory, Webster, TX) in fasting baseline plasma specimens that had been maintained at -70C storage, as previously described [26]. Within-batch and between-batch coefficients of variation (CVs) derived from a control

pool of 214 participants from the present study were 6.9% and 6.0% for IGF-I, 3.5% and 3.1%

for IGFBP-1, and 6.0% and 3.6% for IGFBP-3. While we obtained only single baseline IGF

measurements on most subjects, for 249 randomly-chosen subjects, we conducted repeat

measurements to assess the within-person stability of IGF levels between baseline and year 3.

The correlations were 0.83 for IGF-I and 0.83 for IGFBP-3, similar to those previously reported

[26], suggesting that levels remain correlated within individuals over 3 years. For n=50

individuals, we replicated assays at our laboratory and at two outside laboratories; between-

laboratory correlations for IGF-I levels were r =0.95-0.97.

Statistical methods In cross-sectional analyses conducted among subcohort members, we

computed Pearson's correlations between IGF levels and subclinical CVD measures including

ankle-arm blood pressure index (ABI), common and internal carotid artery intima-media

thickness (IMT), after adjustment for previously-identified predictors [21, 22]. Cox proportional

hazards regression was used in time-to-event analyses to estimate hazard ratios (HRs) and 95%

confidence intervals (CIs) for the associations of IGF levels with incident coronary events and

ischemic stroke, using the Self and Prentice estimators as described by Therneau and Li [27]. In

addition to analyses of total coronary events, we also present separate analyses of the three

components of this endpoint: nonfatal MI, fatal MI and fatal CHD. We rescaled IGF levels

according to standard deviation (SD) units, and present HRs, CIs, and p-values per SD of IGF

variables modeled as linear predictors. We also present results by tertiles to give a more detailed

assessment of associations between IGFs and outcomes. Further analyses used up to 8 categories

to examine for threshold effects, and we also modeled log-transformed data and linear-plus-

quadratic models to assess non-linear associations. Models were adjusted for vascular risk

factors identified in previous CHS analyses [28, 29]: age, sex, treated hypertension (self-reported history plus antihypertensive medication), systolic BP, smoking, creatinine, high density lipoprotein (HDL) cholesterol and race/ethnicity. Using a 10% change in effect estimate criteria, we found that results were affected little with additional adjustment for marital status, education, general health status, annual income, diastolic BP, alcohol use, physical activity level, lipid-lowering medications, total cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, fibrinogen, white blood cell count and C-reactive protein (CRP). We chose *a priori* not to adjust for diabetes (defined as insulin or oral hypoglycemic therapy, or fasting glucose >=126 mg/dl), impaired fasting glucose (IFG) status (fasting glucose 110-125 mg/dl), diabetes medication (oral drugs and insulin), fasting glucose and insulin levels, body mass index (BMI), or waist circumference in our primary models because prior data suggest that glucose intolerance may mediate possible effects of IGF-I or IGFBP levels on risk of CVD events [18]. However, adjustment of the final models for these variables did not alter the study findings. Using subgroup analyses and modeling of interaction terms, we examined whether the association between IGF levels and events differed by age (<70, 70-75, >75), race, sex, CRP, diabetes/IFG, general health status, BMI, and subclinical CVD (defined as ABI<0.9, internal carotid artery IMT>80th percentile, common carotid artery IMT>80th percentile, carotid stenosis>25%, major ECG abnormalities, angina, or claudication) [30]. Sensitivity analyses confirmed that results were similar after stratifying by follow-up time. We did not detect evidence of violations of the assumption of proportional hazards. Data were missing for 2% or fewer of subjects for all variables except income (missing =6%), and hot-deck methods were used to impute missing values.

RESULTS

Subject characteristics The present analyses included 534 CHS subjects with incident coronary events (316 nonfatal MIs, 48 fatal MIs, and 170 fatal CHD events), 370 subjects with incident ischemic stroke, and 1,122 subjects in the randomly-selected subcohort (Table 1). Mean (median, maximum) follow-up time was 6.7 (7.0, 11.9) years for subjects who sustained coronary events, 5.6 (5.9, 11.9) years for subjects who sustained strokes, and 9.3 (11.1, 12,1) years for controls. Mean age at baseline (range) was 73.7 (65-94) among coronary event cases, 74.1 (65-93) among ischemic stroke cases and 72.4 (64-92) among subcohort members. Seventy-seven subjects were included in this study as both a coronary event and as a stroke case.

Correlations were r =-0.23 (p<0.0001) between total IGF-I and IGFBP-1; r = 0.62 (p<0.0001) between total IGF-I and IGFBP-3; and r =-0.14 (p<0.0001) between IGFBP-1 and IGFBP-3. Age had an inverse correlation with IGF-I (r =-0.11, p<0.001) and IGFBP-3 (r =-0.17, p<0.001) and a direct correlation with IGFBP-1 (r =0.20, p<0.001).

IGF levels and subclinical CVD In cross-sectional analyses, after adjustment for age, sex, race, diabetes, smoking, systolic blood pressure, low density lipoprotein cholesterol, high density lipoprotein cholesterol, body mass index, C-reactive protein, hypertension, fibrinogen and creatinine, low IGF-I and IGFBP-3 levels were independently correlated with lower ABI (IGF-I, r = 0.10, p<0.01; IGFBP-3, r = 0.08, p<0.01). Low IGFBP-3 levels were correlated with higher common and internal carotid IMT in unadjusted analyses, but not in adjusted analyses.

IGF levels and incident coronary events   No association was present between level of total IGF-I and risk of incident coronary events (nonfatal MI, fatal MI, or fatal CHD) (Table 2).   In age- and sex-adjusted analyses, low IGFBP-3 level was significantly (p=0.02) associated with increased risk of coronary events. This finding remained significant (p=0.05) in multivariate-adjusted analyses; compared with subjects in the first (lowest) IGFBP-3 tertile, the adjusted HR (95% CI) was 0.69 (0.53, 0.90) for subjects in the second IGFBP-3 tertile and 0.80 (0.61, 1.05) for subjects in the third IGFBP-3 tertile. IGFBP-1 level, IGF-I:IGFBP-1 molar ratio, or IGF-I:IGFBP-3 molar ratio were not associated with risk of coronary events.

IGF levels and fatal vs. nonfatal coronary events   We repeated the analyses after subclassifying coronary endpoints according into the three component types of events: nonfatal MI, fatal MI and fatal CHD. Lower IGF-I level was a significant predictor of nonfatal MI (p=0.04). Compared with the lowest IGF-I tertile, the adjusted HR (95% CI) for nonfatal MI was 0.88 (0.63, 1.23) for the second IGF-I tertile and 0.69 (0.48, 0.99) for the third IGF-I tertile. In contrast, IGF-I did not predict fatal MI (versus tertile 1, HR [95% CI] =0.80 [0.34, 1.93] for tertile 2 and 1.10 [0.48, 2.51] for tertile 3, p=0.28) or fatal CHD (versus tertile 1, HR [95% CI] =1.02 [0.64, 1.62] for tertile 2 and 1.00 [0.62, 1.61] for tertile 3, p=0.67). Similarly, for IGFBP-3, analyses suggested a significant association with nonfatal MI (versus tertile 1, HR [95% CI] =0.60 [0.43, 0.84] for tertile 2 and 0.64 [0.45, 0.91] for tertile 3, p<0.01), but no association with fatal MI or fatal CHD. IGFBP-1 levels and IGF-I:IGFBP molar ratios were not associated with either nonfatal MI, fatal MI or fatal CHD.

IGF levels and incident stroke   IGF-I, IGFBP-1, IGFBP-3, and IGF-I:IGFBP molar ratios were not significantly associated with ischemic stroke. The adjusted HR of stroke was 0.99 (95% CI=0.87, 1.12, p=0.88) per SD of IGF-I level, 0.96 (95% CI=0.84, 1.10, p=0.84) per SD of IGFBP-1 level, and 0.95 (95% CI=0.83, 1.09, p=0.45) per SD of IGFBP-3 level.

Subgroup analyses Associations between IGF-I or IGFBPs and CVD events were similar across subgroups of age, sex, CRP, diabetes/IFG, general health status, BMI, and subclinical CVD (all p for interaction >0.01). Additionally, results were similar when analyses were limited to white, non-Hispanic subjects, although the number of events other race/ethnicity subgroups were too small to make any subgroup-specific analyses.

DISCUSSION

This large prospective study suggested that total IGF-I level did not predict incident coronary events or ischemic stroke among men and women 65 years of age and older. In exploratory findings that examined components of the endpoint of coronary events, we found that low IGF-I levels were significantly associated with higher risk of nonfatal MI, but not with risk of fatal MI or fatal CHD. Subjects with low IGFBP-3 levels had higher risk of incident coronary events. As was found for IGF-I, further analyses suggested that the association with IGFBP-3 was present for nonfatal MI but not fatal CHD or fatal MI. Low IGFBP-3 level and IGF-I level were also independently associated with low ABI in cross-sectional analyses. IGFBP-3 level was not associated with risk of incident stroke, and IGFBP-1 did not predict incident coronary events or stroke.

Prior population-based studies have identified low total IGF-I level as an independent vascular

risk factor. In the Rancho Bernardo cohort, the risk of death from ischemic heart disease was

increased by 38% for every 40 ng/ml decrease in baseline IGF-I level (95% CI = 9%, 76%

increase in risk, p=0.005) [12]. A nested case-control study from the Dan-MONICA (Danish

multinational MONItoring of trends and determinants in CArdiovascular disease) cohort found

relative risks for incident ischemic heart disease of approximately 2 comparing the lowest and

highest quartiles of IGF-I [11]. Another nested case-control study of Danish men and women,

the Diet, Cancer and Health study, found that those in the bottom quartile of IGF-I levels were at

increased risk of incident ischemic stroke compared to those in the top quartile (odds ratio =2.06,

95% CI=1.05, 4.03) [13]. Given these prior observations, the lack of an overall association

between IGF-I level and incident CVD events in our study was unexpected.

Exploratory analyses suggested that associations between IGF-I level and coronary events

differed according to whether events were fatal or nonfatal. Low IGF-I level was a significant

predictor of nonfatal MI, which was consistent with our initial hypothesis. In contrast, IGF-I

level was not associated with fatal MI or fatal CHD. This suggests that maintaining high IGF-I

levels among aging adults may have favorable effects on the pathophysiology of nonfatal

atherothrombotic events, possibly by enhancing myocardial glucose uptake, tolerance of

myocytes to ischemic insult, or VSMCs survival [3-6], but may also have other effects that

increase risk of coronary death. Pro-arrhythmic effects of IGF-I may be the mechanism

responsible for this putative adverse effect. In patients with dilated cardiomyopathy, GH

treatment has been associated with worsening ventricular arrhythmia [31], although this effect of

GH treatment has not been seen in GH-deficient adults without preexisting heart disease [32].

Effects of IGF-I on cardiac arrhythmia in vulnerable patients may in turn promote sudden cardiac death and other fatal coronary events, counterbalancing any favorable effects of high IGF-I on atherothrombosis or survival of myocytes exposed to ischemia. It is also possible that these exploratory analyses may represent a chance finding.

In this study, subjects with low IGFBP-3 level tended to have increased risk of incident coronary events. As with IGF-I, this association was present for nonfatal MI but not for fatal MI or fatal CHD. Low IGFBP-3 level was also correlated with markers of prevalent subclinical CVD. Some prior data are consistent with these findings, although studies relating to IGFBP-3 and CVD have been conflicting. Low IGFBP-3 was identified as an independent predictor of more severe coronary atherosclerosis in an angiographic series of German men <70 years old [14]. The Diet, Cancer, and Health study reported that subjects in the bottom IGFBP-3 quartile had an odds ratio of 2.29 (95% CI = 1.17, 4.49) for incident ischemic stroke compared with those in the highest quartile [13]. In contrast, low IGFBP-3 levels predicted decreased rather than increased risk of ischemic heart disease events among participants in the Dan-MONICA study [11]. A study of elderly Dutch men reported no association between IGFBP-3 level and carotid IMT [15], while two studies of Japanese middle-aged men reported an association between high IGFBP-3 and subclinical carotid disease [16, 17].

IGF-I bioactivity is determined by the interplay among IGF-I, IGFBPs, and IGFBP proteases [2]. IGFBP-3 may either potentiate or inhibit IGF-I effects. In plasma, IGF-I binding to IGFBP-3 prolongs the half-life of circulating IGF-I and blocks interactions of IGF-I with IGF-IR. At the tissue level, binding of IGFBP-3 to cells reduces affinity of IGFBP-3 for IGF-I, permitting

interactions between IGF-I and its receptors [33]. IGFBP-3 may also have IGF-I-independent

effects on glucose metabolism (inhibition of insulin-stimulated glucose uptake in adipocytes)

[34] or cell survival (antiproliferative and proapoptotic effects) [35, 36]. The conflicting results

from the present and prior studies [11, 13-17] are consistent with a complex relationship between

IGFBP-3 and risk of vascular disease. IGF-I and IGFBPs are expressed ubiquitously in tissues

including myocardium, endothelium, and VSMCs. Circulating levels may not reflect IGF effects

mediated through autocrine or paracrine mechanisms. This is a limitation of the present study.

Novel laboratory techniques for measuring IGF-I and IGFBPs [15, 37-39] may better capture the

relevant biological effects of the IGF system than those employed here.

This study is the largest prospective investigation to date on IGF levels and CVD events.

Included were 534 incident coronary events, 370 incident strokes, and over 1100 comparison

subjects. Prior studies have all included approximately 250 or fewer vascular events [11-13].

While all coronary events and strokes in this study were identified through several sources of

information (including self-report and databases containing discharge diagnoses), and later

confirmed by medical record review, previous studies of IGFs have relied upon hospital

discharge databases or death certificates to identify vascular events [11, 12]. Previously-studied

populations have also had younger age distributions compared with the present study. All of our

CHS study participants were 65 years or older upon enrollment. The Dan-MONICA [11] and

Diet, Cancer and Health cohorts [13] consisted of middle-aged populations (baseline age 30-60

years old, and 50-64 years old, respectively). Because IGF-I and IGFBP-3 levels decline with

aging, the associations with CVD risk may be age-dependent. While our study showed that IGF

levels were highly correlated within individuals over 3 years (r =0.83), it is unclear whether

levels among older individuals accurately reflect IGF activity during the development of

atherosclerosis in earlier life. Like our CHS study, the Rancho Bernardo cohort included elderly

adults (mean age 74 years), although there were several notable methodological differences. IGF

levels were assessed in relation to ischemic heart disease mortality but not nonfatal CVD in

Rancho Bernardo, and events were identified from death certificates rather than medical record

review.

In summary, in this prospective study of older adults, total IGF-I levels and IGFBP-1 levels did

not predict risk of incident coronary events or ischemic stroke. Exploratory analyses that

examined individual components of the endpoint of incident coronary events suggested that low

IGF-I level predicted higher risk of nonfatal MI, but was unassociated with fatal MI or fatal

CHD. Low IGFBP-3 level appeared to correlate with subclinical vascular disease and higher

risk of incident coronary events, particularly nonfatal MI.   Whether the age-associated changes

in circulating total IGF-I and IGFBP levels have an appreciable impact on vascular disease risk

among older adults remains uncertain.

References

1. Thrailkill, K.M., T. Quattrin, L. Baker, J.E. Kuntze, P.G. Compton, and P.M. Martha, Jr. Cotherapy with recombinant human insulin-like growth factor I and insulin improves glycemic control in type 1 diabetes. RhIGF-I in IDDM Study Group. *Diabetes Care* 1999; 22: 585-592.
2. Le Roith, D. Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. *N Engl J Med* 1997; 336: 633-640.
3. Friehs, I., C. Stamm, H. Cao-Danh, F.X. McGowan, and P.J. del Nido. Insulin-like growth factor-1 improves postischemic recovery in hypertrophied hearts. *Ann Thorac Surg* 2001; 72: 1650-1656.
4. Li, B., M. Setoguchi, X. Wang, A.M. Andreoli, A. Leri, A. Malhotra, J. Kajstura, and P. Anversa. Insulin-like growth factor-1 attenuates the detrimental impact of nonocclusive coronary artery constriction on the heart. *Circ Res* 1999; 84: 1007-1019.
5. Anwar, A. Tumor Necrosis Factor-a Regulates Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor Binding Protein-3 Expression in Vascular Smooth Muscle. *Circulation* 2002; 105: 1220-1225.
6. Digicaylioglu, M., G. Garden, S. Timberlake, L. Fletcher, and S.A. Lipton. Acute neuroprotective synergy of erythropoietin and insulin-like growth factor I. *Proc Natl Acad Sci U S A* 2004; 101: 9855-9860.
7. Donahue, L.R., S.J. Hunter, A.P. Sherblom, and C. Rosen. Age-related changes in serum insulin-like growth factor-binding proteins in women. *J Clin Endocrinol Metab* 1990; 71: 575-579.
8. Kaplan, R.C., H.D. Strickler, T.E. Rohan, R. Muzumdar, and D.L. Brown. Insulin-like growth factors and coronary heart disease. *Cardiol Rev* 2005; 13: 35-39.
9. Rosen, T. and B.A. Bengtsson. Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet* 1990; 336: 285-288.
10. Pfeifer, M., R. Verhovec, B. Zizek, J. Prezelj, P. Poredos, and R.N. Clayton. Growth hormone (GH) treatment reverses early atherosclerotic changes in GH-deficient adults. *J Clin Endocrinol Metab* 1999; 84: 453-457.
11. Juul, A., T. Scheike, M. Davidsen, J. Gyllenborg, and T. Jorgensen. Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation* 2002; 106: 939-944.
12. Laughlin, G.A., E. Barrett-Connor, M.H. Criqui, and D. Kritz-Silverstein. The prospective association of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-1 levels with all cause and cardiovascular disease mortality in older adults: the Rancho Bernardo Study. *J Clin Endocrinol Metab* 2004; 89: 114-120.
13. Johnsen, S.P., H.H. Hundborg, H.T. Sorensen, H. Orskov, A. Tjonneland, K. Overvad, and J.O. Jorgensen. Insulin-like growth factor (IGF) I, -II, and IGF binding protein-3 and risk of ischemic stroke. *J Clin Endocrinol Metab* 2005; 90: 5937-5941.
14. Schuler-Luttmann, S., G. Monnig, A. Enbergs, H. Schulte, G. Breithardt, G. Assmann, S. Kerber, and A. von Eckardstein. Insulin-like growth factor-binding protein-3 is associated with the presence and extent of coronary arteriosclerosis. *Arterioscler Thromb Vasc Biol* 2000; 20: E10-15.

15. van den Beld, A.W., M.L. Bots, J.A. Janssen, H.A. Pols, S.W. Lamberts, and D.E. Grobbee. Endogenous hormones and carotid atherosclerosis in elderly men. *Am J Epidemiol* 2003; 157: 25-31.

16. Kawachi, S., N. Takeda, A. Sasaki, Y. Kokubo, K. Takami, H. Sarui, M. Hayashi, N. Yamakita, and K. Yasuda. Circulating insulin-like growth factor-1 and insulin-like growth factor binding protein-3 are associated with early carotid atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005; 25: 617-621.

17. Watanabe, T., M. Itokawa, Y. Nakagawa, T. Iguchi, and T. Katagiri. Increased levels of insulin-like growth factor binding protein-3 in hypertensive patients with carotid atherosclerosis. *Am J Hypertens* 2003; 16: 754-760.

18. Sandhu, M.S., A.H. Heald, J.M. Gibson, J.M. Cruickshank, D.B. Dunger, and N.J. Wareham. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet* 2002; 359: 1740-1745.

19. Perls, T.T., N.R. Reisman, and S.J. Olshansky. Provision or distribution of growth hormone for "antiaging": clinical and legal issues. *Jama* 2005; 294: 2086-2090.

20. Fried, L.P., N.O. Borhani, P. Enright, C.D. Furberg, J.M. Gardin, R.A. Kronmal, L.H. Kuller, T.A. Manolio, M.B. Mittelmark, A. Newman, D. O'Leary, B.M. Psaty, P. Rautaharju, R.P. Tracy, and P.G. Weiler. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol* 1991; 1: 263-276.

21. Newman, A.B., L. Shemanski, T.A. Manolio, M. Cushman, M. Mittelmark, J.F. Polak, N.R. Powe, and D. Siscovick. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. The Cardiovascular Health Study Group. *Arterioscler Thromb Vasc Biol* 1999; 19: 538-545.

22. O'Leary, D.H., J.F. Polak, R.A. Kronmal, P.J. Savage, N.O. Borhani, S.J. Kittner, R. Tracy, J.M. Gardin, T.R. Price, and C.D. Furberg. Thickening of the carotid wall. A marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. *Stroke* 1996; 27: 224-231.

23. Ives, D.G., A.L. Fitzpatrick, D.E. Bild, B.M. Psaty, L.H. Kuller, P.M. Crowley, R.G. Cruise, and S. Theroux. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. *Ann Epidemiol* 1995; 5: 278-285.

24. Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP Cooperative Research Group. *Jama* 1991; 265: 3255-3264.

25. Longstreth, W.T., Jr., C. Bernick, A. Fitzpatrick, M. Cushman, L. Knepper, J. Lima, and C.D. Furberg. Frequency and predictors of stroke death in 5,888 participants in the Cardiovascular Health Study. *Neurology* 2001; 56: 368-375.

26. Chan, J.M., M.J. Stampfer, J. Ma, P. Gann, J.M. Gaziano, M. Pollak, and E. Giovannucci. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J Natl Cancer Inst* 2002; 94: 1099-1106.

27. Therneau, T.M. and H. Li. Computing the Cox model for case cohort designs. *Lifetime Data Anal* 1999; 5: 99-112.

28. Psaty, B.M., C.D. Furberg, L.H. Kuller, D.E. Bild, P.M. Rautaharju, J.F. Polak, E. Bovill, and J.S. Gottdiener. Traditional risk factors and subclinical disease measures as predictors of first myocardial infarction in older adults: the Cardiovascular Health Study. *Arch Intern Med* 1999; 159: 1339-1347.

29. Lumley, T., R.A. Kronmal, M. Cushman, T.A. Manolio, and S. Goldstein. A stroke prediction score in the elderly: validation and Web-based application. *J Clin Epidemiol* 2002; 55: 129-136.

30. Kuller, L., N. Borhani, C. Furberg, J. Gardin, T. Manolio, D. O'Leary, B. Psaty, and J. Robbins. Prevalence of subclinical atherosclerosis and cardiovascular disease and association with risk factors in the Cardiovascular Health Study. *Am J Epidemiol* 1994; 139: 1164-1179.

31. Frustaci, A., N. Gentiloni, and M.A. Russo. Growth hormone in the treatment of dilated cardiomyopathy. *N Engl J Med* 1996; 335: 672-673; discussion 673-674.

32. Tanriverdi, F., N.K. Eryol, H. Atmaca, K. Unluhizarci, I. Ozdogru, I. Sarikaya, F. Bayram, and F. Kelestimur. The effects of 12 months of growth hormone replacement therapy on cardiac autonomic tone in adults with growth hormone deficiency. *Clin Endocrinol (Oxf)* 2005; 62: 706-712.

33. McCusker, R.H. and D.R. Clemmons. Use of lanthanum to accurately quantify insulin-like growth factor binding to proteins on cell surfaces. *J Cell Biochem* 1997; 66: 256-267.

34. Chan, S.S., S.M. Twigg, S.M. Firth, and R.C. Baxter. Insulin-like growth factor binding protein-3 leads to insulin resistance in adipocytes. *J Clin Endocrinol Metab* 2005; 90: 6588-6595.

35. Franklin, S.L., R.J. Ferry, Jr., and P. Cohen. Rapid insulin-like growth factor (IGF)-independent effects of IGF binding protein-3 on endothelial cell survival. *J Clin Endocrinol Metab* 2003; 88: 900-907.

36. Rajah, R., K.W. Lee, and P. Cohen. Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-alpha-induced apoptosis: role of Bcl-2 phosphorylation. *Cell Growth Differ* 2002; 13: 163-171.

37. Janssen, J.A., M.L. Jacobs, F.H. Derkx, R.F. Weber, A.J. van der Lely, and S.W. Lamberts. Free and total insulin-like growth factor I (IGF-I), IGF-binding protein-1 (IGFBP-1), and IGFBP-3 and their relationships to the presence of diabetic retinopathy and glomerular hyperfiltration in insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1997; 82: 2809-2815.

38. Frystyk, J., K. Hojlund, K.N. Rasmussen, S.P. Jorgensen, M. Wildner-Christensen, and H. Orskov. Development and clinical evaluation of a novel immunoassay for the binary complex of IGF-I and IGF-binding protein-1 in human serum. *J Clin Endocrinol Metab* 2002; 87: 260-266.

39. Chen, J.W., T. Ledet, H. Orskov, N. Jessen, S. Lund, J. Whittaker, P. De Meyts, M.B. Larsen, J.S. Christiansen, and J. Frystyk. A highly sensitive and specific assay for determination of IGF-I bioactivity in human serum. *Am J Physiol Endocrinol Metab* 2003; 284: E1149-1155.

Table 1. Characteristics of coronary heart disease cases, ischemic stroke cases, and controls

	Coronary heart disease n=534	Ischemic stroke n=370	Random subcohort (controls) n=1122
		Percent	
Age <75 years old	59.7*	57.0*	69.1
Age >75 years old	40.3	43.0	30.9
Male	47.9*	37.0	35.4
Female	52.1	63.0	64.6
Non-Hispanic White	83.3	83.5	81.6
Other race/ethnicity	16.7	16.5	18.5
General Health Status			
Excellent, very good, or good	73.8*	72.1*	79.7
Poor or fair	26.2	27.9	20.1
Income <$25,000	69.4*	64.8	57.0
Former smoker	44.6*	38.1	39.8
Current smoker	14.2	13.5	11.8
Hypertension	55.4*	62.4 *	47.9
Treated hypertension	43.6*	51.4*	37.3
Impaired fasting glucose	16.0	17.9*	13.2
Diabetes	21.4*	20.7*	13.4
Untreated diabetes	38.6	47.4	50.1
Oral hypoglycemic therapy	48.3	36.8	37.3
Insulin therapy	11.4	13.2	9.3
Oral and insulin therapy	1.8	2.6	2.7
Lipid lowering medication use	3.9	5.1	5.5

Table 1 (continued)

	mean ± SD		
BMI, kg/m^2	27.4 ± 5.0	26.8 ± 4.5	26.9 ± 5.1
Systolic blood pressure, mmHg	141.1 ± 21.7*	142.8 ± 23.4*	137.0 ± 22.0
Diastolic blood pressure, mmHg	72.3 ± 12.7	72.6 ± 12.1	71.6 ± 11.6
Ankle arm blood pressure index (ABI)	1.03 ± 0.19*	1.04 ± 0.17*	1.08 ± 0.16
Total cholesterol, mg/dl	211.0 ± 39.0	214.9 ± 39.4	214.5 ± 28.3
HDL-cholesterol, mg/dl	51.9 ± 14.0*	55.1 ± 17.4	55.3 ± 15.3
LDL-cholesterol, mg/dl	130.9 ± 35.6	132.1 ± 35.7	131.7 ± 35.0
Triglycerides, mg/dl	143.9 ± 74.0	144.3 ± 73.6	139.7 ± 72.7
Glucose, mg/dl	116.3 ± 39.3*	115.1 ± 37.5*	109.6 ± 36.8
IGF-I, μg/L	151.5 ± 60.2	150.3 ± 58.3	152.2 ± 58.3
IGFBP-1, μg/L	31.4 ± 19.5	31.8 ± 20.2	31.4 ± 20.2
IGFBP-3, μg/L	3919.7 ± 911.8*	3974.3 ± 912.2	4044.5 ± 903.2

* Indicates significant difference between case group and controls (p<0.05)

Conversion factors for SI units are: [mg/dl to mmol/L] x38.6 for LDL, HDL and total cholesterol; [μg/L to nmol/L] x0.13 for IGF-I, x0.22 for IGFBP-1, and x0.035 for IGFBP-3.

MI, myocardial infarction; SD, standard deviation; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IGF-I, insulin-like growth factor I; IGFBP, insulin-like growth factor binding protein

Table 2. Hazard ratios for incident myocardial infarction or fatal coronary heart disease by IGF-I, IGFBP-1, and IGFBP-3

	Tertile Categories of IGF-I			**Per SD increase in IGF-I**	**Linear p-value****
	1	2	3		
N events	178	191	165		
Age- and gender- adjusted					
HR	1	0.90	0.82	0.95	
95% CI	*(reference)*	*(0.70, 1.17)*	*(0.63, 1.18)*	*(0.85, 1.06)*	0.36
Multivariate-adjusted*					
HR	1	0.91	0.81	0.94	
95% CI	*(reference)*	*(0.70, 1.19)*	*(0.61, 1.07)*	*(0.84, 1.05)*	0.29
	Tertile Categories of IGFBP-1			**Per SD increase in IGFBP-1**	**Linear p-value**
	1	2	3		
N events	176	176	181		
Age- and gender- adjusted					
HR	1	0.87	0.99	1.01	
95% CI	*(reference)*	*(0.67, 1.13)*	*(0.76, 1.29)*	*(0.90, 1.12)*	0.93
Multivariate-adjusted*					
HR	1	0.97	1.08	1.04	
95% CI	*(reference)*	*(0.74, 1.26)*	*(0.81, 1.43)*	*(0.92, 1.17)*	0.57
	Tertile Categories of IGFBP-3			**Per SD increase in IGFBP-3**	**Linear p-value**
	1	2	3		
N events	215	153	166		
Age- and gender- adjusted					
HR	1	0.68	0.80	0.87	
95% CI	*(reference)*	*(0.52, 0.88)*	*(0.61, 1.04)*	*(0.78, 0.98)*	0.02
Multivariate-adjusted*					
HR	1	0.69	0.80	0.88	
95% CI	*(reference)*	*(0.53, 0.90)*	*(0.61, 1.05)*	*(0.78, 1.00)*	0.05

*Adjusted for sex, race, age, treated hypertension, systolic blood pressure, smoking, creatinine and high-density lipoprotein cholesterol
** P-values derived from model relating IGF measures, as linear terms scaled per SD, to outcomes.

Tertile cut-points defined as: IGF-I: 121.14 and 170.89 µg/L; IGFBP-1: 19.75 and 36.88 µg/L; IGFBP-3: 3650.80 and 4419.69 µg/L

HR, hazard ratio; SD, standard deviation; IGF-I, insulin-like growth factor I; IGFBP, insulin-like growth factor binding protein