Short Title: IGF and incident CVD events

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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 asse

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

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

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

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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/eth2sn analystsnaly(n)-10a(o w)2oJ -434.123.4-

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

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

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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. 7[(i)-2(nt)-215 ts, and over 1100 7.6 Td [(r)3(e)4-2(ve)4(i)-2(nve)4(s1310(]TJ -35(i)-2(ne)400 7.6 5l

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 st(s)-16ng34(r)3(na)-10(e)4(ve)4(nt)- reclof inpE07.5 m

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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/p0vs1.08 ± 0.16			

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