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Serum Insulin-Like Growth Factor 1 and the risk of Ischemic Stroke: The Framingham Study

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Abstract

Background—Low insulin-like growth factor 1 (IGF-1) have been associated with increased risk of atherosclerosis and atrial fibrillation in cross-sectional studies. Yet, prospective data linking IGF-1 levels to the development of ischemic stroke remain inconclusive. We examined prospectively the association between serum IGF-1 levels and incident ischemic stroke.

Methods—We measured serum IGF-1 levels in 757 elderly individuals (mean age 79±5, 62% women), free of prevalent stroke, from the Framingham original cohort participants at the 22nd examination cycle (1990-1994) and were followed up for the development of ischemic stroke. Cox models were used to relate IGF-1 levels to the risk for incident ischemic stroke, adjusted for potential confounders.

Results—During a mean follow-up of 10.2 years, 99 individuals developed ischemic stroke. After adjustment for age, sex and potential confounders, higher IGF-1 levels were associated with a lower risk of incident ischemic stroke with subjects in the lowest quintile of IGF-1 levels having a 2.3-fold higher risk of incident ischemic stroke (95% CI: 1.09-5.06, p=0.03) as compared to the top quintile. We observed an effect modification by diabetes and waist-hip ratio (WHR) for the association between IGF-1 and ischemic stroke (p<0.1). In subgroup analyses, the effects were restricted to diabetics and those in top WHR quartile, in whom each standard deviation increase in

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IGF-1 was associated with a 61% (HR: 0.39, 95% CI: 0.20-0.78, p=0.007), and 41% (HR: 0.59, 95% CI: 0.37-0.95, p=0.031) lower risk of incident ischemic stroke, respectively.

Conclusions—IGF-1 levels were inversely associated with ischemic stroke, especially among persons with insulin resistance.

Keywords

IGF-1; Ischemic Stroke; Insulin-resistance; Risk

Introduction

Recent experimental and clinical studies indicate physiologic benefits of insulin-like growth factor-1 (IGF-1) on vascular functions. In recent basic science studies, IGF-1 has been shown to reduce atherosclerosis burden through anti-apoptotic (1) and anti-inflammatory (2) properties, and protect vascular function by promoting nitric oxide (NO) production from the endothelium and vascular smooth muscle cells (3). IGF-1 also enhances insulin sensitivity (4) and induces vasodilation (5), thereby, regulating glucose homeostasis, blood pressure and cerebral blood flow (6,7). Additionally, low circulating IGF-1 levels have been associated with increased carotid intima-media thickness (8) and atrial fibrillation (9) in an elderly population. Lower levels of IGF-1 have been found to be associated with the presence of traditional vascular risk factors, particularly obesity (10), insulin resistance and diabetes (4,11). Hence, the effects of IGF-1 levels on vascular events may also be mediated through pathways of insulin resistance.

The association of IGF-1 with the risk of incident ischemic stroke, however, remains unclear. Two previous prospective observational studies that have examined this association were inconsistent, with one study showing a protective role for IGF-1 and another study showing no association with incident ischemic stroke (12,13). On the other hand, higher IGF-1 levels have been associated with early carotid artery atherosclerosis (14). While it has been shown that the physiologic effects of IGF-1 on vasculature may be altered in persons with insulin resistance, no previous study has examined this association in insulin-resistant states.

To clarify these gaps in knowledge, we prospectively related circulating IGF-1 levels to the risk of ischemic stroke in a large, community-based cohort of elderly individuals. We hypothesized that higher circulating IGF-1 levels would be associated with a lower risk of ischemic stroke, particularly in insulin-resistant states.

Methods

The design and selection criteria of the Framingham Heart Study original cohort have been described elsewhere (15). Briefly, between 1948 and 1953, a total of 5209 participants were recruited and have been examined approximately every two years. IGF-1 levels were measured in 796 participants from generation 1 of a total of 1116 persons who attended the 22nd examination cycle (1990–1994). The remaining persons did not have sufficient serum drawn to permit IGF-1 assay. Thirty-nine participants were excluded for prevalent stroke

(Supplemental figure I). The study protocol was approved by the Institutional Review Board of the Boston University Medical Center and all participants provided written informed consent prior to assessments.

Measurement of Serum IGF-I

At the baseline examination, 3 mL of serum from each participant was obtained from subjects in a supine, non-fasting state in the afternoon and stored at -80° C. Serum IGF-1 was measured by radioimmunoassay after acid-ethanol extraction in 3 batches (16); the intra-assay coefficient of variation was less than 4%. Insulin-like growth factor binding protein (IGFBP) levels were not measured.

Endpoints

All Framingham Study participants are under continuous surveillance for stroke. We have previously outlined our screening and surveillance methods for the development of stroke (17). In brief, stroke was defined as an acute onset focal neurological deficit of presumed vascular pathogenesis lasting ≥24 hours. Ischemic stroke was diagnosed if a focal neurological deficit was documented in the presence of a normal CT scan, CT or MRI showed an infarct that correlated with the clinical deficit, or an infarct was documented at autopsy. Using this information, it was possible to classify stroke subtypes as large vessel atherothrombotic and lacunar infarction, and cardioembolic from a documented cardiac source.

Statistical Analysis

Serum IGF-1 levels were standardized within sex. Multivariable-adjusted Cox proportional hazards regression models were used to assess the associations between serum IGF-1 and incident ischemic stroke after confirming that assumptions of proportional hazards were met. Primary analysis was performed using quintiles of IGF-1 levels. We used three models. The first model was adjusted for age and sex. Model 2 was additionally adjusted for systolic blood pressure, smoking, anti-hypertensive treatment and prevalent cardiovascular disease (CVD). Prior data suggest that glucose intolerance, abdominal obesity, and atrial fibrillation may mediate the association between IGF-1 and risk of stroke (4), so we separately adjusted for these covariates in Model 3. We also evaluated for the associations between serum IGF-1 and incident ischemic stroke in major subtypes of ischemic stroke (large artery, cardioembolic and lacunar ischemic stroke). Possible nonlinear associations were evaluated by use of restricted cubic splines. Exploratory analyses were performed using interaction terms to assess effect modification of relations by age, sex, waist-hip ratio (WHR) and diabetes mellitus status, and then stratifying analyses by subgroups when significant. These covariates were selected based on biologic interest and association with exposure in our dataset. WHR was selected as the preferred metric of body fat content and distribution because it is more strongly correlated with stroke risk than body mass index. Significance was set at $p<0.05$ for all models and $p<0.10$ for analyses assessing effect modification. All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics

Participant characteristics are provided in Table 1. The mean sample age was 79 years (72-95 years) and 62% participants were women. Participants who did not have IGF-1 levels measured were more likely to be older (Supplemetal table I). During a mean follow-up period of 10.2 years (0.02-19.28 years), 99 (13%) participants developed ischemic stroke.

IGF-1 and Incident Ischemic Stroke

After adjustment for age and sex, as compared with the fifth (top) quintile, the hazard ratios (and 95% CIs) for ischemic stroke were as follows: first quintile, 2.56 (1.20-5.45); second quintile, 2.12 (0.99 to 4.54); third quintile, 2.43 (1.17 to 5.07); fourth quintile, 2.12 (1.00 to 4.51) (Table 2). After adjusting for additional vascular risk factors in model 2, the associations remained significant for the first: 2.35 (1.09-5.06); and third quintile: 2.48 (1.19-5.17), as compared with the fifth (top) quintile (Table 2). In our analysis, we did not find any significant associations between IGF-1 levels and the risk of the main ischemic stroke subtypes separately (Supplemental tables II-IV). We found no evidence of nonlinear relationships between IGF-1 levels and the outcomes of interest based on restricted cubic spline analysis. Visual inspection of the splines also suggested relatively linear inverse associations of serum IGF-1 with the incidence of ischemic stroke (Supplemental figure II).

Effect modification by Waist-Hip Ratio (WHR)

Our results were suggestive for a potential effect modification by WHR for the association between IGF-1 and incident ischemic stroke (p=0.083). In subsequent stratified analysis, quartiles were used to categorize WHR, consistent with prior publications where we have found the highest quartile of the WHR associated with adverse neurological outcomes (mean value for WHR in the top quartile: 1.02). We observed a 41% lower risk of incident ischemic stroke per one standard deviation increase in IGF-1 (p=0.031) only among subjects who were in the top sex-specific WHR quartile. Additional adjustment with potential confounders (model 2) and mediators (model 3) did not alter these results (Table 3).

Effect modification by Diabetes Mellitus

We observed evidence for effect modification by diabetes for the association between IGF-1 and incident ischemic stroke (p=0.016). In subsequent stratified analysis, there was a 61% lower risk of incident ischemic stroke (p=0.007) per one standard deviation increase in IGF-1 only among subjects with diabetes. Additional adjustment with potential confounders (model 2) and mediators (model 3) did not alter these findings (Table 4).

Discussion

In our prospective community-based cohort of older individuals, circulating IGF-1 levels were associated with risk of ischemic stroke, with subjects in the lowest quintile of IGF-1 levels having a 2.5-fold higher risk of incident ischemic stroke. Associations appeared strongest for diabetic and obese individuals, among whom one standard deviation increase in IGF-1 levels (60 ng/ml) was associated with a nearly 60% lower risk of ischemic stroke in

diabetics and 50% lower risk of ischemic stroke for persons in the top WHR quartile. These associations remained significant after adjustment for traditional stroke risk factors. The lack of associations among each ischemic stroke subtypes can be explained by the presence of strong effect modifications and lack of statistical power in each subtype. Our findings suggest that IGF-1 is related to the risk of incident ischemic stroke and support an emerging hypothesis that low circulating IGF-1 may be an especially important determinant of ischemic stroke events in obese and diabetic individuals.

Earlier studies have suggested that IGF-1 may be atherogenic by inducing vascular smooth muscle cell proliferation. A growing body of evidence indicates, instead, that IGF-1 has protective effects on the vasculature (18,19). IGF-1 can directly oppose endothelial dysfunction and development of ischemic stroke through its effects on NO production, plaque stability, anti-inflammatory actions, increased endothelial cell survival and inhibition of endothelial cell apoptosis (1-5) and indirectly through an inverse association with traditional CVD risk factors (5-7). In vivo human studies have demonstrated that infusion of IGF-1 into human brachial arteries increased blood flow in the forearm by NO-dependent mechanisms (20). Lower circulating IGF-1 levels have been associated with the presence of traditional stroke risk factors, particularly diabetes (4,21) and atrial fibrillation (9). Hence, the effects of IGF-1 levels on cerebrovascular events may be mediated through components of the metabolic syndrome and pathways of atrial fibrillation. Furthermore, adults with growth hormone deficiency and resulting low circulating IGF-1 levels had considerably higher risk of mortality due to cerebrovascular diseases (22) and increased intima-media thickness (23) that was reversible following growth hormone replacement (24).

Yet, the contribution of serum IGF-1 levels to risk of incident ischemic stroke in the general population is unclear. To date, only 2 prospective observational studies have evaluated serum IGF-1 levels and the risk of incident stroke (12,13). In a prospective nested case-control study within a Danish population with 254 cases of incident ischemic stroke, subjects in the bottom quartile of IGF-1 were at increased risk of ischemic stroke [OR: 2.06, 95% confidence interval (CI), 1.05–4.03] when compared with participants in the top quartile (12). In contrast, among older adults within the Cardiovascular Health Study (CHS), no association was found between total IGF-1 levels and the risk of coronary events or ischemic stroke (13). Our epidemiological observations of an inverse association of baseline IGF-1 concentrations with incident ischemic stroke are consistent with previous experimental results. Hence, low IGF-1 may represent an additional independent risk factor for ischemic stroke in insulin-resistant states. While our primary analysis suggested that most of the effect is derived from the difference between top IGF-1 quintile vs the bottom 4 IGF-1 quintiles, there was no evidence of nonlinear relationships between IGF-1 levels and the outcomes of interest based on restricted cubic spline analysis. We were able to demonstrate the presence of a strong effect modification for this association and therefore, computing and interpreting an overall estimate of association may not be reliable. Although our study was observational, the temporal associations and the consistency of results in multiple stratified analyses could suggest the possibility of a casual association. Reports demonstrating lower values of carotid intima-media thickness and high endothelium-dependent vasodilation in individuals with IGF-1 gene polymorphisms associated with higher levels further support a causal relationship (25).

Obesity and type 2 diabetes mellitus are characterized by insulin resistance, reduced NO availability and increased risk of atherothrombosis. Clinical trials have shown that intensive glycemic control does not reduce the risk of macro-vascular events and stroke in type 2 diabetes, suggesting that factors beyond blood glucose mediated vascular risk in these individuals (26,27). IGF-1, like insulin, protects vascular integrity mainly through activation of NO synthase via an Akt-catalyzed phosphorylation (28,29). IGF-1, also, enhances insulin sensitivity and improves glycemic control, lipid profile and body composition in individuals with type 2 diabetes (30). However, recent animal studies demonstrated that IGF-1-induced NO availability is blunted in diabetic and obese mice (31,32). An association between a decline in NO availability and accelerated atherosclerosis is also well established, particularly in those with obesity and type 2 diabetes (33,34). In fact, insulin resistance triggers atherothrombosis mainly through impaired NO activation and an imbalance between NO availability and accumulation of reactive oxygen species, leading to endothelial dysfunction (35). Also, impaired NO synthesis is implicated in the pathogenesis of atrial fibrillation (36). In an experimental study by Cai et al, authors concluded that loss of left atrial NO activity contributes to the thromboembolic events associated with atrial fibrillation (37). Taken together, these experimental and clinical findings suggest that low IGF-1 levels could have detrimental effects on the vasculature, especially in individuals with type 2 diabetes or obesity. Our observation of a substantially lower risk of incident stroke in diabetic and centrally obese individuals with higher serum IGF-1 is consistent with these studies. If our findings are replicated by others, serum IGF-1 levels may serve as an important marker in ischemic stroke risk assessment, particularly in individuals with type 2 diabetes or central obesity. New mechanism-based therapies that improve endothelial dysfunction are required to prevent macro-vascular events and stroke in these individuals, targeting both hyperglycemia and insulin-resistance pathways and simultaneously improving metabolic and vascular functions.

Our investigation had strengths. Incident stroke was prospectively adjudicated using a consistent set of criteria and based on continuous surveillance of all subjects. Furthermore, covariates were assessed comprehensively prior to events. This is a well-established community-based cohort with negligible loss to follow-up.

Our study also had limitations. We did not measure biologically active free IGF-1 and IGF-binding proteins. It is suggested that levels of IGF-binding proteins may affect the actions of circulating IGF-1 on cardiovascular risk (20). Yet, circulating IGF-1 is highly correlated with the ratio of IGF-1 to IGF-binding proteins (38) and therefore, measuring circulating IGF-1 alone may have led to an underestimation of true relationships with incident stroke. IGF-1 levels were measured at baseline, and metabolic fluctuations could have increased exposure misclassification over time. This cohort included older European Americans that may limit generalizability to other ethnicities or younger populations. The observational design cannot exclude residual confounding by unknown or unmeasured factors. Yet, results were robust to adjustment for multiple major risk factors. Finally, it should be noted that the precision of our risk estimates in the stratified analysis was moderate due to the relatively small number of events in subgroups as suggested by the relatively wide confidence intervals, and may lead to overestimation of effect estimates.

Summary

Our findings suggest that circulating IGF-1 levels are linked to lower risk of incident ischemic stroke among adults later in life, with potentially highest impact in individuals with type 2 diabetes or central obesity. These findings suggest the hypothesis that IGF-1 is involved in the pathogenesis of ischemic stroke, particularly in the insulin resistant states. Further studies are warranted to explore the underlying cellular mechanisms and investigate potential clinical implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline Characteristics of entire study sample

N	757
Age, mean ± SD	79±5
Female, n (%)	470 (62)
Systolic Blood Pressure, mean ± SD	143±21
Body Mass Index, mean ± SD	26.78±4.68
Waist-Hip Ratio, mean ± SD	0.93±0.09
Current Smoking, n (%)	62 (8)
Diabetes Mellitus, n (%)	86 (12)
Anti-hypertensive, n (%)	363 (49)
Prevalent CVD, n (%)	241 (32)
Prevalent AF, n (%)	81 (11)
IGF-1, mean ± SD	145±60
Incident Stroke, n (%)	119 (16)
Ischemic Stroke, n (%)	99 (13)

N: Number; CVD: Cardiovascular disease; IGF-1: Insulin like growth factor1, SD: Standard Deviation, AF: Atrial Fibrillation.

Table 2

Hazard ratio of ischemic stroke based on IGF-1 quintiles (N=757, Events=99)

	Quintile of Plasma IGF-1				
	I	**II**	**III**	**IV**	**V**
Ischemic Stroke					
N	151	148	156	150	152
Mean IGF-1 (SD)	69.93(21.18)	109.8(10.02)	138.9(11.69)	170.91(13.82)	232.97(41.04)
Age and sex adjusted	2.56 [1.20-5.45] (0.015)	2.12 [0.99, 4.54] (0.055)	2.43 [1.17, 5.07] (0.018)	2.12 [1.00, 4.51] (0.050)	1.00 (ref)
Multivariate †	2.35 [1.09, 5.06] (0.030)	1.99 [0.93, 4.28] (0.078)	2.48 [1.19, 5.17] (0.016)	2.11 [0.99, 4.49] (0.053)	1.00 (ref)
Multivariate+Mediators ‡	1.86 [0.86, 4.01] (0.113)	1.72 [0.79, 3.76] (0.175)	2.44 [1.17, 5.10] (0.018)	2.15 [1.01, 4.60] (0.048)	1.00 (ref)

Results are HR [95% CI] with (p-values), comparing the top vs. bottom IGF-1 quintiles.

HR: Hazard ratio; CI: Confidence interval; SD: Standard deviation; N: Number of observations.

†Adjusted for age, sex, systolic blood pressure, smoking, anti-hypertensive treatment and prevalent CVD

‡Additionally adjusted for waist-hip ratio and diabetes mellitus, and atrial fibrillation.

Table 3

Stratified analysis by WHR quartiles using an indicator of top sex-specific quartile

	Top Quartile of WHR	Bottom 3 quartiles of WHR
Events/N	27/189	71/564
Mean IGF-1(SD)	144.46(58.42)	144.57(60.56)
Multivariate[†]	0.52 [0.32, 0.85] (0.009)	0.90 [0.69, 1.16] (0.403)
Multivariate+Mediators[‡]	0.60 [0.37, 0.97] (0.035)	0.95 [0.74, 1.22] (0.680)

Hazard ratios, HR [95% CI], (p-values) are estimated for 1 SD (standard deviation) increase in IGF-1 levels in each category.

P-value for interaction = 0.083

HR: Hazard ratio; CI: Confidence interval; SD: Standard deviation; N: Number of observations, IS: Ischemic stroke; WHR: Waist-hip ratio.

[†]Adjusted for age, sex, systolic blood pressure, smoking, anti-hypertensive treatment, prevalent CVD.

[‡]Additionally adjusted for WHR, diabetes mellitus and atrial fibrillation.

Table 4

Stratified analysis by diabetes mellitus status using an indicator of top sex-specific quartile

	Diabetes	No Diabetes
Events/N	11/86	87/655
Mean IGF-1(SD)	152.08(66.35)	144.34(59.22)
Age and sex adjusted*	0.39 [0.20, 0.78] (0.007)	0.88 [0.69, 1.11] (0.277)
Multivariate †	0.42 [0.22, 0.79] (0.007)	0.91 [0.72, 1.16] (0.451)
Multivariate+Mediators‡	0.40 [0.20, 0.81] (0.011)	0.96 [0.77, 1.21] (0.756)

Hazard ratios, HR [95% CI], (p-values) are estimated for 1 SD (standard deviation) increase in IGF-1 levels in each category.

P-value for interaction = 0.016

HR: Hazard ratio; CI: Confidence interval; SD: Standard deviation; N: Number of observations, IS: Ischemic stroke.

† Adjusted for age, sex, systolic blood pressure, smoking, anti-hypertensive treatment, prevalent CVD.

‡ Additionally adjusted for waist-hip ratio and atrial fibrillation.