

The Association between IGF-I and IGFBP-3 and Incident Diabetes in an Older Population of Men and Women in the Cardiovascular Health Study

Chino S. Aneke-Nash, Xiaonan Xue, PhD, Qibin Qi, PhD, Mary L. Biggs PhD, Anne Cappola, MD, ScM, Lewis Kuller MD, DrPH, Michael Pollak MD, Bruce M. Psaty, MD, PhD, MPH, David Siscovick MD, MPH, BA, Kenneth Mukamal MD, MPH, Howard D. Strickler MD MPH, Robert C. Kaplan PhD

IGFBP-3 and Incident Diabetes in the Elderly.

The Association between IGF-I and IGFBP-3 and Incident Diabetes in an Older Population of Men and Women in the Cardiovascular Health Study

Chino S. Aneke-Nash¹, Xiaonan Xue, PhD¹, Qibin Qi, PhD¹, Mary L. Biggs PhD², Anne Cappola, MD, ScM³, Lewis Kuller⁴ MD, DrPH, Michael Pollak⁵ MD, Bruce M. Psaty, MD, PhD, MPH⁶, David Siscovick MD, MPH, BA⁷, Kenneth Mukamal MD, MPH⁸, Howard D. Strickler MD MPH¹, Robert C. Kaplan PhD¹

¹Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY;

²Department of Biostatistics, University of Washington, Seattle, Washington;

³Division of Endocrinology, Diabetes, and Metabolism, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA;

⁴Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA;

⁵Department of Experimental Medicine, McGill University, Montreal, Quebec, Canada; Department of Oncology, McGill University, Montreal, Quebec, Canada;

⁶Cardiovascular Health Research Unit, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, WA, USA, Group Health Research Institute, Group Health Cooperatives, Seattle, WA.

⁷The New York Academy of Medicine, New York, New York

⁸Division of General Medicine and Primary Care, Beth Israel Deaconess Medical Center, Boston, Massachusetts; Harvard Medical School, Boston, Massachusetts; Harvard School of Public Health, Boston, Massachusetts.

Context: Insulin like growth factor-I (IGF-I) has structural/functional similarities with insulin and may play a role in glucose homeostasis, along with IGF binding protein-3 (IGFBP-3), which binds the majority of circulating IGF-I.

Objective: To assess whether IGF-I and IGFBP-3 are associated with higher risk of incident diabetes in older adults.

Design: Participants in the Cardiovascular Health Study (n=3133), a cohort of adults aged ≥ 65 years, were observed for 16 years (n=3133) for the development of incident diabetes. Statistical models were fit separately for men and women due to interactions with sex (p-interaction: IGF-I- 0.02, IGFBP-3- 0.009), and were adjusted for relevant covariates.

Setting: General community

Participants: Individuals who were non-diabetic at baseline and who did not develop diabetes within the first year of follow-up

Interventions: n/a

Main Outcome Measure: Incident diabetes as measured by fasting plasma glucose (FPG) ≥ 126 mg/dl, non-FPG ≥ 200 mg/dl, use of pharmacological treatment for diabetes, or existence of ≥ 2 inpatient or ≥ 3 outpatient or (≥ 1 inpatient and ≥ 1 outpatient) CMS claims using the diagnostic ICD-9-CM code of 250.xx.

Results: In women, higher IGFBP-3 ($HR_{T3 \text{ vs. } T1}=2.30$, $CI=1.55-3.40$, $P\text{-trend} < 0.0001$) was significantly associated with incident diabetes. Total IGF-I was not significantly associated with incident diabetes. In men, neither IGF-I nor IGFBP-3 were significantly associated with incident diabetes.

Conclusions: We confirm a previously reported association between circulating IGFBP-3 and diabetes risk in the older adult population, establishing that this association is present among women but could not be shown to be associated in men.

We studied baseline total IGF-I and IGFBP-3 levels as predictors of incident diabetes. We found that higher IGFBP-3 was associated with a 2-fold higher risk of diabetes in women but not in men.

Introduction

The growth hormone (GH)/insulin-like growth factor (IGF) axis is an evolutionarily conserved axis thought to regulate carbohydrate metabolism (1). IGF-I, the primary mediator of the glucose regulatory function of this axis, circulates in a ternary complex along with an acid labile subunit and one of six IGF binding proteins (IGFBP1-6) (2,3). Studies suggest that some of these binding proteins, particularly IGFBP-3, may independently regulate glucose homeostasis as well (4,5).

While IGF-I and its binding proteins may plausibly be involved in normal carbohydrate metabolism, the relationship between IGF proteins and incident diabetes is not completely understood. GH stimulates the hepatic release of IGF-I, a protein that is structurally similar to insulin and promotes glucose uptake in peripheral tissues (6-8). Interactions of IGF-I with its binding proteins may modulate its biological activity (9). IGFBP-3 bound IGF-I accounts for 90-95% of the IGF-I in the blood (10).

Prior epidemiological studies on the relationship between IGF proteins and incident diabetes report conflicting findings (11-19). In the Nurses Health Study and in the European Prospective Investigation Into Cancer and Nutrition-Potsdam Study, total IGF-I levels were not associated with incident diabetes, while in an analysis including data from the Diabetes Cardiovascular Risk-Evaluation: Targets and Essential Data for Commitment of Treatment (DETECT) study and the Study of Health in Pomerania, total IGF-I had a U-shaped relationship with incident diabetes (14,18,19). These studies did not adequately control for all known confounders, did not evaluate interactions with sex and did not adjust for IGFBP-3 in models of IGF-I and incident diabetes.

This study examines the relationship between total IGF-I and IGFBP-3 on risk of diabetes using data from the Cardiovascular Health Study (CHS), a cohort of community dwelling older adults aged 65 and older.

Materials and Methods

Setting and Participants

The CHS is a population-based, prospective longitudinal study of 5201 community-dwelling adults aged ≥ 65 years who were recruited during 1989 -1990. An additional 687 individuals, most of whom were African Americans, were recruited in 1992-1993 (20). Participants were identified from Medicare eligibility lists at four U.S locations (Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Pittsburgh, PA). Participants were recruited into the CHS if they were not receiving active treatment for cancer, were expected to remain in the study area for 3 years after recruitment and were able to give informed consent without a proxy. Standardized examinations, blood draw and questionnaires were administered annually through 1999, after which information on health status and key disease risk factors were updated through semiannual phone interviews. In 2006, the surviving participants were invited for another study clinic examination.

Laboratory measurements

Following standard procedures, fasting blood samples and a second blood sample obtained 2 hours after ingestion of a 75g glucose load were collected, frozen on-site and deposited at -70°C (21). Levels of IGF-I and IGFBP-3 were measured using stored fasting blood samples from the 1993/1994 CHS clinic visit. IGF-I levels were measured using enzyme-linked immunosorbent assay (ELISA) methods and IGFBP-3 levels were measured using chemiluminescence assays (IDS, Immunodiagnostic Systems Ltd, Boldon Business Park, Boldon, Tyne & Wear, England). A randomly-selected sample of 501 individuals at the 1993/1994 CHS examination had previous measurements of IGF-I using ELISA methods (DSL, Diagnostics Systems Laboratory, Webster, TX). We found high correlation (Pearson's $r = 0.93$) between the IDS and DSL measurements in a study involving split-sample measurements among 1,158 samples. Thus, these additional observations were incorporated to the analysis after performing a proper calibration. Details of this calibration procedure are reported elsewhere (22). Assay coefficient of variation was 3–14% for IGF-I and IGFBP-3. Standard methods were used to measure glucose, insulin, total cholesterol and C-reactive protein (CRP) at the CHS Central Laboratory (21).

Clinical Variables

Diabetes status was determined using data collected during CHS study contacts and clinical encounter data from the Center for Medicare and Medicaid Services (CMS). Of all CHS participants, 77% of them had available data from CMS during the 1993/1994 baseline. The other 23% either were not enrolled in Medicare Part B coverage or did not have any clinic visits during the baseline year. In CHS, fasting glucose was measured approximately every 2 years from enrollment until 1999 and then again in 2006. Use of diabetes medications was ascertained annually using a medication inventory (20,23). Diabetes was defined in our analyses as having fasting plasma glucose (FPG) $\geq 126\text{mg/dl}$, non-fasting plasma glucose $\geq 200\text{mg/dl}$, use of pharmacological treatment for diabetes, or existence of ≥ 2 inpatient or ≥ 3 outpatient or (≥ 1 inpatient and ≥ 1 outpatient) CMS claims during a 2-year time period which included a diagnostic International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code of 250.xx at any position in the medical claim.

Other covariates were obtained from CHS study examinations including body mass index (BMI (kg/m^2)), total cholesterol (mg/dl), smoking status (never vs. ever vs. current), hypertension (normal vs. pre-hypertensive vs. hypertensive), alcohol consumption (number of drinks/week), leisure time physical activity (kcal/week) (24), C-reactive protein (mg/dl), fasting insulin (IU/mL) and adiponectin (mg/L).

Statistical Analysis

The 1993/1994 study visit was established as the baseline for analysis and follow-up of study participants continued until 2009/2010. Participants were included in the current analyses if they had either IGF-I or IGFBP-3 measure available and had diabetes status follow-up either through CHS data alone or through CHS data supplemented by CMS data. Participants were excluded if they had prevalent diabetes at baseline or developed diabetes within the first year after the baseline year. There were 5055 individuals who attended the 1993/1994 visit, 567 had unknown diabetes status, 856 had prevalent diabetes and 21 individuals lacked diabetes follow-up data and were all excluded. An additional 133 individuals who developed diabetes in the first year were excluded from the primary analysis, but were included for sensitivity analysis. From the 3479 individuals remaining, 230 individuals did not have IGF measures available and 116 were missing at least one of the confounding variables included in the study. After all exclusions, 3133 individuals remained for analysis.

The population at risk of diabetes was defined as follows: fasting glucose <126 mg/dl during the 1992/1993 clinic visit, no reported use of diabetes medication or record of diabetes diagnosis using CMS data during the 1993/1994 study visit. Characteristics of study participants at the baseline years were summarized using descriptive statistics. In addition, Pearson correlations were used to assess bivariate relationships between each biomarker and covariates that were continuous variables.

Cox proportional hazards regression models, analyzed separately for men and women, were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the relationship between IGF and IGFBP-3 biomarkers and incident diabetes. Both biomarkers were analyzed in tertiles with the lowest tertile as reference group. P-values for trend were determined by modeling the median value in each tertile as a continuous variable to minimize the effects of extreme values. Covariates in the models were determined apriori based on previous studies and were added sequentially into the model to assess confounding effects(14). The final, fully adjusted models included covariates for age, sex, race, BMI, smoking status, alcohol consumption, cholesterol, hypertension, fasting insulin, adiponectin, C-reactive protein and physical activity. While most covariates were measured contemporaneous with the main risk factors of interest, others were from the prior annual visit if they were unavailable at the baseline study year.

Because of the strong correlation between IGF-I and IGFBP-3 levels, the Cox model for IGF-I was adjusted additionally for IGFBP-3 levels while the model for IGFBP-3 was adjusted for IGF-I levels. Multivariable models for women also included adjustment for use of hormone replacement therapy (current vs none). In the final adjusted models, interactions were formally tested using binary interaction terms, defined as above or below sex-specific medians for age, BMI and fasting insulin levels. The proportional hazards assumption implicit in Cox models was formally tested using Schoenfeld and scaled Schoenfeld residuals and graphically using log-log plots.

Sensitivity analyses were conducted to assess whether similar results were obtained if diabetes cases were only identified using only CHS measured glucose levels and medication use without inclusion of CMS claims data. Analyses were repeated after inclusion of participants who developed diabetes within the first year after baseline, and also, after exclusion of those with fasting glucose between 100mg/dL and 125 mg/dL. All analyses were performed using Stata Version 12.1 (College Station, TX: StataCorp LP).

Results

Baseline Characteristics

During the baseline year (1993/1994 clinic visits), characteristics were similar among the 1254 men and 1879 women included in the study. Mean age was 75 ± 4.9 years, 15% were Black, and 16% of the women were currently using hormone replacement therapy (Table 1). Baseline IGFBP-3 levels were significantly higher in those who had prediabetes when compared to those who were normoglycemic in men ($p=0.01$) and women ($p<0.0001$) (Supplemental Table 1). Among the women, IGF-I levels were significantly higher in the prediabetic group versus normoglycemic groups ($p=0.001$), whereas there was no difference in IGF-I levels in men ($p=0.28$).

Bivariate Relationships with IGF-I and IGFBP-3

IGF-I levels were inversely associated with age, more so in men more than in women. IGF-I was positively associated with total cholesterol and fasting glucose levels in women (Table 2). In

both men and women, IGFBP-3 had a highly significant inverse association with age and adiponectin, and strong positive association with cholesterol levels. In women, IGF-I and IGFBP-3 were positively associated with fasting and 2-hour measures of glucose and insulin levels (r : 0.08-0.15), whereas men displayed weaker or absent associations of IGF-I and IGFBP-3 with glycemic traits.

Associations with Diabetes Risk

During a median 10.6 years of follow-up, 452 incident diabetes cases (men, $n=178$, women, $n=274$) occurred among 3133 individuals studied from baseline.

Associations between IGF-I, IGFBP-3 and incident diabetes varied significantly by sex (p -interaction: IGF-I- 0.02, IGFBP-3- 0.009). In women, total IGF-I was not significantly associated with risk of diabetes (Table 3). After adjustment for age, race, BMI, smoking, alcohol, cholesterol, hypertension, C-reactive protein, physical activity, fasting insulin, adiponectin and total IGF-I levels, participants in the highest IGFBP-3 tertile had an approximate 2-fold increase in risk of diabetes when compared to those in the lowest tertile ($HR_{T3 \text{ vs. } T1}=2.30$, $CI=1.55-3.40$, P -trend <0.0001).

In men, levels of IGF-I and IGFBP-3 were not significantly associated with incident diabetes in fully adjusted models (Table 4).

Sensitivity analyses

In an analysis that limited ascertainment of diabetes to medications and glucose measurements, associations between IGF-I and IGFBP-3 with incident diabetes were generally consistent with the results reported. However, the power of this analysis was much lower because numerous diabetes cases were identified using CMS data. In addition, the strength of the association was lower. Inclusion of those who developed diabetes in the first year resulted in slightly weaker associations between IGF biomarkers and ghrelin with incident diabetes. Additional subgroup analysis in normoglycemic individuals and those with prediabetics at baseline showed results with similar direction of associations, although with wider confidence intervals and larger p -values, with the association between IGFBP-3 and incident diabetes reaching statistical significance in women ($p=0.03$, Supplemental Tables 2-6). Among the women, exclusion of 299 participants who were receiving estrogen/progestin therapy did not alter the outcomes (IGF-I: $HR_{T3 \text{ vs } T1}=0.85$, $CI=0.56-1.30$, p -value for trend= 0.46, IGFBP-3: $HR_{T3 \text{ vs } T1}=1.87$, $CI=1.22-2.87$, p -value for trend= 0.003) (Supplemental Table 7).

Discussion

In this study, we evaluated the association between circulating levels of IGF-I and IGFBP-3, and risk of incident diabetes in a large prospective cohort of older men and women. Sexual dimorphism of the GH/IGF axis have been observed in animal models and human studies which show varying effects of IGF mutations and/or IGF protein levels in males versus females with regards to associations with lifespan, body composition, and bone aging (25-30). Our results were consistent with these previously published data, which varied by sex. Among women, higher levels of IGFBP-3 were associated with higher risk of incident diabetes. In men, IGF-I and IGFBP-3 had no statistically significant associations with diabetes risk. Our findings confirm and highlight long known sex-related differences in animal models with respect to IGFs, glucose metabolism and diabetes and support the hypothesis that IGF proteins may be biomarkers for diabetes risk (31).

High IGFBP-3 levels had a strong association with higher risk of incident diabetes in women, even after additional adjustment for IGF-I levels and an array of potential confounders. Although IGFBP-3 binds 90-95% of circulating IGF-I and may modify diabetes risk by modifying IGF-I activity, IGF-I independent effects of IGFBP-3 on glucose metabolism have also been reported. IGFBP-3 binds to nuclear receptors (RXR-alpha, PPAR-gamma), which play a role in glucose and lipid metabolism (32,33). In transgenic mouse models overexpressing IGFBP-3, circulating IGFBP-3 levels are 5-8 fold higher than in wild type and these mice have numerous metabolic derangements characteristic of Type 2 diabetes(34). Evidence from *in vivo* animal models may not fully represent the biology of IGFBP-3 in humans. However, our findings are consistent not only with these mechanistic observations but also with prior epidemiological studies from the Nurses' Health Study (NHS) (14). Unlike the prior study, which was limited to women, our report was not only able to confirm this previously reported association, but also showed that the association was specific to females. Another study by the European Prospective Investigation Into Cancer and Nutrition (EPIC)- Potsdam Study did not demonstrate an interaction with sex; however, EPIC is a study of middle-aged adults, which may confer different risk profiles given the age-related changes in IGFBP-3 levels (18). Thus, it remains plausible that high levels of IGFBP-3 may be useful to identify older women at increased risk for diabetes who may benefit from preventive measures.

Levels of IGF-I were not significantly associated with incident diabetes in either men or women. Recent studies in the Cardiovascular Health Study has shown that IGFBP-1 and ghrelin levels both predict major health events such as stroke, heart failure and myocardial infarction, as well as death in older adults (35). Given the established relationships between IGF axis proteins and adverse health outcomes, one would expect an association between Total IGF-I and incident diabetes. However, total IGF-I may not reflect the biologically active subfraction, thus variations in total IGF-I may not contain information about derangements in glucose metabolism. Data from animal studies suggest that overexpression of IGF-I is associated with moderate protection from diabetes (36). Findings from epidemiological studies have been conflicting. A functional polymorphism in the promoter region of the IGF-I gene is associated with lower IGF-I levels and a 70% increased risk of diabetes (17), however, a recent study failed to confirm this finding (11). Another study reported an association between low total IGF-I and diabetes risk but only in those with IGFBP-1 below median levels (15). Similarly, data from the Nurses Health Study reported an association of high free-IGF-I with diabetes risk in women with insulin levels below the median but low IGFBP-1 with diabetes risk in women with insulin levels above the median (14). Lewitt et. al reported a positive association with incident diabetes in women, although these findings were not adjusted for confounders (12). One study reported a U-shaped relationship between IGF-I and risk of diabetes but levels of IGFBP-3 were not assessed (19) thus, models were not adjusted for IGFBP-3 levels. Adjusting for IGFBP-3, especially IGFBP-3 that sequesters the majority of IGF-I, is critical in the analysis of IGF-I and diabetes risk in order to identify independent effects of IGF-I. In the Nurses Health Study, high total IGF-I was positively associated with diabetes risk in a multivariate regression model that excluded IGFBP-3; however, addition of IGFBP-3 into the model showed that low IGF-I levels predicted diabetes, although the associations did not reach statistical significance (14). In our analyses, total IGF-I was not significantly associated with incident diabetes in either men and women; however it should be noted that addition of IGFBP-3 into the model did change the direction of the association, even if the p-values were not statistically significant. The reasons for the inconsistencies reported by IGF studies are unknown and further sex-stratified studies that control for confounders as well as

IGFBPs are needed. Given the age-related differences in IGF protein levels, extrapolating results from middle-aged adults to older populations should be done cautiously.

Our analyses adjusted for several confounders of the relationship between IGF proteins and incident diabetes, however, we cannot eliminate the effects of residual confounders that are yet to be identified. In addition, subgroup analyses in only older adults who were normoglycemic and those with prediabetes at baseline showed that IGFBP-3 was directly associated with incident diabetes; however this association reached statistical significance only in the prediabetes group. Possibly, our study was not powered to detect differences in risk in the normoglycemic subgroup. Our study overcomes limitations of many similar prior studies that have lacked assessment of multiple confounders, have used of self-report without repeated glucose measurements for ascertainment of DM status, and lacked sex-specific analyses. Our study is one of the largest to date in older adults, but it is unknown if results are the same in younger adults. While we showed that associations were gender-specific, women in CHS were uniformly postmenopausal and it is unknown whether menopausal status or endogenous sex hormone levels may influence the associations. Another strength was the use of multiple sources to identify diabetes cases including fasting and non-fasting glucose measures, use of diabetes medication as well as use of administrative codes, which are particularly important for frail elders least likely to attend study visits.

Conclusion

In conclusion, circulating levels of IGFBP-3 are associated with risk of diabetes in women but could not be shown to be associated in men. These findings may be informative for determining which patient populations will benefit from diabetes prevention programs.

Acknowledgements

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. Additional support was provided by T32-GM007288.

Disclosure Statement: The authors have nothing to disclose.

References

1. Le Roith D. Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. *N Engl J Med.* 1997;336(9):633-640.
2. Li E, Kim Y, Kim S, Park S. Ghrelin-induced hippocampal neurogenesis and enhancement of cognitive function are mediated independently of GH/IGF-1 axis: lessons from the spontaneous dwarf rats. *Endocrine journal.* 2013;60(9):1065-1075.
3. Shimasaki S, Shimonaka M, Zhang HP, Ling N. Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a

novel IGFBP-5 in rat and human. *The Journal of biological chemistry*. 1991;266(16):10646-10653.

4. Schedlich LJ, Le Page SL, Firth SM, Briggs LJ, Jans DA, Baxter RC. Nuclear import of insulin-like growth factor-binding protein-3 and -5 is mediated by the importin beta subunit. *The Journal of biological chemistry*. 2000;275(31):23462-23470.
5. Yamanaka Y, Wilson EM, Rosenfeld RG, Oh Y. Inhibition of insulin receptor activation by insulin-like growth factor binding proteins. *The Journal of biological chemistry*. 1997;272(49):30729-30734.
6. Clemmons DR. Role of insulin-like growth factor in maintaining normal glucose homeostasis. *Hormone research in paediatrics*. 2004;62 Suppl 1:77-82.
7. Murphy LJ. The role of the insulin-like growth factors and their binding proteins in glucose homeostasis. *J Diabetes Res*. 2003;4(4):213-224.
8. Holt RI, Simpson HL, Sonksen PH. The role of the growth hormone-insulin-like growth factor axis in glucose homeostasis. *Diabet Med*. 2003;20(1):3-15.
9. Bayes-Genis A, Conover CA, Schwartz RS. The insulin-like growth factor axis: A review of atherosclerosis and restenosis. *Circulation research*. 2000;86(2):125-130.
10. Rajpathak SN, McGinn AP, Strickler HD, Rohan TE, Pollak M, Cappola AR, Kuller L, Xue X, Newman AB, Strotmeyer ES, Psaty BM, Kaplan RC. Insulin-like growth factor-(IGF)-axis, inflammation, and glucose intolerance among older adults. *Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society*. 2008;18(2):166-173.
11. Frayling TM, Hattersley AT, McCarthy A, Holly J, Mitchell SM, Gloyn AL, Owen K, Davies D, Smith GD, Ben-Shlomo Y. A putative functional polymorphism in the IGF-I gene: association studies with type 2 diabetes, adult height, glucose tolerance, and fetal growth in U.K. populations. *Diabetes*. 2002;51(7):2313-2316.
12. Lewitt MS, Hilding A, Brismar K, Efendic S, Ostenson CG, Hall K. IGF-binding protein 1 and abdominal obesity in the development of type 2 diabetes in women. *European journal of endocrinology / European Federation of Endocrine Societies*. 2010;163(2):233-242.
13. Lewitt MS, Hilding A, Ostenson CG, Efendic S, Brismar K, Hall K. Insulin-like growth factor-binding protein-1 in the prediction and development of type 2 diabetes in middle-aged Swedish men. *Diabetologia*. 2008;51(7):1135-1145.
14. Rajpathak SN, He M, Sun Q, Kaplan RC, Muzumdar R, Rohan TE, Gunter MJ, Pollak M, Kim M, Pessin JE, Beasley J, Wylie-Rosett J, Hu FB, Strickler HD. Insulin-like growth factor axis and risk of type 2 diabetes in women. *Diabetes*. 2012;61(9):2248-2254.
15. Sandhu MS, Heald AH, Gibson JM, Cruickshank JK, Dunger DB, Wareham NJ. Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance: a prospective observational study. *Lancet*. 2002;359(9319):1740-1745.
16. Teppala S, Shankar A. Association between serum IGF-1 and diabetes among U.S. adults. *Diabetes Care*. 2010;33(10):2257-2259.
17. Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A, Lamberts SW, Oostra BA, Pols HA, van Duijn CM. A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes*. 2001;50(3):637-642.
18. Drogan D, Schulze MB, Boeing H, Pischon T. Insulin-Like Growth Factor 1 and Insulin-Like Growth Factor-Binding Protein 3 in Relation to the Risk of Type 2 Diabetes Mellitus: Results From the EPIC-Potsdam Study. *American journal of epidemiology*. 2016;183(6):553-560.

19. Schneider HJ, Friedrich N, Klotsche J, Schipf S, Nauck M, Volzke H, Sievers C, Pieper L, Marz W, Wittchen HU, Stalla GK, Wallaschofski H. Prediction of incident diabetes mellitus by baseline IGF1 levels. *European journal of endocrinology / European Federation of Endocrine Societies*. 2011;164(2):223-229.
20. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A. The Cardiovascular Health Study: design and rationale. *Annals of epidemiology*. 1991;1(3):263-276.
21. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. *Clin Chem*. 1995;41(2):264-270.
22. Aneke-Nash CS, Dominguez-Islas C, Buzkova P, Qi Q, Xue X, Pollak M, Strickler HD, Kaplan RC. Agreement between circulating IGF-I, IGFBP-1 and IGFBP-3 levels measured by current assays versus unavailable assays previously used in epidemiological studies. *Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society*. 2016;26:11-16.
23. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. *Clinical chemistry*. 1995;41(2):264-270.
24. Soares-Miranda L, Sattelmair J, Chaves P, Duncan GE, Siscovick DS, Stein PK, Mozaffarian D. Physical activity and heart rate variability in older adults: the Cardiovascular Health Study. *Circulation*. 2014;129(21):2100-2110.
25. Geary MP, Pringle PJ, Rodeck CH, Kingdom JC, Hindmarsh PC. Sexual dimorphism in the growth hormone and insulin-like growth factor axis at birth. *J Clin Endocrinol Metab*. 2003;88(8):3708-3714.
26. Liu Z, Mohan S, Yakar S. Does the GH/IGF-1 axis contribute to skeletal sexual dimorphism? Evidence from mouse studies. *Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society*. 2016;27:7-17.
27. Ashpole NM, Logan S, Yabluchanskiy A, Mitschelen MC, Yan H, Farley JA, Hodges EL, Ungvari Z, Csiszar A, Chen S, Georgescu C, Hubbard GB, Ikeno Y, Sonntag WE. IGF-1 has sexually dimorphic, pleiotropic, and time-dependent effects on healthspan, pathology, and lifespan. *Geroscience*. 2017;39(2):129-145.
28. Ashpole NM, Herron JC, Mitschelen MC, Farley JA, Logan S, Yan H, Ungvari Z, Hodges EL, Csiszar A, Ikeno Y, Humphrey MB, Sonntag WE. IGF-1 Regulates Vertebral Bone Aging Through Sex-Specific and Time-Dependent Mechanisms. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2016;31(2):443-454.
29. Ashpole NM, Herron JC, Estep PN, Logan S, Hodges EL, Yabluchanskiy A, Humphrey MB, Sonntag WE. Differential effects of IGF-1 deficiency during the life span on structural and biomechanical properties in the tibia of aged mice. *Age*. 2016;38(2):38.
30. Bokov AF, Garg N, Ikeno Y, Thakur S, Musi N, DeFronzo RA, Zhang N, Erickson RC, Gelfond J, Hubbard GB, Adamo ML, Richardson A. Does reduced IGF-1R signaling in Igf1r^{+/-} mice alter aging? *PLoS one*. 2011;6(11):e26891.
31. Kadowaki T. Insights into insulin resistance and type 2 diabetes from knockout mouse models. *The Journal of clinical investigation*. 2000;106(4):459-465.
32. Angelini A, Bendini C, Neviani F, Bergamini L, Manni B, Trenti T, Rovati R, Neri M. Insulin-like growth factor-1 (IGF-1): relation with cognitive functioning and neuroimaging

marker of brain damage in a sample of hypertensive elderly subjects. *Archives of gerontology and geriatrics*. 2009;49 Suppl 1:5-12.

33. Cukierman T, Gerstein HC, Williamson JD. Cognitive decline and dementia in diabetes--systematic overview of prospective observational studies. *Diabetologia*. 2005;48(12):2460-2469.

34. Silha JV, Gui Y, Murphy LJ. Impaired glucose homeostasis in insulin-like growth factor-binding protein-3-transgenic mice. *American journal of physiology Endocrinology and metabolism*. 2002;283(5):E937-945.

35. Kaplan RC, Strizich G, Aneke-Nash C, Dominguez-Islas C, Buzkova P, Strickler H, Rohan T, Pollak M, Kuller L, Kizer JR, Cappola A, Li CI, Psaty BM, Newman A. Insulinlike Growth Factor Binding Protein-1 and Ghrelin Predict Health Outcomes Among Older Adults: Cardiovascular Health Study Cohort. *J Clin Endocrinol Metab*. 2017;102(1):267-278.

36. Robertson K, Lu Y, De Jesus K, Li B, Su Q, Lund PK, Liu JL. A general and islet cell-enriched overexpression of IGF-I results in normal islet cell growth, hypoglycemia, and significant resistance to experimental diabetes. *American journal of physiology Endocrinology and metabolism*. 2008;294(5):E928-938.

Table 1: Baseline Characteristics of the Cardiovascular Health Study Participants

Characteristic	1993/1994	
	Women	Men
	N=1879	N=1254
	Mean (SD)	Mean (SD)
DEMOGRAPHICS		
Age (years)	75.3 (4.9)	75.9(5.2)
Black Race, n (%)	272 (14.5)	152 (12.1)
ANTHROPOMETRICS		
BMI (kg/m ²)	26.6 (5.0) ^a	26.5 (3.6) ^a
CARDIOVASCULAR		
Systolic BP (mmHg)	134.6 (20.6)	132.3 (18.8)
Diastolic BP (mmHg)	70.0 (10.8)	71.4 (10.8)
Hypertension, n (%)	823 (43.8)	473 (37.7)
Cholesterol (mg/dL)	209.0 (34.0)	188.0 (33.3)
INFLAMMATION		
CRP (mg/L)	5.2 (9.4) ^a	4.1 (7.1) ^a
GLUCOSE METABOLISM		
Fasting glucose (mg/dL)	96.9 (9.5) ^a	98.8 (9.5) ^a
2-hour glucose (mg/dL)	137.5 (44.8)*	127.9 (41.3)*
Fasting Insulin (IU/mL)	14.4 (6.3) ^a	14.4 (6.2) ^a
2-hour Insulin (IU/mL)	85.2 (63.0)*	73.7 (52.7)*
Adiponectin (mg/L)	16.2 (7.9)	11.5 (5.9)
BEHAVIORS		
Physical Act (Kcal/wk)	1321 (1603) ^a	1885 (1980) ^a
Smoking, n (%)	175 (9.3)	106 (8.5)
Alcohol (drinks/week)	1.6 (4.3)	3.5 (7.0)
MEDICATION		
Hormone therapy n (%)	299 (15.9)	n/a
IGF BIOMARKERS		
Total IGF-I (µg/l)	92.7 (30.7)	106.5 (34.2)
IGFBP-3 (µg/l)	3734 (843)	3398 (804)

[†] Variables were log-transformed

* Oral glucose tolerance tests were not performed at the 1993/1994 visits. Thus, values reported are means (sd) from 1996-1997 visits.

^a Values reported are extracted from data collected during the 1992-1993 visits. For BMI, height was measured at the 1992-1993 visits.

IGF-I: insulin-like growth factor-I, IGFBP: insulin-like growth factor binding protein, BP: blood pressure, CRP: C-reactive protein, 2-h: 2-hour measure.

Table 2: Correlations between IGF Variables and Covariates.

	Total IGF-I		IGFBP-3	
	Women	Men	Women	Men
Age	-0.06 ^b	-0.17 ^d	-0.14 ^d	-0.23 ^d
BMI ^e	0.05 ^a	0.05	0.04	0.09 ^d
Systolic BP	-0.03	-0.01	-0.03	0.03
Diastolic BP	0.03	0.04	0.01	0.09 ^d
Cholesterol	0.1 ^d	0.06 ^a	0.21 ^d	0.22 ^d
Fasting glucose ^e	0.11 ^d	0.01	0.15 ^d	0.06 ^d
2-h glucose*	0.08 ^b	0.04	0.14 ^d	0.05
Fasting insulin ^e	0.13 ^d	0.04	0.13 ^d	0.04
2-h Insulin*	0.13 ^d	0.1 ^b	0.15 ^d	0.09 ^b
Adiponectin	-0.05 ^a	-0.13 ^d	-0.11 ^d	-0.15 ^d
Physical Activity ^e (kcal/wk)	-0.02	0.02	0.01	0.05
Alcohol (drinks/wk)	-0.03	-0.04	0.07 ^b	0.08 ^d
CRP ^e	-0.06 ^a	-0.01	0.01	-0.03

^j Variables were log-transformed

* For correlations with total IGF-I and IGFBP-3, measures from 1996/1997 visits were used since oral glucose tolerance tests were not performed at the 1993/1994 visits. IGF-I: insulin-like growth factor-I, IGFBP: insulin-like growth factor binding protein, BP: blood pressure, CRP: C-reactive protein, 2-h: 2-hour measure. Physical Activity was measured in kilo-calories per week and alcohol was measured as number of alcoholic beverages per week.

^e Values reported are extracted from data collected during the 1992-1993 visits. For BMI, height was measured at the 1992-1993 visits.

a: p<0.05

b: p<0.01

c: p<0.001

d: p<0.0001

Table 3: HRs (95 % CI) for Incident Diabetes by tertiles of IGF proteins in women (n=1879)

	Hazard Ratios (95% CI)			
	Tertile 1 (n=627)	Tertile 2 (n=626)	Tertile 3 (n=626)	p-value (trend)
Total IGF-I, (µg/l)	26.5 – 77.4	77.4 – 101.3	101.3 – 281	
Subjects, cases/total	79/627	87/626	102/626	
Adjusted for age, race and BMI	ref	1.04 (0.77 - 1.41)	1.28 (0.95 - 1.72)	0.09
Multivariate adjusted	ref	0.80 (0.57 - 1.12)	0.74 (0.50 - 1.09)	0.171
IGFBP-3, (µg/l)	1425 – 3357	3358 – 4077	4078 – 6841	
Subjects, cases/total	64/627	82/626	122/626	
Adjusted for age, race and BMI	ref	1.30 (0.93 - 1.80)	1.94 (1.43 - 2.64)	0.0001
Multivariate adjusted	ref	1.52 (1.07 - 2.16)	2.30 (1.55 - 3.40)	0.0001

Multivariate adjusted: age, race, BMI, smoking, alcohol, cholesterol, hypertension, C-reactive protein, physical activity adiponectin, and fasting insulin. For IGF-I, Multivariate adjusted models adjusted additionally for IGFBP-3 levels; for IGFBP-3, Multivariate adjusted models adjusted additionally for IGF-I levels.

Table 4: HRs (95 % CI) for Incident Diabetes by Tertiles of IGF Proteins in Men (n=1254)

	Hazard Ratios (95% CI)			
	Tertile 1 (n=418)	Tertile 2 (n=418)	Tertile 3 (n=418)	p-value (trend)
Total IGF-I, (µg/l)	24.9 – 89.6	89.7 – 116.4	116.5 – 412.4	
Subjects, cases/total	62/418	55/418	60/418	
Adjusted for age, race and BMI	ref	0.81 (0.56 - 1.17)	0.85 (0.59 - 1.22)	0.418
Multivariate adjusted	ref	0.78 (0.52 - 1.17)	0.78 (0.49 - 1.25)	0.348
IGFBP-3, (µg/l)	1235 – 3042	3043 – 3727	3730 – 7633	
Subjects, cases/total	54/418	62/420	61/416	
Adjusted for age, race and BMI	ref	1.06 (0.74 - 1.53)	0.95 (0.65 - 1.38)	0.763
Multivariate adjusted	ref	1.14 (0.76 - 1.70)	1.06 (0.66 - 1.71)	0.821

Multivariate adjusted: age, race, BMI, smoking, alcohol, cholesterol, hypertension, C-reactive protein, physical activity adiponectin, and fasting insulin. For IGF-I, Multivariate adjusted adjusts additionally for IGFBP-3 levels; for IGFBP-3, Multivariate adjusted adjusts additionally for IGF-I level.