Published in final edited form as:

Metabolism. 2015 October ; 64(10): 1316–1323. doi:10.1016/j.metabol.2015.07.013.

Associations between metabolic dysregulation and circulating biomarkers of fibrosis: the Cardiovascular Health Study

Isha Agarwal, AB/AM^a, Nicole L. Glazer, PhD^b, Eddy Barasch, MD^c, Luc Djousse, MD DSc^d, John S. Gottdiener, MD^e, Joachim H. Ix, MD MAS^f, Jorge R. Kizer, MD MSc^g, Eric B. Rimm, SD^{a,h}, David S. Siscovick, MD MPHⁱ, George L. King, MD^j, and Ken J. Mukamal, MD MPH^k

^aDepartments of Epidemiology and Nutrition, Harvard School of Public Health, MA, USA

^bDepartment of Medicine, Boston University, MA, USA

^cDepartment of Research and Education, St. Francis Hospital, The Heart Center, SUNY at Stonybrook, NY, USA

^dDepartment of Medicine, Brigham and Women's Hospital, MA, USA

^eDepartment of Medicine, University of Maryland Medical School, MD, USA

^fDepartment of Medicine, University of California San Diego and Veterans Affairs San Diego Healthcare System, CA, USA

^gDepartments of Medicine, Epidemiology, and Population Health, Albert Einstein College of Medicine, NY, USA

^hChanning Division of Network Medicine, Brigham and Women's Hospital, MA, USA

ⁱThe New York Academy of Medicine, New York, NY, USA, and Departments of Medicine and Epidemiology, Cardiovascular Health Research Unit, University of Washington, WA, USA

^jResearch Division, Joslin Diabetes Center and Department of Medicine, Harvard Medical School, MA, USA

^kDepartment of Medicine, Beth Israel Deaconess Medical Center, MA, USA

Abstract

Aim—Fibrosis is one postulated pathway by which diabetes produces cardiac and other systemic complications. Our aim was to determine which metabolic parameters are associated

Corresponding author: Isha Agarwal, ScD, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston MA 02115, Fax: 617.566.7805, Phone: 617.432.1050, Isha_Agarwal@hms.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosure Statement

There are no conflicts of interest to disclose.

Author contributions

I.A. and K.J.M. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. I.A. wrote the manuscript. N.L.G., E.B., M.L.B., L.D., A.L.F., J.S.D., J.H.I., J.R.K., E.B.R., D.S.S., R.P.T, G.L.K, and K.J.M. reviewed and edited the manuscript.

with circulating fibrosis-related biomarkers transforming growth factor- β (TGF- β) and procollagen type III N-terminal propeptide (PIIINP).

Methods—We used linear regression to determine the cross-sectional associations of diverse metabolic parameters, including fasting glucose, fasting insulin, body mass index, fatty acid binding protein 4, and non-esterified fatty acids, with circulating levels of TGF- β ($n = 1,559$) and PIIINP ($n = 3,024$) among community-living older adults in the Cardiovascular Health Study.

Results—Among the main metabolic parameters we examined, only fasting glucose was associated with TGF- β ($P = 0.03$). In contrast, multiple metabolic parameters were associated with PIIINP, including fasting insulin, body mass index, and non-esterified fatty acids ($P < 0.001$, $P < 0.001$, $P = 0.001$, respectively). These associations remained statistically significant after mutual adjustment, except the association between BMI and PIIINP.

Conclusions—Isolated hyperglycemia is associated with higher serum concentrations of TGF- β , while a broader phenotype of insulin resistance is associated with higher serum PIIINP. Whether simultaneous pharmacologic targeting of these two metabolic phenotypes can synergistically reduce the risk of cardiac and other manifestations of fibrosis remains to be determined.

Keywords

fibrosis; growth factors; hyperglycemia; insulin resistance

2. Introduction

Emerging evidence suggests that fibrosis may play a role in mediating the association between diabetes and cardiovascular disease [1, 2]. In *in vitro* models, high glucose concentrations have been shown to activate the diacylglycerol-protein kinase C (DAG-PKC) pathway [3]. This pathway stimulates the production of pro-fibrotic matrix cytokines such as transforming growth factor- β (TGF- β), and the downstream release of collagen byproducts such as procollagen type III N-terminal propeptide (PIIINP). TGF- β and PIIINP are novel and complementary biomarkers of fibrosis [4–6] that have been independently associated with risk of incident heart failure, myocardial infarction, and stroke in multiple large, community-based cohorts [7–10]. In humans, TGF- β levels are elevated in diabetes compared to normoglycemia [11]. Whether this elevation is due to hyperglycemia *per se* or other correlates of metabolic dysregulation is unknown.

Knowledge of the specific metabolic parameters associated with fibrosis could enable a targeted approach to prevent vascular and myocardial fibrosis. To our knowledge, no studies have examined whether metabolic parameters other than hyperglycemia are associated with TGF- β . Two studies, both conducted in middle-aged populations, have attempted to establish the specific metabolic parameters associated with PIIINP [12, 13]; however, the first [12] was conducted in a relatively small cohort of 160 individuals, and the second [13] was conducted without in-depth, fasting and post-challenge measurements of metabolic disturbance. Neither study included a second, complementary biomarker of fibrosis such as TGF- β .

The aim of this study was to determine the cross-sectional associations of diverse indicators of metabolic disturbance, including diabetes status, fasting glucose, fasting insulin, body mass index (BMI), fatty acid binding protein 4 (FABP4), and non-esterified fatty acids (NEFA), with circulating levels of TGF- β and PIIINP among community-living older adults in the Cardiovascular Health Study (CHS). In the subset of our cohort in which measurements were available, we additionally assessed the associations of 2-hour glucose and insulin, Gutt insulin sensitivity index (ISI), and carboxymethyl lysine (CML; a marker of advanced glycosylated end products) with TGF- β and PIIINP.

3. Methods

3.1. Study Design

The design, rationale and details of the examinations in CHS have previously been published [14]. In brief, 5,201 participants were recruited in 1989–1990 from Medicare eligibility lists in four US communities: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. A supplemental cohort of 687 mostly African-American participants was added in 1992–1993 and is also included in this analysis. Eligible individuals were at least 65 years old, living in the community, and expected to remain in that community for at least three years after baseline. Individuals were excluded if they were under active cancer treatment or unable to provide written informed consent. Follow-up interviews to ascertain incident events were conducted at annual study visits through 1998–1999. All participants in our study provided written informed consent, and the institutional review board at each center approved the study protocol.

3.2. Exposure Assessment

We collected serum samples at the 1996–1997 visit after an 8–12 hour overnight fast, and again 2 hours after a 75-g oral glucose tolerance test (OGTT) [15]. Fasting and 2-hour glucose were measured with a standard clinical chemistry analyzer (Eastman Kodak, Rochester, NY). Fasting and 2-hour insulin were measured by competitive radioimmunoassay (Diagnostic Products Corporation, Malvern, PA) [16]. We defined impaired fasting glucose as fasting glucose 100–125 mg/dL, and diabetes as fasting glucose ≥ 126 mg/dL or use of insulin or oral hypoglycemic medications. We calculated the Gutt ISI ($\text{mg} \cdot \text{L}^2 / \text{mmol} \cdot \text{mU} \cdot \text{min}$) as $\text{insulin sensitivity} = m / (G \times I)$, where m is a measure of glucose uptake during the OGTT calculated from body weight and fasting and 2-hour glucose, G is the mean of fasting and 2-hour glucose, and I is a log10 transformation of the mean of fasting and 2-hour insulin [17]. We used technician-measured height and weight from the 1996–1997 visit to calculate BMI (the weight in kilograms divided by the square of the height in meters). CML was measured from 1996–1997 serum samples using a photometric enzyme-linked immunosorbent assay (ELISA) (Microcoat, Penzberg, Germany) [18]. Because 1996–1997 data were unavailable, we measured plasma FABP4 and NEFA from plasma collected at the 1992–1993 visit. FABP4 concentration was measured using ELISA (Biovendor ELISA); NEFA concentration was measured by the Wako enzymatic method [19].

3.3. Outcome Assessment

TGF- β and PIIINP were measured from stored 1996–1997 EDTA plasma samples in 2011–2012. TGF- β was measured by ELISA (Quantikine Human TGF- β 1 Immunoassay; R&D Systems, Minneapolis, MN). PIIINP was measured by the UniQ Intact N terminal Propeptide of Type III Procollagen radioimmunoassay kit (Orion Diagnostics, Fountain Hills, AZ). Inter- and intra-assay coefficients of variation (CVs) for TGF- β were between 1.9–2.9% and 6.4–9.3%, respectively. For PIIINP, both CVs were less than 7.2%.

In pilot studies, we identified probable platelet contamination resulting in artificially elevated levels of TGF- β [20] at two of our four CHS sites. Hence, we measured TGF- β only at the two remaining sites, *a priori*. Of the two sites at which TGF- β was not measured, one site (Washington County, Maryland) did not enroll an African American cohort in 1992–1993. However, participant characteristics other than race were similar between sites where TGF- β was and was not measured (eTable 1). Our analysis included 1,559 individuals with measured levels of TGF- β and 3,024 individuals with measured levels of PIIINP.

3.4. Covariate Assessment

When possible, covariate measurements were taken from the 1996–1997 visit, which is baseline for these analyses. If 1996–1997 data was unavailable, we used data from the 1995–1996 visit, or earlier visits depending on data availability. Participants self-reported age, sex, race, smoking history, and use of statins, anti-hypertensive medications (including diuretics, β -blockers, ACE inhibitors, calcium channel blockers, and other anti-hypertensive medications), oral hypoglycemic agents, and insulin using a validated medication inventory [21]. Trained study personnel measured systolic blood pressure (SBP) using standardized protocols. Blood and urine samples were obtained from participants to measure total cholesterol, C-reactive protein (CRP), cystatin-C based estimated glomerular filtration rate (eGFR), and serum albumin using standard methods.

3.5. Statistical Analysis

We examined the distribution of TGF- β and PIIINP by the above listed covariates and calculated Spearman correlations and bivariate associations using the Kruskal-Wallis test, categorizing categorical variables according to clinically-relevant cutpoints.

We used multivariable linear regression with robust standard errors to assess the associations of diabetes status (normoglycemic, impaired fasting glucose, diabetes) with TGF- β and PIIINP, using normoglycemia as the reference category. Among individuals not using insulin or oral hypoglycemic agents, we evaluated the associations of diabetes-related metabolic parameters (fasting glucose and insulin, BMI, FABP4, and NEFA) with TGF- β and PIIINP. Several metabolic parameters were not available in the full cohort, therefore we separately evaluated associations of 2-hour glucose and insulin, Gutt ISI, and CML with TGF- β and PIIINP. Although the distributions of TGF- β and PIIINP were both right skewed, we chose not to ln-transform TGF- β or PIIINP in our main analysis to enhance interpretability. However, we present robust standard errors to accommodate non-normality of our outcome variables. To improve model fit, we ln-transformed several metabolic parameters, including

fasting and 2-hour insulin, NEFA, and Gutt ISI. As a sensitivity analysis, we re-estimated our models after ln-transforming TGF- β and PIIINP.

We created sequential models, choosing covariate forms based on graphical analysis and model likelihood parameters. Model 1 adjusted for sociodemographic variables including age, sex, race, and clinic site. Model 2 additionally adjusted for potential confounders including pack years, smoking status (never, former, current), SBP (quintiles), statins, use of medications for hypertension, total cholesterol (quintiles), ln(CRP), eGFR (quintiles), and albumin. In sensitivity analysis, we explored mutual adjustment for our main metabolic parameters (fasting glucose and insulin, BMI, FABP4, and NEFA).

For metabolic parameters with data available at more than one time point, we explored differences in the associations of prior (1992–1993) vs. current (1996–1997) measurements with TGF- β and PIIINP from 1996–1997. Where we observed interactions between prior and current measurements of metabolic parameters, we conducted stratified analyses dichotomized at the median of the prior (1992–1993) measurements.

All analyses were conducted in SAS (SAS Institute, Cary, North Carolina). *P*-values < 0.05 were considered statistically significant for all analyses, including interaction terms.

4. Results

Measured levels of both TGF- β and PIIINP were higher among non-blacks, among individuals with higher CRP, and among individuals with worse renal function (Table 1). TGF- β levels were weakly positively correlated with PIIINP levels (Spearman $r=0.07$, $P=0.007$).

4.1. Diabetes Status

Graphically, we observed a graded, positive relationship between categories of fasting glucose (normoglycemia, impaired fasting glucose, diabetes) and TGF- β (Figure 1). In contrast, PIIINP levels were similar except among individuals with diabetes, in whom they were elevated.

4.2. Diabetes-related Metabolic Parameters

Among the main metabolic parameters we examined (Table 2), only fasting glucose was associated with TGF- β ($P=0.03$). Multiple metabolic parameters were associated with PIIINP, including fasting insulin, body mass index, and non-esterified fatty acids ($P<0.001$, $P<0.001$, $P=0.001$, respectively). These associations remained statistically significant after mutual adjustment, except the association between BMI and PIIINP, which was attenuated (eTable 2). Among the metabolic parameters associated with PIIINP, the standardized association of fasting insulin with PIIINP was largest in magnitude (eTable 2). Our conclusions were similar after ln-transformation of TGF- β or PIIINP in sensitivity analysis (eTable 3).

4.3. Secondary Analysis

In our secondary analysis conducted in a limited cohort in whom measurements were available (1,422 individuals with TGF- β and 2,759 individuals with PIIINP), 2-hour glucose was associated with TGF- β ($P = 0.01$), as was Gutt ISI ($P = 0.006$), which is calculated based on glucose measurements (Table 3). Diverse metabolic phenotypes were associated with PIIINP including 2-hour glucose, 2-hour insulin, and Gutt ISI ($P = 0.02$, $P = 0.002$, $P = 0.001$ respectively). Our conclusions were similar after ln-transformation of TGF- β or PIIINP in sensitivity analysis (eTable 3).

4.4. Current and Prior Metabolic Exposures

With the exception of BMI, associations between current (1996–1997) measurements of metabolic phenotypes and fibrosis-related biomarkers were generally stronger than associations between prior (1992–1993) measurements of metabolic phenotypes and fibrosis-related biomarkers (Table 4). We observed an interaction between current and prior fasting insulin for PIIINP (P -interaction = 0.007), and conducted stratified analyses accordingly. Strata were dichotomized at the median level of prior fasting insulin, 2.52 ln(IU/mL). The association between current fasting insulin and PIIINP was larger in magnitude among individuals with higher prior fasting insulin than among individuals with lower prior fasting insulin, suggesting that a longer duration of elevated fasting insulin is associated with higher levels of PIIINP. Other interactions between current and prior measurements were not statistically significant (all P -interaction > 0.05).

5. Discussion

TGF- β and PIIINP are two complementary biomarkers of fibrosis that have been independently associated with risk of incident heart failure, myocardial infarction, and stroke [7–10]. To our knowledge, our study is the first of its size to evaluate the associations of diverse, in-depth metabolic parameters with both TGF- β and PIIINP. We found that only hyperglycemia was associated with TGF- β , while multiple measures of insulin resistance were associated with PIIINP. Our findings suggest that elevations in TGF- β and PIIINP may represent distinct patterns of metabolic disturbances (Figure 2).

5.1. TGF- β

We observed that hyperglycemia is positively associated with TGF- β . Our finding is supported by previous experimental studies, which have observed an increase in mRNA and protein expression of TGF- β in cells exposed to high glucose concentrations [22–24]. In rodent models of diabetes, mRNA and protein levels of TGF- β have been shown to increase three days after the onset of overt hyperglycemia [25]. Limited data are available in human cohorts. However, in one experimental study of normoglycemic, middle-aged volunteers there was a significant increase in urinary TGF- β levels after 120 minutes of glucose exposure using a glucose-clamp procedure [26]. In our study, we observed a graded, positive association between categories of glycemic control (normoglycemic, impaired fasting glucose, diabetes) and circulating TGF- β concentrations. Additionally, fasting and 2-hour glucose were associated with circulating TGF- β , as well as Gutt ISI, which is calculated based on fasting and 2-hour glucose measurements. These observations support the

experimental evidence that hyperglycemia *per se* may increase the expression of TGF- β via activation of the DAG-PKC pathway [27].

5.2. PIIINP

Multiple metabolic parameters related to insulin resistance were associated with higher serum PIIINP concentrations, including higher fasting insulin, BMI, NEFA, 2-hour glucose and insulin, and Gutt ISI. Two previous studies have investigated associations between metabolic parameters and serum PIIINP levels, with results that are consistent with our study. In one hospital-based study conducted among 160 non-diabetic obese individuals, circulating PIIINP was correlated with glucose concentration, insulin sensitivity, insulin, and parameters associated with the insulin resistance syndrome (high-density lipoprotein cholesterol and fat trunk/fat leg ratio) [12]. In a second study conducted among 967 community-based individuals in the Framingham Heart Study, BMI was positively associated with circulating PIIINP (fasting and 2-hour laboratory measurements were not collected) [13]. Our study found that BMI was associated with PIIINP; however this association was no longer significant after mutual adjustment for fasting insulin, NEFA, and other metabolic parameters. One possible explanation for this is that insulin may mediate the association of BMI with PIIINP. Taken together, our results suggest that a broad insulin resistance phenotype may drive collagen synthesis and, downstream, PIIINP production.

5.3. Implications

Given our finding that two distinct patterns of metabolic disturbance (isolated hyperglycemia and a broader phenotype of insulin resistance) are associated with TGF- β and PIIINP, future efforts to prevent the cardiovascular complications of diabetes may need to include multi-pronged lifestyle and pharmacological strategies that target both hyperglycemia and metabolic disturbances such as elevated fasting insulin and NEFA. Dietary modifications may play an important role; one recent study in mice has demonstrated a causal relationship between a high fat diet and cardiac fibrosis [28]. Simultaneous targeting of multiple metabolic pathways could synergistically reduce the risk of fibrosis and downstream cardiovascular complications. Additional interventional studies will be required to investigate this hypothesis.

Given the differences in the metabolic parameters correlated with TGF- β and PIIINP, it is tempting to speculate that these biomarkers may be differentially associated with type 1 and type 2 diabetes risk. Type 1 diabetes is characterized by glycemic dysregulation, while type 2 diabetes is characterized by a broader phenotype of insulin resistance. Aggressive glycemic control has been shown to reduce the risk of microvascular and cardiovascular disease among individuals with type 1 diabetes [29], while among individuals with type 2 diabetes, the benefit of intensive therapy has been demonstrated for microvascular but not cardiovascular disease [30]. Based on the strong associations that we observed between glycemic dysregulation and TGF- β , it is possible that TGF- β may be a type 1 diabetes-selective marker of cardiovascular disease risk. In contrast, PIIINP may be a better marker of cardiovascular disease risk among individuals with type 2 diabetes, in whom multiple metabolic factors contribute to cardiovascular disease risk [31, 32]. Studies conducted

specifically among individuals with type 1 or type 2 diabetes are required to evaluate these preliminary hypotheses.

5.4. Strengths and Limitations

Strengths of our study include a large, well-characterized, community-based population and the availability of a diverse range of adjustment variables to minimize confounding in multivariable-adjusted models. We focused our work on older adults, a population in which the metabolic correlates of fibrosis have not previously been studied. Age-associated changes in glucose metabolism, body composition, comorbidity, and lifestyle distinguish the clinical picture of diabetes in older adults from other age groups. Rather than focusing exclusively on diabetes status in our study, we examined diverse indicators of metabolic disturbance including fasting and 2-hour glucose and insulin, BMI, FABP 4, NEFA, insulin sensitivity, and CML. This detailed phenotypic information allowed us to elucidate specific metabolic parameters associated with TGF- β and PIIINP. Furthermore, we were able to examine the full range of glucose homeostasis, given the population-based design of our study. Finally, we investigated both TGF- β and PIIINP, two biomarkers that provide complementary measures of fibrosis.

Our study is not without limitations. The metabolic phenotypes in our analysis cannot be interpreted as risk factors for fibrosis given the lack of temporality in our dataset. Ultimately, our results require corroboration by prospective studies to infer temporality. Also, we measured TGF- β on a smaller sample of participants, and thus had more limited statistical power to detect associations of metabolic phenotypes with TGF- β . It is possible that pre-analytical variability in levels of TGF- β precluded our ability to detect associations between TGF- β and other study variables, though we were able to detect an association between TGF- β and glucose. Additionally, we were unable to assess the association between hemoglobin A1c and fibrosis-related biomarkers as hemoglobin A1c was not available for study participants. Furthermore, we were unable to directly evaluate the association between diabetes duration and fibrosis-related biomarkers. Where available, we did evaluate associations of both current and prior measurements of metabolic parameters with fibrosis-related biomarkers; however, the high correlations between these repeated measures limited our ability to make distinctions.

5.5. Summary

There is growing recognition that fibrosis may play a fundamental role in the development of the cardiovascular complications of diabetes. Our findings provide evidence that multiple metabolic parameters are associated with circulating biomarkers of fibrosis, beyond hyperglycemia alone. Early lifestyle and pharmacologic intervention to control these diverse metabolic disturbances may improve our ability to prevent fibrosis and its downstream effects on vascular and myocardial disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

None.

Funding

This research was supported by contracts HL118775, HL094555, HHSN268201200036C, HHSN268200800007C, N01 HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant HL080295 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 AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

Abbreviations

TGF-β	transforming growth factor-β
PIIINP	procollagen type III N-terminal propeptide
DAG-PKC	diacylglycerol-protein kinase C
BMI	body mass index
FABP-4	fatty acid binding protein 4
NEFA	non-esterified fatty acids
CHS	Cardiovascular Health Study
ISI	insulin sensitivity index
CML	carboxymethyl lysine
OGTT	oral glucose tolerance test
ELISA	enzyme-linked immunosorbent assay
CV	coefficient of variation
SBP	systolic blood pressure
CRP	c-reactive protein
eGFR	estimated glomerular filtration rate
NHLBI	National Heart, Lung, and Blood Institute
NINDS	National Institute of Neurological Disorders and Stroke
NIA	National Institute on Aging

References

1. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol. 2000; 35:569–582. [PubMed: 10716457]

2. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N Engl J Med.* 1994; 330:1431–1438. [PubMed: 8159199]
3. Koya D, Jirousek MR, Lin YW, Ishii H, Kuboki K, King GL. Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest.* 1997; 100:115–126. [PubMed: 9202063]
4. Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. *Cardiovasc Res.* 2007; 74:196–206. [PubMed: 17376414]
5. Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF)-beta signaling in cardiac remodeling. *J Mol Cell Cardiol.* 2011; 51:600–606. [PubMed: 21059352]
6. Lopez B, Gonzalez A, Diez J. Circulating biomarkers of collagen metabolism in cardiac diseases. *Circulation.* 2010; 121:1645–1654. [PubMed: 20385961]
7. Barasch E, Gottdiener JS, Aurigemma G, et al. The relationship between serum markers of collagen turnover and cardiovascular outcome in the elderly: the Cardiovascular Health Study. *Circ Heart Fail.* 2011; 4:733–739. [PubMed: 21900186]
8. Barasch E, Gottdiener JS, Aurigemma G, et al. Association between elevated fibrosis markers and heart failure in the elderly: the cardiovascular health study. *Circ Heart Fail.* 2009; 2:303–310. [PubMed: 19808353]
9. Velagaleti RS, Gona P, Sundstrom J, et al. Relations of biomarkers of extracellular matrix remodeling to incident cardiovascular events and mortality. *Arterioscler Thromb Vasc Biol.* 2010; 30:2283–2288. [PubMed: 20798380]
10. Agarwal I, Glazer NL, Barasch E, et al. Fibrosis-related biomarkers and risk of total and cause-specific mortality: the cardiovascular health study. *Am J Epidemiol.* 2014; 179:1331–1339. [PubMed: 24771724]
11. Pfeiffer A, Middelberg-Bisping K, Drewes C, Schatz H. Elevated plasma levels of transforming growth factor-beta 1 in NIDDM. *Diabetes Care.* 1996; 19:1113–1117. [PubMed: 8886558]
12. Quilliot D, Alla F, Bohme P, et al. Myocardial collagen turnover in normotensive obese patients: relation to insulin resistance. *Int J Obes (Lond).* 2005; 29:1321–1328. [PubMed: 16116494]
13. Wang TJ, Larson MG, Benjamin EJ, et al. Clinical and echocardiographic correlates of plasma procollagen type III amino-terminal peptide levels in the community. *Am Heart J.* 2007; 154:291–297. [PubMed: 17643579]
14. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol.* 1991; 1:263–276. [PubMed: 1669507]
15. Thacker EL, Psaty BM, McKnight B, et al. Fasting and post-glucose load measures of insulin resistance and risk of ischemic stroke in older adults. *Stroke.* 2011; 42:3347–3351. [PubMed: 21998054]
16. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. *Clin Chem.* 1995; 41:264–270. [PubMed: 7874780]
17. Gutt M, Davis CL, Spitzer SB, et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract.* 2000; 47:177–184. [PubMed: 10741566]
18. Whitson HE, Arnold AM, Yee LM, et al. Serum Carboxymethyl-Lysine, Disability, and Frailty in Older Persons: The Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci.* 2013
19. Djousse L, Khawaja O, Bartz TM, et al. Plasma fatty acid-binding protein 4, nonesterified fatty acids, and incident diabetes in older adults. *Diabetes Care.* 2012; 35:1701–1707. [PubMed: 22584136]
20. Fredericks S, Holt DW. TGF-beta quantitation can be tricky. *Transplantation.* 1999; 68:468–469. [PubMed: 10480399]
21. Psaty BM, Lee M, Savage PJ, Rutan GH, German PS, Lyles M. Assessing the use of medications in the elderly: methods and initial experience in the Cardiovascular Health Study. The Cardiovascular Health Study Collaborative Research Group. *J Clin Epidemiol.* 1992; 45:683–692. [PubMed: 1607909]
22. Rocco MV, Chen Y, Goldfarb S, Ziyadeh FN. Elevated glucose stimulates TGF-beta gene expression and bioactivity in proximal tubule. *Kidney Int.* 1992; 41:107–114. [PubMed: 1593845]

23. Ziyadeh FN, Sharma K, Ericksen M, Wolf G. Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by autocrine activation of transforming growth factor-beta. *J Clin Invest.* 1994; 93:536–542. [PubMed: 8113392]
24. Hoffman BB, Sharma K, Zhu Y, Ziyadeh FN. Transcriptional activation of transforming growth factor-beta1 in mesangial cell culture by high glucose concentration. *Kidney Int.* 1998; 54:1107–1116. [PubMed: 9767526]
25. Sharma K, Ziyadeh FN. Renal hypertrophy is associated with upregulation of TGF-beta 1 gene expression in diabetic BB rat and NOD mouse. *Am J Physiol.* 1994; 267:F1094–F2001. [PubMed: 7810696]
26. McGowan TA, Dunn SR, Falkner B, Sharma K. Stimulation of urinary TGF-beta and isoprostanes in response to hyperglycemia in humans. *Clin J Am Soc Nephrol.* 2006; 1:263–268. [PubMed: 17699215]
27. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab.* 2013; 17:20–33. [PubMed: 23312281]
28. Wang Z, Li L, Zhao H, Peng S, Zuo Z. Chronic high fat diet induces cardiac hypertrophy and fibrosis in mice. *Metabolism.* 2015; 64:917–925. [PubMed: 25982698]
29. Nathan DM, Bayless M, Cleary P, et al. Diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: advances and contributions. *Diabetes.* 2013; 62:3976–3986. [PubMed: 24264395]
30. Hemmingsen B, Lund SS, Gluud C, et al. Targeting intensive glycaemic control versus targeting conventional glycaemic control for type 2 diabetes mellitus. *Cochrane Database Syst Rev.* 2013; 11:CD008143. [PubMed: 24214280]
31. Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circ Res.* 2010; 106:1319–1331. [PubMed: 20431074]
32. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care.* 1991; 14:173–194. [PubMed: 2044434]

Figure 1.

Multivariable-adjusted associations of diabetes status with TGF-β (ng/L) and PIIINP (ng/L), using normoglycemia as the reference category

FG: Fasting Glucose, PIIINP: Procollagen type III N-terminal propeptide, TGF-β: Transforming growth factor-β

* $P = 0.06$ for TGF-β, $P = 0.01$ for PIIINP

P -trend = 0.057 for TGF-β, P -trend = 0.032 for PIIINP

Adjusted for age, sex, race, clinic, pack years, smoking status, systolic blood pressure, statins, use of hypertension medications, total cholesterol, C-reactive protein, estimated glomerular filtration rate, and serum albumin

Figure 2.

Schematic diagram depicting a single pathway from hyperglycemia to TGF-β and multiple pathways from insulin resistance to PIIINP.

BMI: Body Mass Index, PIIINP: Procollagen type III N-terminal propeptide, PKC: Protein Kinase C, TGF-β: Transforming growth factor-β

Squares indicate fibrosis biomarkers of interest.

Baseline characteristics of the Cardiovascular Health Study participants and bivariate associations with TGF- β and PIIINP, 1996–1997 study visit

Table 1

Risk factor	TGF- β , ng/mL			PIIINP, ng/mL		
	N	Median (IQ range)	P-value ^a	N	Median (IQ range)	P-value ^a
Age:						
<80	1,115	3.4 (2.0, 6.1)	0.29	2,095	4.4 (3.7, 5.4)	<0.001
80	481	3.6 (2.1, 6.2)		989	4.7 (3.8, 5.7)	
Sex:						
female	936	3.5 (2.0, 6.3)	0.70	1,824	4.4 (3.6, 5.4)	<0.001
male	660	3.5 (2.1, 6.0)		1,260	4.6 (3.8, 5.6)	
Race:						
black	328	3.4 (2.0, 5.8)	<0.001	2,626	4.4 (3.7, 5.4)	<0.001
non-black	1,268	4.0 (2.1, 7.4)		458	4.8 (3.9, 6.0)	
Clinic:						
NC	769	2.4 (1.7, 3.9)	<0.001	730	4.6 (3.8, 5.7)	<0.001
CA	0			861	4.5 (3.7, 5.5)	
MD	0			671	4.2 (3.5, 5.2)	
PA	827	4.7 (3.1, 7.6)		822	4.5 (3.8, 5.5)	
Smoke:						
never	707	3.4 (1.9, 6.1)	0.26	1,452	4.4 (3.7, 5.5)	0.02
former	741	3.5 (2.2, 6.0)		1,392	4.5 (3.8, 5.5)	
current	148	3.7 (1.8, 7.4)		240	4.3 (3.4, 5.4)	
Statins:						
no	1,426	3.5 (2.0, 6.1)	0.82	2,784	4.5 (3.7, 5.5)	0.98
yes	170	3.5 (2.1, 6.0)		300	4.6 (3.7, 5.5)	
Anti-hypertensive meds:						
no	677	3.3 (2.0, 6.0)	0.16	1,294	4.3 (3.6, 5.3)	<0.001
yes	919	3.6 (2.0, 6.2)		1,790	4.6 (3.8, 5.6)	
C-reactive protein (mg/L):						
<3	913	3.4 (2.0, 5.8)	0.03	1,775	4.4 (3.7, 5.4)	0.004
3	683	3.6 (2.1, 6.7)		1,309	4.6 (3.8, 5.6)	
eGFR (mL/min/1.73m ²):						
<60	397	3.8 (2.3, 6.3)	0.007	826	5.0 (4.2, 6.2)	<0.001
60	1,199	3.3 (1.9, 6.1)		2,258	4.3 (3.6, 5.2)	
Albumin (g/dL)						
<3.5	152	3.2 (1.9, 5.6)	0.20	357	4.8 (3.9, 5.8)	<0.001
3.5	1,444	3.5 (2.0, 6.2)		2,727	4.4 (3.7, 5.4)	

eGFR: estimated glomerular filtration rate, PIIINP: Procollagen type III N-terminal propeptide, TGF- β : Transforming growth factor- β ^aKruskal-Wallis rank test

Table 2

Multivariable-adjusted associations of hyperglycemia and other metabolic exposures with TGF- β and PIIINP

	TGF- β (ng/L) (N=1,422)				PIIINP (ng/L) (N=2,759)			
	B	SE	P	β	B	SE	P	
Fasting glucose (mg/dL)								
Age, sex, race, clinic-adjusted	15.6	6.1	0.01	5.5	2.3	0.02		
Multivariable-adjusted ^a	14.4	6.5	0.03	4.2	2.3	0.07		
Log-fasting insulin (IU/mL)								
Age, sex, race, clinic-adjusted	324.9	336.2	0.33	750.7	103.7	< 0.001		
Multivariable-adjusted ^a	249.8	384.7	0.52	605.7	107.1	< 0.001		
Body mass index (kg/m ²)								
Age, sex, race, clinic-adjusted	15.5	23.6	0.51	46.7	7.7	< 0.001		
Multivariable-adjusted ^a	2.3	26.4	0.93	31.7	8.5	< 0.001		
Fatty acid binding protein 4 (ng/mL)								
Age, sex, race, clinic-adjusted	13.1	6.8	0.05	15.1	2.3	< 0.001		
Multivariable-adjusted ^a	11.2	7.5	0.14	3.0	2.8	0.28		
Log-non-esterified fatty acids (mEq/L)								
Age, sex, race, clinic-adjusted	472.8	238.5	0.05	264.9	86.8	0.002		
Multivariable-adjusted ^a	394.3	237.5	0.09	277.8	86.5	0.001		

PIIINP: Procollagen type III N-terminal propeptide, TGF- β : Transforming growth factor- β Associations are calculated among individuals not taking insulin or oral hypoglycemic agents.

^a Adjusted for age, sex, race, clinic, pack years, smoking status, systolic blood pressure, statins, use of hypertension medications, total cholesterol, C-reactive protein, estimated glomerular filtration rate, and serum albumin

Table 3

Multivariable-adjusted associations of metabolic exposures not available in the full cohort with TGF- β and PIINP

	TGF- β (ng/L) (N=1,080)			PIINP (ng/L) (N=2,095)		
	β	SE	P	β	SE	P
2-hour glucose (mg/dL)						
Age, sex, race, clinic-adjusted	6.0	2.0	0.003	2.2	0.8	0.006
Multivariable-adjusted ^a	5.2	2.1	0.01	1.9	0.8	0.02
Log-2-hour insulin (IU/mL)						
Age, sex, race, clinic-adjusted	249.5	157.4	0.11	231.2	65.6	< 0.001
Multivariable-adjusted ^a	203.4	166.2	0.22	199.8	65.6	0.002
Log-Gutt (standard deviation units)						
Age, sex, race, clinic-adjusted	-323.7	100.9	0.001	-157.5	38.5	< 0.001
Multivariable-adjusted ^a	-292.6	106.5	0.006	-137.0	39.1	< 0.001
Carboxymethyl lysine (ng/mL)						
Age, sex, race, clinic-adjusted	0.1	0.5	0.91	0.4	0.2	0.07
Multivariable-adjusted ^a	-0.1	0.6	0.88	0.1	0.2	0.45

PIINP: Procollagen type III N-terminal propeptide, TGF- β : Transforming growth factor- β Associations are calculated among individuals not taking insulin or oral hypoglycemic agents.

^a Adjusted for age, sex, race, clinic, pack years, smoking status, systolic blood pressure, statins, use of hypertension medications, total cholesterol, C-reactive protein, estimated glomerular filtration rate, and serum albumin

Table 4
Multivariable-adjusted associations of prior and current metabolic exposures with TGF- β and PIINP

	TGF- β (ng/L) (1996–1997)					PIINP (ng/L) (1996–1997)				
	N	β	SE	P	P _{int}	N	β	SE	P	P _{int}
Fasting glucose (mg/dL)										
Prior (1992–1993)	1,380	4.3	5.8	0.46		2,685	0.4	2.4	0.86	
Current (1996–1997)	1,380	14.9	6.6	0.02	0.66	2,685	4.8	2.3	0.04	0.41
Log-fasting insulin (IU/mL)										
Prior (1992–1993)	1,380	–23.3	271.9	0.93		2,685	285.0	100.3	0.005	
Current (1996–1997)	1,380	306.1	390.1	0.43	0.86	2,685	618.2	107.2	< 0.001	0.007
+ Prior < Median						1,352	515.7	210.3	0.01	
+ Prior Median						1,333	806.4	143.5	< 0.001	
Body mass index (kg/m ²)										
Prior (1992–1993)	1,380	19.9	27.3	0.47		2,685	36.1	8.8	< 0.001	
Current (1996–1997)	1,380	8.4	27.1	0.76	0.72	2,685	33.0	8.6	< 0.001	0.67

PIINP: Procollagen type III N-terminal propeptide, TGF- β : Transforming growth factor- β Associations are calculated among individuals not taking insulin or oral hypoglycemic agents, and with exposure data from 1992–1993 and 1996–1997 available. Median prior log-Fasting insulin = 2.52 ln(IU/mL).

Adjusted for age, sex, race, clinic, pack years, smoking status, systolic blood pressure, statins, use of hypertension medications, total cholesterol, C-reactive protein, estimated glomerular filtration rate, and serum albumin