THE EPIDEMIOLOGY OF NONTRADITIONAL BIOMARKERS OF HYPERGLYCEMIA AND THEIR PROGNOSTIC VALUE

by
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Abstract

This dissertation focuses on the utility of nontraditional biomarkers of hyperglycemia (fructosamine, glycated albumin, and 1,5-anhydroglucitol [1,5-AG]) for research and clinical purposes. We have undertaken several studies in the Atherosclerosis Risk in Communities (ARIC) Study to assess the following: the short-term variability of these biomarkers, racial comparisons of associations with long-term microvascular and macrovascular complications, and their ability to improve performance of risk prediction in the setting of diabetes.

The first chapter is a review of the utility of nontraditional biomarkers of hyperglycemia for diagnosis, prognosis, and management of diabetes. There has been growing interest in the use of fructosamine, glycated albumin, and 1,5-AG in clinical practice but their epidemiology is relatively uncharacterized. We evaluated the recent literature and summarized findings in regard to associations of these biomarkers with complications; use of these biomarkers for monitoring of glycemic control, diabetes screening, and diagnosis, and in special populations; and discussed limitations of current studies, as well as potential avenues of interest for further study.

The second chapter is a repeatability study that we conducted using a subset of participants who attended the fifth examination of the ARIC Study (from 2011 to 2013) and a second examination approximately 6 weeks after the first. We quantified the short-term variability of both traditional and nontraditional biomarkers of hyperglycemia in persons with and without diagnosed diabetes, and compared results across biomarkers in the persons who had blood collected at both of these examinations. The within-person

coefficient of variation was highest for fasting glucose (9.6% and 5.3% in persons with and without diabetes, respectively) and lowest for hemoglobin A1c (HbA1c) (2.0% and 1.5%, respectively); and was intermediate between fasting glucose and HbA1c for fructosamine (3.7% and 3.4%, respectively), glycated albumin (3.8% and 2.7%, respectively), and 1,5-AG (5.7% and 2.9%, respectively). For each biomarker, the within-person coefficient of variation was greater in persons with diagnosed diabetes as compared to those without diagnosed diabetes. HbA1c and nontraditional biomarkers of hyperglycemia tracked well over six weeks and they had lower within-person variability than fasting glucose.

The third chapter is a prospective study that compares in whites and blacks the association of traditional and nontraditional biomarkers of hyperglycemia with incident cardiovascular disease (CVD) and end-stage renal disease (ESRD) over ~20 years of follow-up. Previous studies have reported racial differences in HbA1c, which has spurred recent debate over the use of HbA1c as a diagnostic test. We found that levels of hyperglycemia and incidence rates of CVD and ESRD were higher in blacks than whites. However, the relative associations of HbA1c and nontraditional biomarkers of hyperglycemia were similar in whites and blacks (all p-values for interaction >0.15). Our findings support the use of similar cut-points for HbA1c in whites and blacks.

The fourth chapter examines the association of large changes or sustained elevations in hs-CRP over a six-year time period with incident diabetes, cardiovascular events, and mortality over a median of 14 years of follow-up. CRP is a non-specific biomarker of inflammation, which has been associated with diabetes. Although the

pathway by which inflammation may be involved in the development of insulin resistance and diabetes is not entirely clear, hs-CRP may be a good indicator of diabetes risk. We found that the more proximal measure of hs-CRP was associated with incident diabetes, regardless of hs-CRP measured six years earlier. Compared to persons with sustained low or moderate hs-CRP, those with increased or sustained elevated hs-CRP had an increased risk of incident diabetes (HRs [95% CIs]: 1.56 [1.38, 1.76] and 1.39 [1.25, 1.56], respectively). Persons with sustained elevations in hs-CRP were at the highest risk of CVD and mortality. Compared to persons with sustained low or moderate hs-CRP, those with sustained elevated hs-CRP had an increased risk of CVD events and mortality (HRs ranged from 1.51 to 1.70 and all had $P<0.05$).

The fifth chapter presents a risk prediction model for 10-year risk of a combined endpoint of major complications (CVD, CKD, or lower extremity hospitalizations) in persons with diagnosed diabetes, considering death due to another cause as a competing risk. We developed a risk prediction model using traditional demographic and clinical variables, and then tested the addition of 13 biomarkers of hyperglycemia, cardiac function, kidney function, liver function, and inflammation. The addition of HbA1c, beta-2 microglobulin, N-terminal probrain natriuretic peptide, and high-sensitivity cardiac troponin T improved model discrimination (c-statistic 0.679 vs. 0.716, $P<0.001$).

In conclusion, we determined that the epidemiology of fructosamine, glycated albumin, and 1,5-AG is relatively uncharacterized, and that few prospective studies have been sufficiently conducted to address the utility of these biomarkers for diabetes diagnosis, prognosis, and management. This dissertation addressed some of these gaps in

the literature. We found that nontraditional biomarkers of hyperglycemia had good six-week reliability, especially compared to fasting glucose, in persons with and without diabetes. The similar diagnostic and prognostic value of HbA1c and nontraditional biomarkers of hyperglycemia in whites and blacks suggests similar cut-points for HbA1c across race. Furthermore, biomarkers of hyperglycemia, cardiac damage, kidney function, liver function, and inflammation may improve risk prediction of diabetes and its complications. The most recent measurement of hs-CRP was the best indicator of future diabetes risk. However, two measurements of hs-CRP were better than one at identifying persons at highest risk of cardiovascular outcomes and death. Combinations of biomarkers may help improve risk stratification and identify high-risk persons to target for intensive therapy. In particular, fructosamine, glycated albumin, and 1,5-AG may be important as adjuncts or alternatives to fasting glucose and HbA1c.

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Abbreviations

1,5-AG	1,5-anhydroglucitiol
AGE	Advanced glycation endproduct
ADA	American Diabetes Association
ALT	Alanine transaminase
AST	Aspartate transaminase
ARIC	Atherosclerosis Risk in Communities Study
B2M	Beta-2 microglobulin
BMI	Body mass index
CI	Confidence interval
CHD	Coronary heart disease
CKD	Chronic kidney disease
CV	Coefficient of variation
CV_A	Analytical coefficient of variation
CV_G	Between-person coefficient of variation
CV_I	Biological coefficient of variation
CV_W	Within-person coefficient of variation
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DM	Diabetes mellitus
EASD	European Association for the Study of Diabetes
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
FG	Fasting glucose
GGT	Gamma-glutamyl transpeptidase
HbA1c	Hemoglobin A1c
HDL-c	High-density lipoprotein cholesterol
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
hs-cTnT	High-sensitivity cardiac troponin T
ICC	Intraclass correlation coefficient
ICD-9	International Classification of Disease, 9^{th} Revision
ICD-10	International Classification of Disease, 10^{th} Revision
IDI	Integrated discrimination index
IQR	Interquartile range
LDL-c	Low-density lipoprotein cholesterol
MI	Myocardial infarction
NRI	Net reclassification index
NT-proBNP	N-terminal probrain natriuretic peptide
OGTT	Oral glucose tolerance test
r	Correlation coefficient
RCT	Randomized controlled trial
SD	Standard deviation
UKPDS	United Kingdom Prospective Diabetes Study

UMN	University of Minnesota
USRDS	United States Renal Data System
WHO	World Health Organization

Introduction

This dissertation explores the role of nontraditional biomarkers of hyperglycemia in persons with and without diabetes, and contemplates their potential utility in both clinical and research settings. It addresses variability of these biomarkers, racial comparisons of associations with microvascular and macrovascular complications, and utility for risk prediction for major complications in persons with diabetes.

Pre-diabetes, Diabetes and its Complications

Type 2 diabetes accounts for approximately 90-95% of adult diabetes cases.[1] It is characterized by high blood sugar as the result of insulin resistance. As type 2 diabetes progresses, persons may additionally develop beta cell dysfunction, which manifests as the inability to produce sufficient amounts of insulin.[1] Pre-diabetes and diabetes are defined clinically based on levels of hyperglycemia. Most commonly, they are defined using thresholds of either fasting glucose or HbA1c. Persons with pre-diabetes are at increased risk of developing diabetes.

The burden of diabetes is enormous and has continued to grow over the past two decades. Recent estimates of the prevalence of diabetes and pre-diabetes in the US are 8-11% and 12-29%, respectively, depending on the definition used.[2] Compared to persons without diabetes, medical costs for those with diabetes are more than twice as high. The total cost of diabetes in 2012, including both direct medical costs and lowered productivity, has been estimated at nearly $250 billion.[1] The number of hospitalizations

for cardiovascular events is higher in persons with diabetes than in those without. Additionally, diabetes is the top cause of kidney failure in the US.[1]

There are multiple biological pathways that have been proposed leading from elevated levels of circulating blood glucose to development of microvascular and macrovascular complications. Hyperglycemia causes chronic insult to the endothelial tissue in both small and large vessels and can lead to the formation of advanced glycation endproducts (AGEs), which bind to receptors in the endothelial wall and are part of the signaling pathway that leads to inflammation and endothelial dysfunction, ultimately resulting in plaque formation and atherosclerosis.[3] AGEs can also accumulate in the kidneys, which signals this same pathway in local renal tissues, and can ultimately result in reduced renal function, chronic kidney disease, and end-stage renal disease.[4,5] Furthermore, high circulating glucose levels result in the release of vasoactive factors, that cause hemodynamic changes and increased blood pressure that lead to hyperfiltration in the kidney, which may ultimately result in general damage to the kidneys and the vessel walls.[6]

Traditional Biomarkers of Hyperglycemia

Traditional tests of hyperglycemia--fasting or random glucose, 2-hour glucose measured using an oral glucose tolerance test (OGTT) and HbA1c--are currently used for classification of pre-diabetes and diabetes.[7] Current American Diabetes Association (ADA) guidelines define pre-diabetes as fasting glucose of 100-125 mg/dL, HbA1c of 5.7-6.4%, or OGTT of 140-199 mg/dL; and diabetes as fasting glucose of ≥126 mg/dL,

HbA1c ≥6.5%, OGTT≥ 200 mg/dL, or random glucose ≥200 mg/dL. A confirmatory test on a separate occasion is recommended for diagnosis.[7,8] For decades, HbA1c has also been used clinically to monitor glycemic control in persons with diabetes. In general, ADA guidelines have suggested a glycemic target of HbA1c<7%.[8]

Throughout this dissertation, we will consider fasting glucose and HbA1c to be traditional biomarkers of hyperglycemia. Whereas both tests are used for diagnosis, they measure different aspects of glycemia, and therefore provide unique and complementary information about one's glycemic status. Glucose is the most direct measure of current glycemic status, and measures the level of glucose that is circulating in the bloodstream at the time of a blood draw. It is measured in the fasting state to better assess the body's ability to maintain stable glucose levels, as opposed to after eating, at which time glucose levels may more closely reflect glucose derived from a meal.[9] HbA1c is an indirect measure of circulating glucose levels, and is formed when glucose irreversibly attaches to hemoglobin in red blood cells.[9,10] It is a measure of the proportion of hemoglobin that is glycated (attached to glucose), and measures average glycemia over the past 2-3 months.[10]

Since fasting glucose and HbA1c measure different facets of glycemia, there is some amount of discordance in persons identified as having elevated levels of glycemia using one or the other biomarker. Previous studies have shown that persons with both elevated HbA1c and fasting glucose have a higher risk of subsequent diabetes compared to those with high HbA1c but not elevated fasting glucose, which suggests that the use of both tests in conjunction may be useful for prediction of diabetes.[11] The ability to more

comprehensively measure glycemic status could provide insight into the underlying biologic pathway of the development of diabetes and its complications.

Nontraditional Biomarkers of Hyperglycemia

Fasting glucose and HbA1c have inherent limitations. For instance, fasting glucose requires a patient to arrive at a visit fasting, has higher biological and pre-analytical variability compared to HbA1c, and can be affected by acute factors such as stress and illness.[10] HbA1c may be affected by hemoglobin characteristics and changes in red blood cell turnover, and requires whole blood for measurement, which is problematic in settings where only plasma or serum samples are available (i.e., epidemiologic and other clinical research studies).[10]

There has been recent interest in the clinical and research utility of nontraditional biomarkers of hyperglycemia (fructosamine, glycated albumin and 1,5-anhydroglucitol [1,5-AG]) to overcome some of these limitations of traditional biomarkers of hyperglycemia. These biomarkers all measure extracellular glycemia, and are therefore not affected by alterations in hemoglobin or red blood cells; can be measured in serum or plasma samples, which could be particularly useful in research studies; and do not require a patient to be fasting.[12] Importantly, they may also provide potentially useful complementary information about glycemic status and could be useful as adjuncts to fasting glucose and HbA1c in some settings. Fructosamine measures the concentration of total glycated serum proteins. Glycated albumin measures the proportion of albumin that is glycated. Both measure average glycemia over the past 2-3 weeks.[13] 1,5-AG is a

monosaccharide that is derived mainly from the diet, and is excreted in the presence of high circulating glucose levels (>180 mg/dL). It measures average glycemia over the past 2-14 days.[14–18]

It is currently unclear whether nontraditional biomarkers of hyperglycemia may prove useful if used in conjunction with or as alternatives to traditional biomarkers. Little is known about the variability of nontraditional biomarkers of hyperglycemia. Older studies of these nontraditional biomarkers have reported relatively high within-person variability. However, we suspect this is largely due to older, less accurate assays, that were not comparable across laboratories.[19] Assays that are currently available and were used for this study have since improved.

Few prospective studies have examined the associations of these biomarkers with important clinical outcomes. The few that have been conducted have largely shown associations of these biomarkers with microvascular and macrovascular complications,[20–23] and is therefore promising for their use in risk prediction and stratification. The potential utility of nontraditional biomarkers for risk stratification extends to other biomarkers, including those that capture aspects of cardiac damage (high-sensitivity cardiac troponin T [hs-cTnT] and N-terminal probrain natriuretic peptide [NT-proBNP]), kidney function (creatinine, cystatin c, and beta-2 microglobulin [B2M]), liver function (alanine transaminase [ALT], aspartate transaminase [AST], and gamma-glutamyl transpeptidase [GGT]), and inflammation (high-sensitivity C-reactive protein [hs-CRP]). For instance, hs-cTNT has been shown to significantly improve risk prediction of cardiovascular outcomes in the general population[24] and in persons with diabetes.[25] NT-

proBNP has been shown to be a strong independent risk factor for stroke.[26] Markers of cardiac damage have also been shown to independently predict risk of ESRD in persons with diabetes and CKD.[27] B2M has been associated with all-cause and cardiovascular mortality.[28] Using cystatin C in addition to creatinine improved risk stratification for ESRD.[29] Inflammation is postulated to be on the pathway to diabetes.[30,31] In fact, hs-CRP, a non-specific marker of inflammation, has been associated with CVD,[32–38] incident diabetes[36,39–47] and mortality.[34,35] It is currently unknown whether the combined use of these biomarkers could improve prediction of diabetes and its complications and result in better risk stratification of persons with diabetes.

Racial Differences

Racial differences in the discordance of traditional biomarkers of hyperglycemia are well-documented.[48–53] Among persons with similar levels of fasting glucose, blacks tend to have higher HbA1c compared to whites, which has led to controversy over the use of HbA1c as a diagnostic test. Proponents of race-specific cut-points for HbA1c have argued that HbA1c is artificially high in blacks due to non-glycemic factors (e.g., low red cell turnover, high red cell glycation rate), and that the use of HbA1c may lead to over-diagnosis of diabetes in blacks.[54] However, there is currently no evidence of racial differences in non-glycemic factors, and it seems likely that black and white persons may have differences in dietary patterns, physical activity or glucose metabolism, resulting in higher average circulating levels of glycemia in black persons.[55] If this were the case,

then blacks may actually have a higher absolute risk of diabetes and glycemia-related outcomes than whites. However, this debate is far from settled.

Recent studies have shown similar associations of fasting glucose and HbA1c with prevalent retinopathy,[56,57] incident chronic kidney disease (CKD), and cardiovascular disease (CVD)[58] in blacks and whites. This suggests that the utility of these biomarkers for prognosis is similar across these ethnic groups. Similar associations by race also imply that the variation in discordance is due to glycemic factors, and that higher HbA1c in blacks is reflective of a true heightened state of hyperglycemia. Prospective associations of nontraditional biomarkers of hyperglycemia with microvascular and macrovascular complications by race have not been characterized. Nontraditional biomarkers of hyperglycemia, which are extracellular measures of glycation that are unaffected by red blood cell and hemoglobin characteristics, could help address this controversy.

Research Question

This dissertation addresses the following overarching research question:

What is the role of nontraditional biomarkers of hyperglycemia (fructosamine, glycated albumin, and 1,5-AG) for diagnosis and prognosis of diabetes?

Study Aims

This dissertation was designed to address the following specific aims:

Aim 1: To review the current literature regarding the utility of nontraditional biomarkers of hyperglycemia for diagnosis, prognosis, and management of diabetes; and to summarize strengths and weaknesses of the literature, and identify potential areas for future research.

Aim 2: To quantify and compare the short-term within-person variability of traditional and nontraditional biomarkers of hyperglycemia

Aim 3: To compare, in whites and blacks, the associations of traditional and nontraditional biomarkers of hyperglycemia with incident CVD and ESRD (in persons with and without diabetes) to address major controversy regarding racial differences in performance of biomarkers of hyperglycemia

Aim 4: To characterize and compare the associations of six-year change in hs-CRP (focusing on large increases and sustained elevations) with incident diabetes, cardiovascular events and mortality

Aim 5: To develop a risk prediction equation for 10-year risk of major complications in persons with diabetes, using demographic and clinical information and a panel of traditional and nontraditional biomarkers

Conceptual Framework

This dissertation is guided by the conceptual framework displayed in **Figure 1**, which shows diabetes as our main construct of interest. The five biomarkers of hyperglycemia included in this dissertation are empirical indicators of hyperglycemia and are each measured with some inherent amount of error. Aim 1 addresses the existing literature surrounding the role of nontraditional biomarkers of hyperglycemia. Aim 2 intends to quantify and compare the within-person variability of these biomarkers. Aim 3 addresses the potential effect modification of race in the association of these biomarkers with incident microvascular and macrovascular complications. Aim 4 investigates the association of changes in or sustained elevations in inflammation (as measured by hs-CRP) with incident diabetes, cardiovascular events, and mortality. Aim 5 addresses the potential additional utility of these biomarkers in the prediction of 10-year risk of major complications in persons with diabetes. Major potential confounders of this main relationship of biomarkers of hyperglycemia with microvascular and macrovascular complications include age, sex, body mass index, lipids, cholesterol-lowering and antihypertensive medication use, hypertension, estimated glomerular filtration rate, family history of diabetes, education level, alcohol consumption, cigarette smoking status, and physical activity.

Organization of Dissertation

This dissertation is comprised of 5 publishable papers. The first chapter is a review paper that is a survey of the recent literature on the utility of nontraditional

biomarkers of hyperglycemia for diagnosis, prognosis, and management of diabetes. This review was published in *Current Diabetes Reports* in September 2014 (*Curr Diab Rep* 2014;14(11):548).[59]

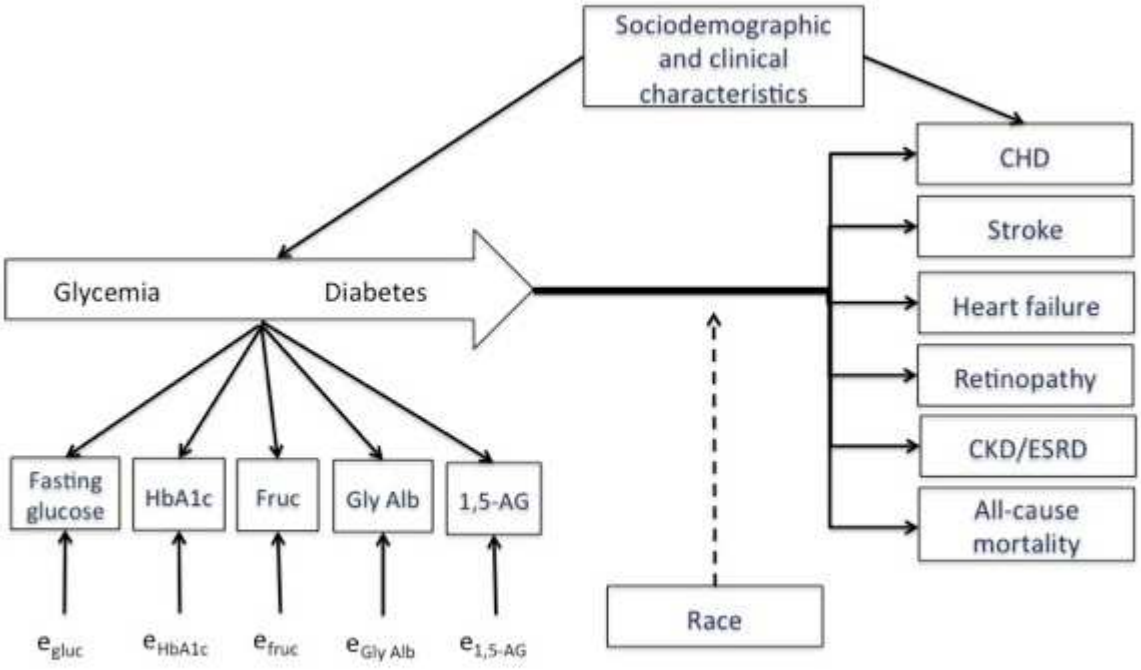
The second chapter is a study of the short-term (~6 week) variability of traditional and nontraditional biomarkers of hyperglycemia. It is currently under review at a peer-reviewed journal.

The third chapter is a study of the comparison by race of associations of traditional and nontraditional biomarkers of hyperglycemia with incident CVD and ESRD. It is currently under review at a peer-reviewed journal.

The fourth chapter is a study of the association of changes and sustained elevations in hs-CRP over six years with incident diabetes, cardiovascular events, and mortality. It is in press at the *American Heart Journal*.[60]

The fifth chapter is a study of the development of a prediction model for 10-year risk of major complications in persons with diabetes, and the assessment of whether the addition of traditional and nontraditional biomarkers of hyperglycemia, cardiac damage, kidney function, liver function, and inflammation improve the predictive ability of the model.

Figure 1. Conceptual Framework



Chapter 1: Beyond HbA1c and glucose: the role of nontraditional glycemic markers in diabetes diagnosis, prognosis, and management

Curr Diab Rep 2014;14(11):548

Abstract

Fasting glucose and hemoglobin A1c (HbA1c) are the standard measures for diagnosis and monitoring of diabetes. There has been recent interest in nontraditional markers of hyperglycemia, including fructosamine, glycated albumin and 1,5-anhydroglucitol (1,5-AG), as alternatives or adjuncts to standard measures. There is a growing literature linking these nontraditional markers with microvascular and macrovascular complications. Fructosamine and glycated albumin have also been shown to improve identification of persons with diabetes. However, long-term prospective studies with clinical outcomes are lacking. Some modern laboratory assays for fructosamine, glycated albumin and 1,5-AG have excellent performance. Expanded use of these tests has the potential to improve diabetes care as these measures may overcome limitations of HbA1c in certain patients, complement traditional measures by providing additional information on shorter-term glycemic control, and improve risk stratification for diabetes and its complications. Nonetheless, studies are needed to demonstrate if their routine use will benefit patients and improve outcomes.

Introduction

Hemoglobin A1c (HbA1c) has long been the standard measure used to monitor glycemic control in clinical practice and is routinely measured in all persons with diabetes. In addition to fasting glucose and 2-hour glucose, the American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD), the World Health Organization (WHO), and other diabetes organizations now recommend the use of HbA1c for diagnosis of diabetes.[1–6] First recommended in 2009, the addition of HbA1c to diagnostic criteria for diabetes has been controversial, largely attributable to limitations of the HbA1c test.[7–9] There is growing interest in serum biomarkers of hyperglycemia, including fructosamine, glycated albumin, and 1,5-anhydroglucitol (1,5-AG), to be used as alternatives to or in conjunction with traditional measures[10–13] These markers can overcome limitations of HbA1c in certain patients, could complement traditional measures in the clinic by providing additional information on shorter-term glycemic control, and may improve risk stratification for diabetes and its complications.

Markers of hyperglycemia

Traditional markers of hyperglycemia

Diabetes is a condition defined by elevations in glucose. Historically, glucose measured in the fasting state or glucose measured two hours after a carbohydrate challenge (oral glucose tolerance test) have been the standard measures used to diagnose diabetes and identify people at risk for diabetes (frequently termed “prediabetes”). HbA1c has been used widely since the 1980s and is the standard measure used for monitoring glycemic control in clinical practice.[5] In red blood cells, HbA1c is

hemoglobin that has glucose attached to the N-terminal valine of the beta chain and is reported as a proportion of total hemoglobin. Because the lifespan of red blood cells is approximately 120 days, HbA1c therefore reflects average glycemia over the past two to three months (since it is weighted towards the more recent months).[13] Advantages of HbA1c include the lack of participant preparation (fasting is not necessary); high within-person reliability;[14,15] and excellent standardization of the assay in most countries.[16–18] Nonetheless, disadvantages of HbA1c include limited interpretability in the setting of altered red blood cell lifespan—levels are affected by changes in duration of red blood cell exposure to circulating blood glucose levels—and interference of some HbA1c assays by hemoglobin variants and several rare conditions (**Table 1**).[7,19] These disadvantages have brought into focus possible roles for nontraditional glycemic markers in the clinic.

Nontraditional markers of hyperglycemia

Fructosamine and glycated albumin are both ketoamines, which are formed as the result of a non-enzymatic process that binds glucose to serum proteins. In states of abnormally high glucose concentrations, as in persons with diabetes, serum proteins are exposed to greater concentrations of glucose and therefore experience increased glycation.[20] Fructosamine assays measure total glycated serum protein (mostly albumin, but also immunoglobulins and other circulating proteins), whereas glycated albumin is reported as a proportion of total albumin. The half-life of albumin and other serum proteins is shorter than that of red blood cells; thus measurements of fructosamine and

glycated albumin reflect average glycemia over a shorter duration, approximately two to three weeks.[20]

1,5-AG is a 6-carbon monosaccharide obtained mainly from dietary sources, that reflects average glycemia over approximately the past 2-14 days.[21–25] In states of normal glycemia, nearly 100% of 1,5-AG is reabsorbed by the renal tubule. However, at very high levels of glycemia (above the renal threshold, ~160-180 mg/dl), glucose competes with 1,5-AG for reabsorption by the renal tubule, and 1,5-AG is excreted in the urine, resulting in a drop in circulating 1,5-AG levels in the blood. Therefore, there is an inverse association between high levels of glucose and 1,5-AG[21]. Soybeans have particularly high levels of 1,5-AG, and certain foods such as rice, bread and beef contain modest levels; it is unclear to what extent dietary intake may affect circulating 1,5-AG levels and the interpretation of this test.[21,22]

Correlations of traditional markers of hyperglycemia with fructosamine, glycated albumin, and 1,5-anhydroglucitol

Fructosamine and glycated albumin are strongly associated with HbA1c and fasting glucose,[26–30] and all four measures have been shown to be similarly correlated with mean glucose from continuous glucose monitoring over about 5 days in persons with diabetes.[31] In settings where HbA1c testing is known to be problematic, fructosamine or glycated albumin may be a useful substitute. A difficulty, however, is that there are no established clinical cut-points and these assays are not standardized across instruments. Conversion equations can help estimate the ranges of fructosamine and glycated albumin test results that are similar to HbA1c targets. Various equations

have been developed to convert fructosamine and glycated albumin to an "HbA1c equivalent". For example, previous reports demonstrated that glycated albumin values in the range of 16% to 22%,[27,32–34] and fructosamine levels around 312 μmol/L as reported by one study,[27] are approximately equivalent to an HbA1c value of 7%. 1,5-AG is strongly inversely associated with HbA1c and fasting glucose in persons with diagnosed diabetes,[27] but appropriate clinical targets are unclear. It should be noted that 1,5-AG is poorly correlated with fasting glucose and HbA1c in persons without diagnosed diabetes--the strongest correlations are observed at the highest glucose concentrations.[27,35] This suggests the utility of 1,5-AG may primarily be limited to persons with overtly elevated glucose.

Since these markers of hyperglycemia are measured on different scales, both clinicians and patients may benefit from being provided with equivalents. However, conversion equations for nontraditional glycemic markers have typically relied on single measurements (which may vary considerably over time, particularly in diabetic patients) and may differ depending on the underlying population from which they are derived, with uncertain generalizability. Furthermore, none of these markers are perfectly correlated, a function of differences in the physiology of each biomarker including the duration of glycemia reflected and other sources of biological and analytical variability. In fact, the discordance across traditional and nontraditional glycemic markers may suggest the complementary nature of these biomarkers. A benefit to the use of multiple measures is that they may each provide unique insight into different aspects of hyperglycemia and diabetes physiology.

Associations of nontraditional markers of hyperglycemia with complications

Cross-sectional studies

Cross-sectional studies have linked nontraditional markers of hyperglycemia with both microvascular and macrovascular complications. Fructosamine and glycated albumin have both been linked to prevalent retinopathy.[36–38] In a recent analysis of 12,306 persons (958 with diabetes) in the Atherosclerosis Risk in Communities (ARIC) Study, we found an independent association of glycated albumin and fructosamine with retinopathy, with patterns of association very similar to those observed for HbA1c (**Figure**).[29] In a Japanese cohort of more than 2,500 participants, the performance of glycated albumin and 1,5-AG to identify cases of retinopathy—as measured by the C-statistic—was shown to be comparable to fasting glucose and HbA1c.[39] In a study of 1,575 Japanese adults without diagnosed diabetes, glycated albumin was associated with carotid artery intima-media thickness, a measure of subclinical atherosclerosis.[40] Glycated albumin has been associated with prevalent kidney outcomes,[41–43] and cardiovascular disease.[43–50] Few studies have assessed the relationship of 1,5-AG to complications, although lower 1,5-AG concentrations have been linked to both prevalent coronary heart disease [51] and retinopathy[52] in persons with diabetes. 1,5-AG has also been associated with measures of atherosclerosis and cardiovascular disease in a population without a history of diabetes.[53]

Prospective studies

Limited evidence from prospective studies suggests nontraditional markers may be useful for identification of persons at risk of developing microvascular and

macrovascular complications. In addition to the associations with retinopathy in the above-mentioned ARIC Study, we found that both fructosamine and glycated albumin strongly predicted incident chronic kidney disease (CKD) over two decades of follow-up. The observed associations of fructosamine and glycated albumin with incident CKD were of similar magnitude to those observed for HbA1c.[29] Analyses conducted in the DCCT/EDIC study of persons with type 1 diabetes also reported that glycated albumin was similarly associated with retinopathy and nephropathy as compared to HbA1c.[28] Additionally, in an analysis of 84 persons with type 1 diabetes from the Wisconsin Diabetes Registry Study, fructosamine was associated with incident retinopathy.[54] By contrast, in a Brazilian cohort of persons with diabetes, fasting glucose was associated with microvascular outcomes over about 5 years of follow-up, but fructosamine was not.[55] In a recent prospective study in 2,095 Japanese persons (including approximately 100 with diabetes), 1,5-AG was associated with incident cardiovascular events during 11 years of follow-up.[56]

Clinical utility of nontraditional markers of hyperglycemia

For monitoring of short-term glycemic control

Nontraditional markers of hyperglycemia are not formally incorporated into clinical guidelines in the United States. However, various organizations in multiple countries, including the US, India, Australia and the United Kingdom, have suggested fructosamine as a useful alternative to HbA1c for monitoring glycemic control in persons with conditions that may interfere with the interpretation of the HbA1c test.[11,12,57–62]

Glycated albumin is used frequently in China, Japan and South Korea for monitoring intermediate glycemic control.[63] Several assays have been developed to measure glycated albumin but the assays are not standardized, and therefore not necessarily equivalent. Some early studies raised serious concerns regarding the validity and reliability of fructosamine assays[64], although second-generation assays had improved technical performance .[65] Modern automated assays for fructosamine have shown high correlations with glucose and HbA1c, strong prognostic value, and very low CVs (approximately 3% in recent studies).[29,31,66]

Whereas HbA1c reflects long-term, 2-3 month glycemic control, fructosamine and glycated albumin reflect hyperglycemia over the past 2 to 3 weeks. Thus, both have been proposed as useful markers of intermediate glycemic control. In clinical practice, HbA1c is typically measured at minimum every 6 months and more frequently (quarterly) in persons with recent therapy changes who are not meeting treatment goals.[1,67]

Fructosamine and glycated albumin may be quite useful to evaluate earlier response to changes in treatment. Glycated albumin has been shown to change faster than HbA1c in response to changes in medication or exercise.[68,69] Compared to HbA1c, glycated albumin is more strongly correlated with continuous glucose measurements over 1 to 2 days, [70,71] and may more accurately reflect long-term glycemic variability and glucose excursions.[72,73]

1,5-AG is thought to reflect hyperglycemia over the past 2 weeks and is recommended by the manufacturer for use in persons with diabetes and HbA1c <8% to help identify patients with frequent hyperglycemic excursions.[74,75] Indeed, 1,5-AG has

been shown to be correlated with postprandial hyperglycemia in persons with diabetes and HbA1c<7%;[76] and to be more strongly correlated with glucose variability as compared to HbA1c, fructosamine or glycated albumin over 2 to 3 days in persons with moderate glycemic control (HbA1c<8%).[77,78]

For diabetes screening or diagnosis

There is evidence that nontraditional markers of hyperglycemia may help to more accurately identify persons with diabetes. In several studies, fructosamine and glycated albumin had similar performance for the identification of persons with diabetes as compared to either fasting glucose or HbA1c.[27,30,79–81] Furthermore, compared to using either test individually, sensitivity to identify cases of diabetes defined by 2-hour glucose was improved when glycated albumin was used in combination with either fasting glucose or HbA1c.[81,82]

A large proportion of persons identified as having pre-diabetes do not go onto develop diabetes, highlighting the need for strategies that will accurately identify persons who will progress to overt diabetes.[83] It is possible that fructosamine or glycated albumin may be useful in early identification of high-risk persons. Recent studies have shown that both fructosamine and glycated albumin are associated with future risk of diabetes, independent of fasting glucose and HbA1c.[29,84] 1,5-AG has also been associated with future development of diabetes, but observed associations were lower in magnitude as compared to other markers of hyperglycemia and were not present in persons with fasting glucose or HbA1c in the non-diabetic range.[84] Nonetheless, the evidence linking nontraditional biomarkers with future diabetes risk is sparse.

Utility of nontraditional markers in special populations

A focus in the literature has been the potential utility of fructosamine or glycated albumin for monitoring glycemic control in the setting of certain populations where HbA1c is thought to inaccurately reflect glycemia, including severe kidney disease.[85] Recent studies have shown that, compared to HbA1c, glycated albumin is more strongly correlated with glucose in dialysis patients.[86–93] Fructosamine and glycated albumin may also be useful for prediction of complications in persons with kidney failure. Indeed, fructosamine and glycated albumin have been both cross-sectionally and prospectively associated with microvascular, macrovascular and all-cause morbidity and mortality in dialysis patients, whereas many studies have reported no association of HbA1c with these outcomes.[66,94–101] Nonetheless, despite their associations with clinical outcomes, fructosamine and glycated albumin may also be limited in this setting, since proteinuria and altered serum protein turnover may affect interpretation of these tests.[102–104]

1,5-AG has not been well studied in the setting of chronic kidney disease or dialysis. Because lowered plasma concentrations of 1,5-AG result from accelerated urine excretion due to competitive inhibition of glucose by the renal tubules, 1,5-AG may have a problematic interpretation in the setting of reduced kidney function. 1,5-AG has been correlated with fasting glucose and HbA1c in persons with diabetes and mild to moderate CKD, but not in those with end stage renal disease (ESRD) (stages 4-5 CKD).[105]

There is also evidence to support the use of nontraditional markers of hyperglycemia in persons with other conditions that may decrease the lifespan of red blood cells. Fructosamine and glycated albumin have been shown to better reflect glucose

levels in the setting of anemia, autologous blood donations and HIV, which may all result in artificially low HbA1c.[106–109] There is also interest in whether fructosamine, glycated albumin, or 1,5-AG testing may play a role in the management of diabetes in patients with liver disease, but evidence for their performance in this setting is inconsistent.[110–112] During pregnancy, glycated albumin may better reflect average glucose compared to HbA1c, which may be artificially elevated due to iron deficiency.[113,114] Furthermore, measures of shorter-term glycemia may be especially important in gestational diabetes given the importance of frequent monitoring and strong associations between diabetes control in pregnancy and maternal and fetal outcomes.[1,115]

Conclusions

Nontraditional markers of hyperglycemia, fructosamine, glycated albumin, or 1,5-AG, may be useful for monitoring of glycemic control when short-term changes are of interest or as alternatives to HbA1c in settings in which HbA1c may be problematic. Fructosamine and glycated albumin may also aid in early identification of persons at future risk for diabetes. In clinical or epidemiologic studies where fasting glucose or HbA1c measurements are not available but where serum or plasma specimens were collected, fructosamine or glycated albumin may be particularly useful to identify persons with undiagnosed hyperglycemia. Furthermore, in resource-intensive randomized clinical trials of short duration (<6 months), fructosamine and glycated albumin may be useful to evaluate responses to glucose-lowering interventions. Additionally, the complementary nature of these different tests of hyperglycemia warrants exploration into the potential

utility of fructosamine, glycated albumin, and/or 1,5-AG in the development of risk prediction models for diabetes and its complications.

Additional studies of fructosamine, glycated albumin and 1,5-AG could help address uncertainty in this area. First, prospective associations of these three nontraditional glycemic markers with clinical complications are largely uncharacterized. Large epidemiologic population-based cohort studies are needed to fully characterize long-term risk associations and to better establish the prognostic value of these biomarkers. Such studies would inform relevant clinical cut-points, performance of these markers for risk stratification, and comparative predictive ability. Second, clinical studies with repeat assessments of glucose and HbA1c and those involving continuous glucose monitoring studies are needed to rigorously characterize associations with average glucose in persons with type 1 and type 2 diabetes. Such studies may help establish construct validity and utility of nontraditional markers for monitoring glycemic control. Finally, randomized clinical trials can determine whether use of these tests can improve care and outcomes for persons with diabetes. It is possible that one or more of these biomarkers may be an efficient and appropriate alternative to HbA1c in some patients and strategies that combine multiple tests for glycemia may be beneficial in certain settings.

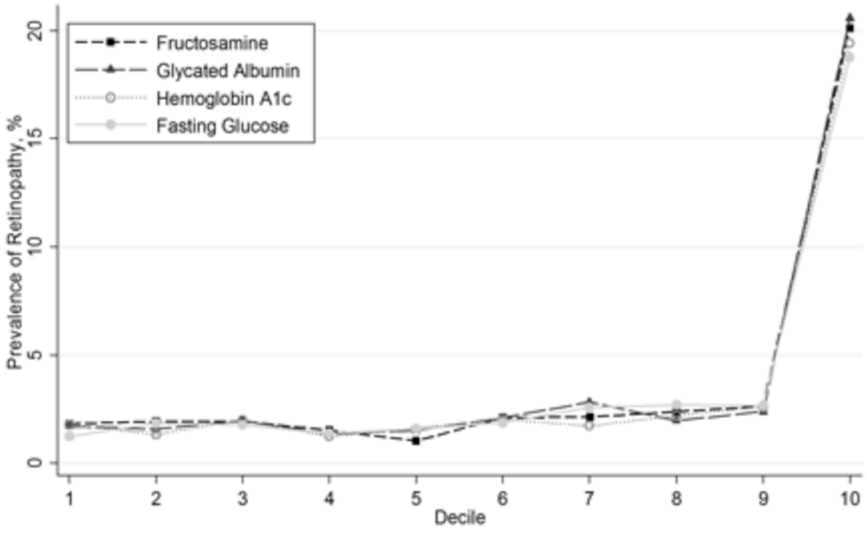
Table 1. Characteristics of traditional and nontraditional markers of hyperglycemia

	Brief Description	Duration of glycemia reflected	Strengths	Limitations
Traditional markers of hyperglycemia				
Fasting glucose	Direct measure of circulating blood glucose	Acute/ immediate	Direct measure Widely accepted Inexpensive	Requires fasting; affected by acute illness and stress; pre-analytical issues (sample stability)*; moderate within-person variability
HbA1c	Proportion of hemoglobin that is glycated	2-3 months	Reflects 2-3 month control Low within-person variability; no patient preparation needed; not affected by acute illness, stress or recent activity levels	Affected by alterations in red cell turnover; some methods for measurement can give inaccurate results in the presence of certain hemoglobin variants**; requires whole blood; cost
Nontraditional markers of hyperglycemia				
Fructosamine	Total serum protein glycation	2-3 weeks	Does not require fasting; highly reliable automated methods are widely available; can be measured in serum or plasma; inexpensive	Affected by changes in serum protein metabolism (mostly albumin) and thyroid dysfunction; limited evidence linking to outcomes
Glycated albumin	Proportion of albumin that is glycated	2-3 weeks	Does not require fasting; can be measured in serum or plasma	Affected by changes in albumin metabolism and thyroid dysfunction; method performance may vary; availability in the US is limited; limited evidence linking to

				outcomes
1,5-AG	Monosaccharide filtered by the kidney and normally reabsorbed; at high levels of glycemia, reabsorption is inhibited and it is excreted, so serum levels drop	2-14 days	Does not require fasting; can be measured in serum or plasma; test is available from major laboratories in the US; expense	Affected by changes in renal threshold for glucose, dialysis or stage 4 or 5 kidney disease, pregnancy; limited evidence linking to outcomes

* See: Gambino R. Clin Chem. 2007 Dec;53(12):2040-1.
** See: www.ngsp.org for comprehensive list

Figure. Prevalence of retinopathy by deciles of fructosamine, glycated albumin, HbA1c, and fasting glucose, the Atherosclerosis Risk in Communities Study, N=9,445



Fructosamine, umol/L										
Mean	195.4	208.6	215.5	220.9	225.9	230.8	236.3	243.1	254.3	335.1
[Min, Max]	[89, 204]	[205, 212]	[213, 218]	[219, 223]	[224, 228]	[229, 233]	[234, 239]	[240, 247]	[248, 264]	[264, 706]
Glycated Albumin, %										
Mean	10.7	11.5	11.9	12.2	12.6	12.9	13.3	13.7	14.5	21.1
[Min, Max]	[5.6, 11.2]	[11.2, 11.7]	[11.7, 12.1]	[12.1, 12.4]	[12.4, 12.7]	[12.7, 13.1]	[13.1, 13.5]	[13.5, 14.1]	[14.1, 15.2]	[15.2, 51.5]
Hemoglobin A1c, %										
Mean	4.8	5.0	5.2	5.3	5.3	5.4	5.6	5.7	6.0	8.2
[Min, Max]	[3.5, 4.9]	[5.0, 5.0]	[5.1, 5.2]	[5.3, 5.3]	[5.3, 5.3]	[5.4, 5.5]	[5.6, 5.6]	[5.7, 5.8]	[5.9, 6.3]	[6.4, 15.9]
Fasting glucose, mg/dL										
Mean	86.6	92.1	95.0	98.0	100.5	103.4	106.9	111.6	121.2	193.5
[Min, Max]	[52, 90]	[91, 93]	[94, 96]	[97, 99]	[100, 101]	[102, 105]	[106, 108]	[109, 115]	[116, 129]	[130, 500]

Source: Selvin E, Rawlings AM, Grams M, et al. Fructosamine and glycated albumin for risk stratification and prediction of incident diabetes and microvascular complications: a prospective cohort analysis of the Atherosclerosis Risk in Communities (ARIC) study. Lancet Diabetes Endocrinol. 2014;2(4):279–288. doi:10.1016/S2213-8587(13)70199-2.

Chapter 2: Short-term total variability in biomarkers of hyperglycemia in older adults

Abstract

Introduction: With the short-term variability of nontraditional markers of hyperglycemia, specifically fructosamine, glycated albumin, and 1,5-anhydroglucitol (1,5-AG) relatively uncharacterized, we quantified the total short-term variability of nontraditional biomarkers of hyperglycemia and compared them to traditional biomarkers (fasting glucose, HbA1c) in a sample of adults >65 years with and without diabetes.

Methods: We included 153 participants (50 with and 103 without diagnosed diabetes, mean age of 76 years) who attended visit 5 of the ARIC Study (2011-13) and participated in a repeatability study with two measurements each of fasting glucose, HbA1c, fructosamine, glycated albumin, and 1,5-AG obtained a mean of 6 weeks apart. In persons with and without diagnosed diabetes separately, we compared the total variability of these biomarkers using the within-person coefficient of variation (CV_W), Spearman's rank correlation coefficient (r), intraclass correlation coefficient (ICC), and index of individuality for each biomarker.

Results: The CV_W was highest for fasting glucose (9.6% and 5.3% in persons with and without diabetes, respectively) and lowest for HbA1c (2.0% and 1.5%). For each biomarker, the CV_W was greater in persons with diagnosed diabetes as compared to those without diagnosed diabetes. The ICC and r were lowest for fasting glucose and fructosamine, and were highest for HbA1c and 1,5-AG. All biomarkers had a favorably low index of individuality (<0.6).

Conclusions: HbA1c and nontraditional biomarkers of hyperglycemia track well over approximately 6 weeks, and have lower within-person variability than fasting glucose. These results demonstrate that nontraditional biomarkers of hyperglycemia have good reliability for monitoring glycemic control.

Introduction

There has been growing interest in the clinical utility of nontraditional biomarkers of hyperglycemia—fructosamine, glycated albumin and 1,5-anhydroglucitol (1,5-AG)—to complement traditional biomarkers (fasting glucose and hemoglobin A1c [HbA1c]) for diagnosis, prognosis and management of diabetes. Fasting glucose has played a central role in defining diabetes for decades, whereas HbA1c is a standard measure for monitoring of glucose control in clinical practice, and more recently has been recommended for diagnosis. Fasting glucose is a direct measure of current glycemia, whereas HbA1c is a measure of average glycemia over the past two to three months.[1] Fructosamine measures total serum protein glycated by glucose, and glycated albumin measures the proportion of total albumin that is glycated. Both fructosamine and glycated albumin measure average glycemia over the past two to three weeks.[2,3] 1,5-AG is a monosaccharide that is derived mainly from the diet, and is excreted at very high levels of glycemia. It reflects glycemia over the past 2-14 days.[3–8]

The within-person variability in HbA1c and fasting glucose have been previously characterized but very little is known about the variability of nontraditional biomarkers of hyperglycemia in the general population.[3] An older study of fructosamine reported relatively high within-person variability (i.e. within-person CV>10% in persons with diabetes comparing multiple samples taken over 2 days).[9] However, early generation fructosamine assays were highly problematic in terms of interlaboratory comparisons.[10] Newer fructosamine assays have excellent performance.[11] Recent studies using modern

assays for fructosamine, glycated albumin, and 1,5-AG have reported relatively low inter-assay CVs.[12–14]

A recent study reported low within-person biological variability (<3%) of fructosamine and glycated albumin.[15] However, this study included a small number of participants without diabetes (n=18), and was therefore unable to assess variability over a wide range of glycemia. The objective of our study was to quantify and compare the total 6-week variability of fasting glucose, HbA1c, fructosamine, glycated albumin and 1,5-AG in older adults with and without diabetes.

Methods

Study population

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based cohort of 15,792 participants recruited from four communities in the United States (Forsyth County, North Carolina; Jackson, Mississippi; the suburbs of Minneapolis, Minnesota; and Washington County, Maryland).[16] Visits 1 through 5 took place during 1987-89, 1990-92, 1993-95, 1996-98, and 2011-13, respectively. There were 200 participants (approximately 50 from each field center) who attended the initial visit 5 exam and returned for a repeat visit for biospecimen collection, intended to take place 4-8 weeks later. Participants were asked to fast for 8 hours prior to the blood draw, and were selected to try to represent the age and gender distribution of the larger cohort of persons

who attended ARIC visit 5. Institutional review boards at each site approved all procedures, and all study participants provided written informed consent.

Measurement of biomarkers of hyperglycemia

Glucose was measured in 2011-13 from plasma at Baylor College of Medicine using the Beckman Olympus 480 analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) using a hexokinase method. HbA1c was measured in 2011-13 from whole blood at the University of Minnesota's (UMN) Advanced Research and Diagnostic Laboratory (ARDL) which is one of the National Glycohemoglobin Standardization Program's Secondary Reference Laboratories using the Tosoh G7 analyzer (Tosoh Medics, Inc., San Francisco, CA, USA) using a high performance liquid chromatography method, and was standardized to the Diabetes Control and Complications Trial (DCCT) assay. For glucose and HbA1c, the assay for each person was run shortly after the clinic visit. Fructosamine, glycated albumin and 1,5-AG were measured at UMN's ARDL in 2014 from stored serum using the Roche Cobas 6000 (Roche Diagnostics Corporation, Indianapolis, IN, USA). Fructosamine was measured using a colorimetric method (Roche Diagnostics Corporation, Indianapolis, IN, USA). Glycated albumin (Asahi Kasei Lucica GA-L, Tokyo, Japan) and 1,5-AG (GlycoMark, New York, NY, USA) were measured using enzymatic methods. Glycated albumin was expressed as a percentage of total albumin, calculated using the follow equation from the manufacturer: [(glycated albumin concentration in g/dL / serum albumin concentration in g/dL) / 1.14*100] + 2.9. All blood samples were stored at -70 degrees Celsius prior to measurement. The inter-assay

CVs from internal laboratory quality control materials were 2.7% for glucose at a mean concentration of 121.7 mg/dL, 1.9% for HbA1c at 5.36%, 3.2% for fructosamine at 220.3 μmol/L, 4.4% for glycated albumin at 0.45 g/dL, and 0.9% for 1,5-AG at 18.0 μg/mL.

Exclusion criteria

We excluded participants who did not have complete data available for both the original and repeat measurements for all five biomarkers of hyperglycemia or were not fasting for at least 8 hours at both visits (N=26). We additionally excluded participants who had any value that was considered to be an outlier for any of the five biomarkers (N=21 additional) (**Supplemental Figure S1**). An iterative outlier removal approach was used to identify outlying data points that were likely due to error processes unrelated to the laboratory method (Parrinello et al,– currently in progress). Analyses for this study using the ARIC visit 5 repeatability subsample therefore consisted of a total of 153 participants.

Other variables

The following variables were self-reported at the original visit 5 exam, unless otherwise specified: age (years; reported date of birth at visit 1, from which age at visit 5 was calculated); sex (male, female; reported at visit 1); race (white, black, Asian, Native American; reported at visit 1); smoking status (current, former, never); current use of glucose-lowering medication (yes, no). Prevalent cardiovascular disease was defined as self-reported history of coronary heart disease (CHD) or stroke at visit 1, or any CHD,

stroke, or heart failure event between visits 1 and 5. We used serum creatinine measured at visit 5 (Jaffe method on a Beckman Olympus 480 analyzer) to calculate estimated glomerular filtration rate (eGFR) using the CKD-EPI equation.[17] Body mass index (BMI) was calculated as weight (measured in kilograms) divided by the square of height (measured in meters). Hypertension was defined as a mean systolic blood pressure ≥ 140 mmHg, mean diastolic blood pressure ≥ 90 mmHg, and/or use of antihypertensive medication. Diagnosed diabetes was defined as self-reported physician diagnosis of diabetes or glucose-lowering medication use at one or more of the ARIC visits or during any of the annual telephone calls. (Self-reported physician diagnosis of diabetes was not asked at visit 5.)

Statistical analysis

All analyses were conducted separately in persons with and without diagnosed diabetes. For each biomarker, we calculated the mean at the original exam, the mean at the repeat exam, the mean difference (repeat – original), and the overall mean (mean of both measurements). We created scatterplots of the original versus the repeat measurements and calculated the following measures of variability with corresponding 95% confidence intervals: within-person coefficient of variation (CV_W), intraclass correlation coefficient (ICC), index of individuality, and Spearman's rank correlation coefficient (r).

To partition the total variance of the repeated measurements into the between-subject variance (σ_{BS}^2) and within-subject variance (σ_{WS}^2), we used linear mixed effects models with each biomarker as the dependent variable and the participant as a random effect. We calculated the between-person coefficient of variation (CV_G) as follows: $\left[\left(\sqrt{\sigma_{BS}{}^2}\right)/\mu\right] * 100$, where μ is the mean of all values (both original and repeat measurements). Similarly, we calculated the within-person coefficient of variation (CV_W): $\left[\left(\sqrt{\sigma_{WS}{}^2}\right)/\mu\right] * 100$. The CV_W is a function of the within-person biological coefficient of variation (CV_I) and the analytical coefficient of variation (CV_A) (or each method's CV reported by the laboratory): $CV_W = \sqrt{CV_A{}^2 + CV_I{}^2}$. We then calculated the index of individuality as follows: $\left(\sqrt{CV_A{}^2 + CV_I{}^2}\right)/CV_G$ or equivalently, CV_W/CV_G.[18,19] We also calculated the ICC as: $\sigma_{BS}{}^2/(\sigma_{BS}{}^2 + \sigma_{WS}{}^2)$. We bootstrapped the 95% confidence intervals for estimates of CV_W, ICC, and index of individuality, using 200 replications.

All statistical analyses were conducted using Stata, version 13.0 (StataCorp, College Station, Texas, USA).

Results

Among the 153 participants in our study population (50 with and 103 without diagnosed diabetes), the mean age was 76 years, 39% were male, and 74% were white (**Table 1**).

The mean time between the original and repeat examinations was 45 days (SD, 16 days) (**Table 1**) (range of 23-102 days). The characteristics of the participants in the repeatability study subsample were similar to those of the entire cohort who attended visit 5 (**Supplemental Table S1**).

As expected, in persons with versus without diabetes, mean measures of all biomarkers of hyperglycemia were greater (except for 1,5-AG, which is inversely related to the other biomarkers, and was lower), and standard deviations were larger (**Table 2**). There was generally good agreement between original and repeat measurements for all biomarkers, although within-person variability was higher for fasting glucose (**Figure 1 and Table 2**). For all biomarkers, the short-term within-person variability, as measured by CV_W, was greater in persons with diagnosed diabetes (60% of whom reported taking glucose-lowering medication(s)), as compared to those without diagnosed diabetes. Compared to all other biomarkers, HbA1c had the lowest within-person variability in persons with and without diagnosed diabetes (CV_W = 2.0% and 1.5%, respectively), whereas glucose had the highest (CV_W = 9.6% and 5.3%, respectively) (**Table 2**). We observed intermediate levels of within-person variability for the nontraditional biomarkers of hyperglycemia (fructosamine, glycated albumin, and 1,5-AG) – lower than fasting glucose, but higher than HbA1c (**Table 2**). The ICC was lowest for fasting glucose and fructosamine, and highest for HbA1c and 1,5-AG (**Table 2**). Patterns of Spearman's rank correlation coefficients were similar to those of ICCs (**Table 2**). All biomarkers had an index of

individuality < 0.5, with fasting glucose and fructosamine having the highest index of individuality, and HbA1c and 1,5-AG having the lowest index of individuality (**Table 2**).

In a sensitivity analysis in which we did not exclude any outliers, compared to the main analysis, variability was greater for each of the biomarkers. In general, we observed similar patterns for results compared to the main analysis (**Supplemental Table S2**). In persons without diabetes, variability of HbA1c was similar to that of the nontraditional biomarkers of hyperglycemia. However, the reliability of all biomarkers was generally still better compared to fasting glucose.

Discussion

Among the five traditional and nontraditional biomarkers of hyperglycemia included in this study, the 6-week within-person variability was highest for fasting glucose and lowest for HbA1c, and fructosamine, glycated albumin, and 1,5-AG were intermediate between the two. Furthermore, for each biomarker, the within-person variability was greater in persons with diagnosed diabetes compared to those without diagnosed diabetes. In general, estimates of within-person reliability were largely similar, except for fasting glucose, for which it was the lowest. All biomarkers had a low index of individuality (which suggests high individuality), regardless of whether calculated in persons with or without diagnosed diabetes.

In regard to the traditional biomarkers of hyperglycemia, we confirmed previous findings, in which we reported that the 2-week within-person variability of fasting glucose was higher than HbA1c.[20] The within-person variability of fructosamine and glycated albumin was similar to that reported in another previous study.[15] Our results confirm the reliability of fructosamine, glycated albumin, and 1,5-AG as intermediate-term biomarkers of hyperglycemia.[3]

Correlations and ICCs provided similar results to one another. Notably, 1,5-AG had very high correlations and estimates of reliability, which may be attributed to the relatively wide range of values for this biomarker. In particular, the remarkably high ICC for 1,5-AG was notable, and suggests that relative to the between-person variance, the within-person variance was low.

The index of individuality (ratio of total within-person CV to between-person CV) can help one detect significant changes in a biomarker of interest. A low index of individuality, typically considered to be <0.6, indicates high individuality.[21] All five biomarkers of hyperglycemia had a low index of individuality, which suggests that comparing serial measurements is particularly useful to assess changes over time within an individual.[19]

Quantifying the short-term variability of these biomarkers of hyperglycemia is important for their use in research studies. High within-person variability can lead to false positive results at the individual level, and substantial overestimates of disease prevalence at the population level, especially if the biomarker is only measured once.[20,22–24] Using only one measurement and not accounting for random error in measurement can also lead to regression dilution, which can result in associations with outcomes that are biased toward the null, or weaker than the "true" association.

Although the mean time between the original and repeat visits was ~6 weeks, this was not consistent across participants, and there was a relatively wide range of time between visits. Ideally, all measurements would have taken place over the same time period. Nonetheless, this study is among the first to comprehensively assess and compare short-term within-person variability of both traditional and nontraditional biomarkers of hyperglycemia. There were several strengths of this study. In this general population of older adults, we had a high prevalence of diabetes, and for a study of repeated measurements, we had a relatively large sample size, allowing for stratification by diabetes status. This allowed us to examine variability across a wide range of glycemia. Including adults >65 years of age is of particular importance, given the high prevalence of diabetes in this age group.[25] For each biomarker, assays for the samples from the original and repeat visits were conducted in the same laboratory and with the same instruments, which helped us isolate within-person variability from other sources of

variability that could arise from storage time, freeze-thaw, machine calibration, and lot-to-lot variability of reagents.

In summary, we found that HbA1c and nontraditional biomarkers of hyperglycemia track well over approximately 6 weeks, and had lower within-person variability and higher reliability than fasting glucose. These data should help inform the use of these biomarkers in research and clinical settings.

Table 1. Characteristics of study participants from the repeatability subsample, the ARIC Study, Visit 5 (2011-13)

Characteristic	**Mean (SD) or %**
N	153
Age, years	76.3 (4.9)
Male	39.2%
Race	
White	73.9%
Black	25.5%
Asian	0.6%
BMI, kg/m^2	28.7 (4.4)
Diabetes	32.7%
Time between visits, days	44.5 (15.9)

Table 2. Total variability in biomarkers of hyperglycemia in older adults with and without diabetes, the Atherosclerosis Risk in Communities Study, 2011-13, N=153

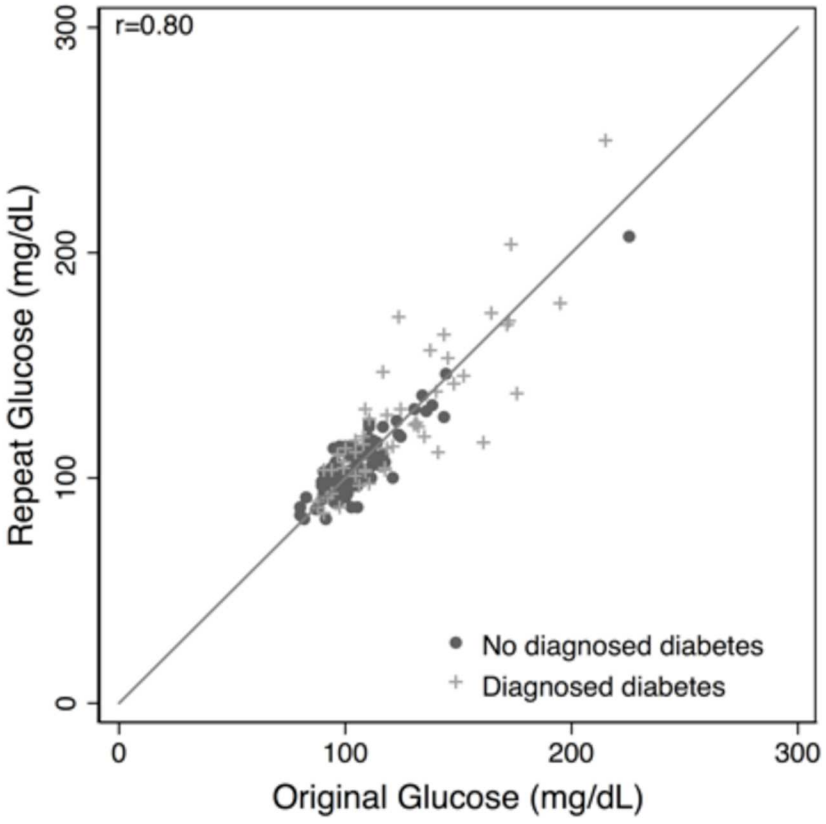
	Original exam Mean (SD)	Repeat exam Mean (SD)	Difference (Repeat-Original) Mean (SD)	CV_W (95% CI)†	ICC (95% CI)†	r (95% CI)	Index of Individuality (95% CI)†
No Diagnosed Diabetes (N=103)							
Fasting glucose, mg/dL	104.7 (17.6)	104.5 (15.9)	-0.18 (7.9)	5.3% (4.6, 6.0)	0.89 (0.85, 0.93)	0.72 (0.61, 0.80)	0.35 (0.27, 0.44)
HbA1c, %	5.7 (0.4)	5.7 (0.4)	-0.01 (0.1)	1.5% (1.3, 1.7)	0.95 (0.95, 0.96)	0.95 (0.92, 0.96)	0.22 (0.19, 0.25)
Fructosamine, µmol/L	241.5 (22.9)	239.4 (21.0)	-2.10 (11.3)	3.4% (2.9, 3.8)	0.86 (0.83, 0.89)	0.83 (0.76, 0.88)	0.40 (0.34, 0.46)
Glycated albumin, %	13.8 (1.5)	13.7 (1.7)	-0.04 (0.5)	2.7% (2.3, 3.0)	0.95 (0.94, 0.96)	0.91 (0.88, 0.94)	0.24 (0.20, 0.27)
1,5-AG, µg/mL	17.5 (6.0)	17.6 (6.1)	0.04 (0.7)	2.9% (2.7, 3.2)	0.99 (0.99, 0.99)	0.99 (0.99, 0.99)	0.09 (0.08, 0.09)
Diagnosed Diabetes (N=50)							
Fasting glucose, mg/dL	125.5 (29.8)	125.3 (32.7)	-0.14 (17.1)	9.6% (7.3, 11.8)	0.85 (0.80, 0.90)	0.84 (0.73, 0.90)	0.42 (0.31, 0.53)
HbA1c, %	6.3 (0.8)	6.3 (0.8)	0.04 (0.2)	2.0% (1.5, 2.5)	0.98 (0.97, 0.98)	0.96 (0.92, 0.98)	0.16 (0.12, 0.19)
Fructosamine, µmol/L	263.2 (39.8)	261.8 (38.7)	-1.46 (13.6)	3.7% (3.0, 4.3)	0.94 (0.92, 0.96)	0.84 (0.73, 0.90)	0.25 (0.20, 0.31)
Glycated albumin, %	15.5 (2.8)	15.7 (2.9)	0.2 (0.8)	3.8% (2.9, 4.7)	0.95 (0.94, 0.97)	0.91 (0.85, 0.95)	0.22 (0.17, 0.27)
1,5-AG, µg/mL	15.1 (6.5)	15.1 (6.5)	0.04 (1.2)	5.7% (4.2, 7.2)	0.98 (0.98, 0.99)	0.98 (0.97, 0.99)	0.13 (0.10, 0.17)

Abbreviations: CI, confidence interval; CV_W, within-person coefficient of variation; ICC, intraclass correlation coefficient; r, Spearman's rank correlation coefficient; SD, standard deviation

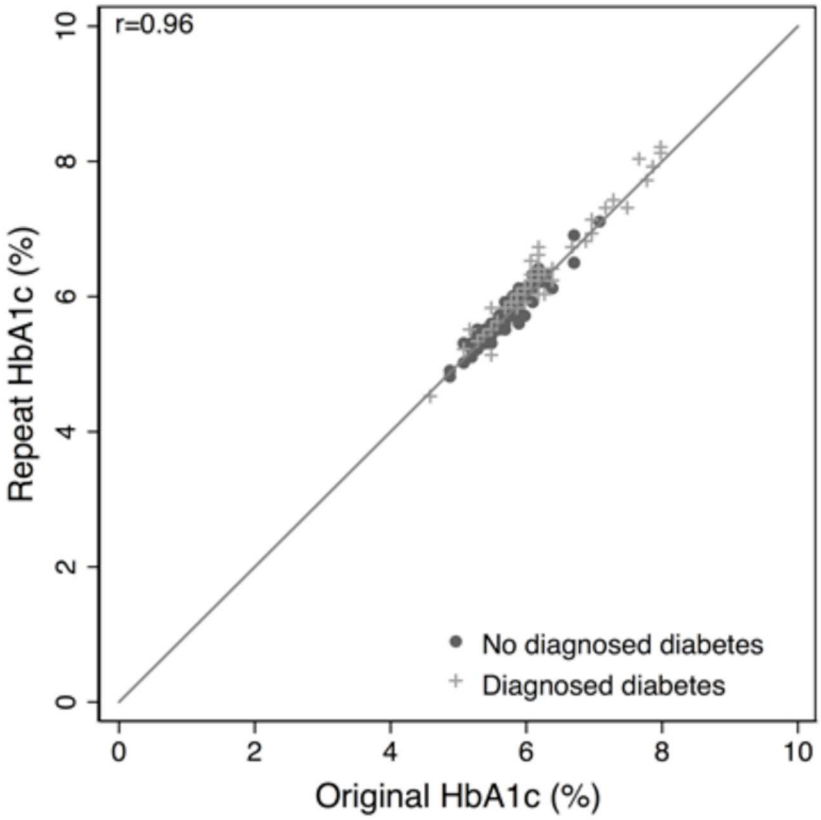
†95% CIs were bootstrapped using 200 replications

Figure 1. Scatterplots of original and repeat measurements of biomarkers of hyperglycemia conducted ~6 weeks apart in persons with and without diagnosed diabetes. The solid line is the line of equality (Y=X). The Spearman correlation coefficient is presented.

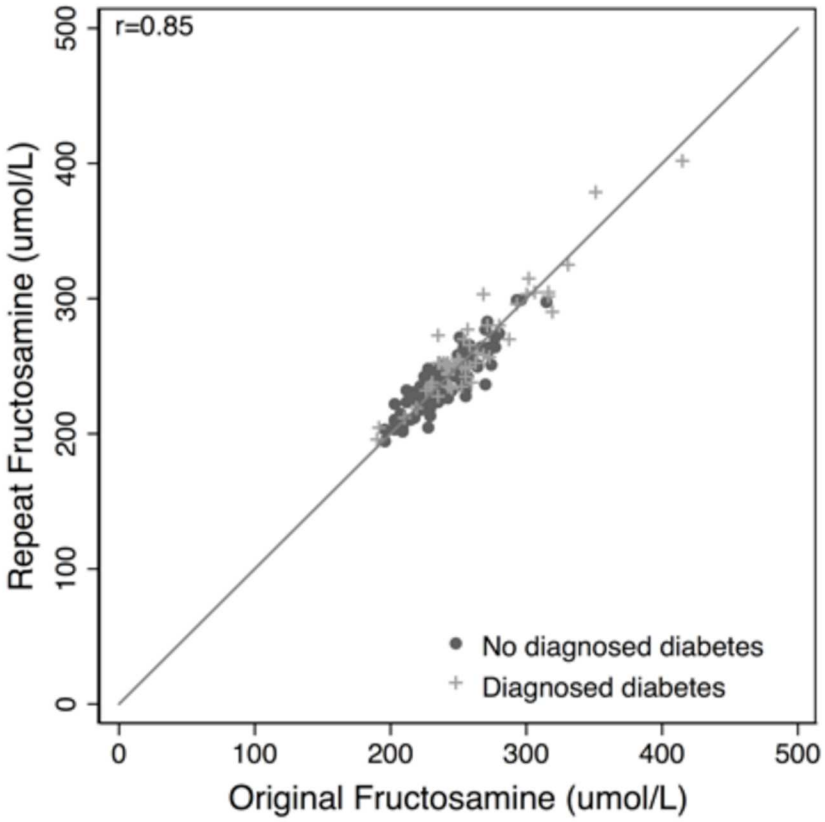
A. Glucose



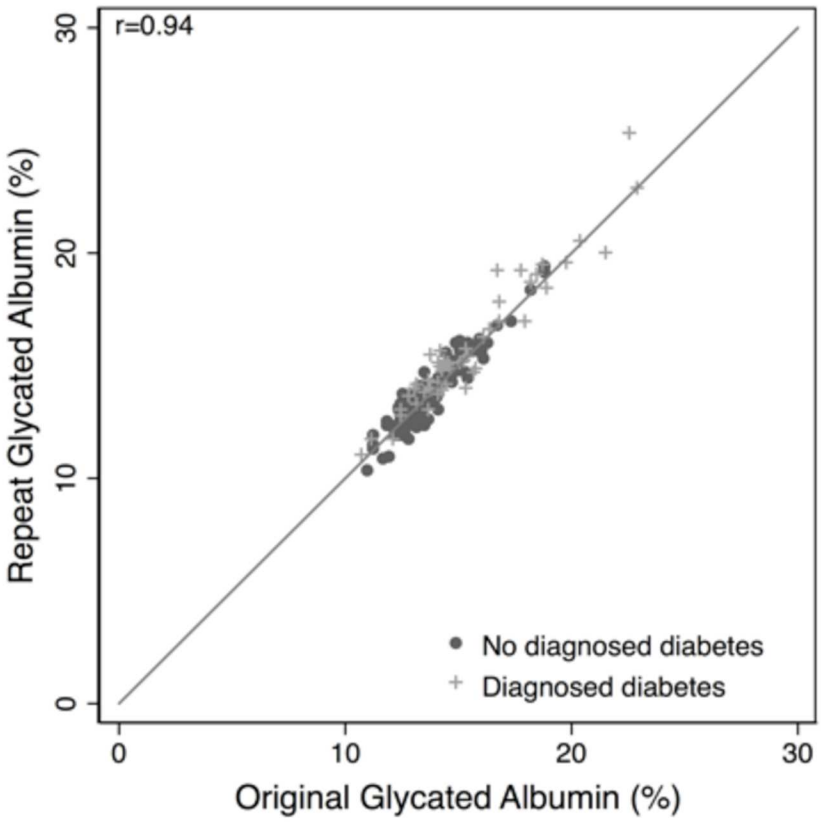
B. Hemoglobin A1c



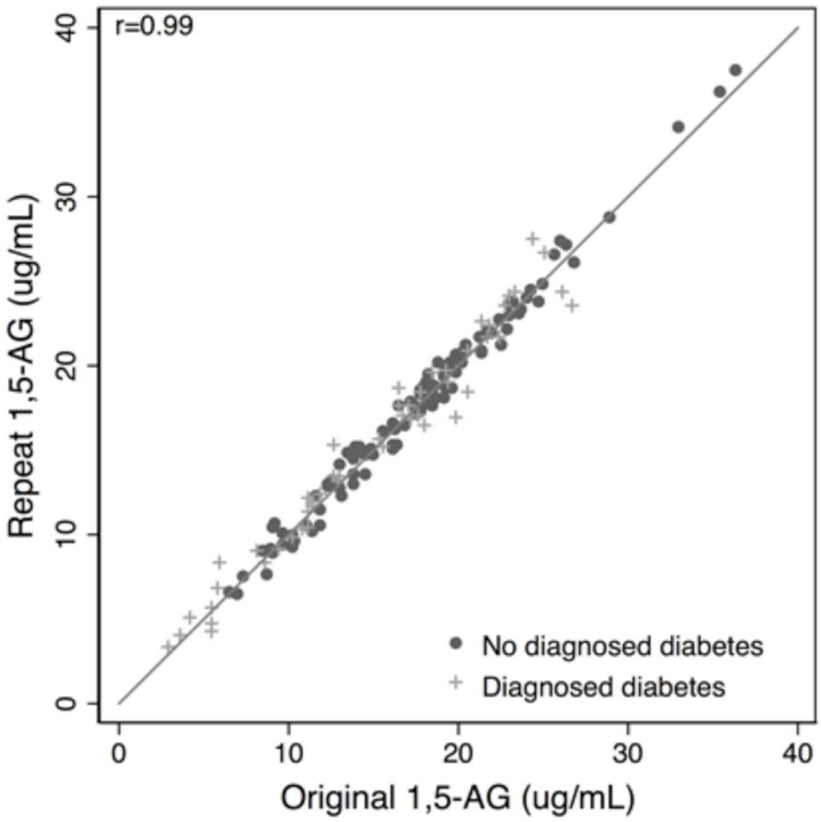
C. Fructosamine



D. Glycated Albumin



E. 1,5-Anhydroglucitol



Chapter 3: Racial differences in hyperglycemia: Comparative prognostic value of traditional and nontraditional biomarkers of hyperglycemia in persons with and without diabetes

Abstract

Background: Associations of nontraditional biomarkers of hyperglycemia with clinical outcomes in blacks and whites are not well-characterized and could inform the debate over race-specific cut-points for hemoglobin A1c (HbA1c).

Objective: To compare, in blacks versus whites, the associations of traditional (fasting glucose, HbA1c) and nontraditional (fructosamine, glycated albumin and 1,5-anhydroglucitol [1,5-AG]) biomarkers of hyperglycemia with incident cardiovascular disease (CVD) and end-stage renal disease (ESRD).

Design: Prospective cohort.

Setting: Atherosclerosis Risk in Communities (ARIC) Study; baseline visit 2 (1990-92) through 2011.

Participants: We included 10,375 participants without (8,096 white, 2,279 black) and 728 with diagnosed diabetes (426 white, 302 black), with baseline measures of biomarkers of hyperglycemia.

Measurements: We used Poisson and Cox regression to compare absolute risks (incidence rates) and relative risks (hazards ratios) of CVD and ESRD in blacks and whites. We tested for the interaction of each biomarker with race.

Results: Median values of biomarkers were higher in blacks versus whites (all $p<0.001$). Age- and sex-adjusted incidence rates for CVD and ESRD were higher in blacks than whites in persons without diagnosed diabetes (both $p<0.001$). Similar patterns were observed in persons with diagnosed diabetes. Relative risks for each biomarker with incident CVD and ESRD were similar by race (all p-values for interaction >0.15).

Limitations: Single measurements of biomarkers; possibility that race effects could be due to geography; potential residual confounding.

Conclusions: Associations of HbA1c, fructosamine, glycated albumin, and 1,5-AG with CVD and ESRD risk were similar by race, even though blacks had higher levels of hyperglycemia and higher incidence rates of CVD and ESRD than whites. Our results support similar clinical utility in black and whites of HbA1c and nontraditional biomarkers of hyperglycemia.

Introduction

Higher hemoglobin A1c (HbA1c) values in blacks and other racial and ethnic minorities compared to whites are well-documented.[1–6] Even among persons with similar levels of fasting glucose, blacks tend to have higher HbA1c levels compared to whites.[1,4] The racial disparity in HbA1c has led to controversy over the use of HbA1c as a diagnostic test in blacks. Proponents of race-specific cut-points for HbA1c have suggested that HbA1c is artificially high in blacks due to non-glycemic factors such as hemoglobin glycation or red cell turnover, and that the use of HbA1c may lead to overdiagnosis of diabetes in minority populations.[7–11]

Nontraditional biomarkers of hyperglycemia (fructosamine, glycated albumin, and 1,5-anhydroglucitol [1,5-AG]) have emerged as possible adjuncts to the traditional biomarkers, fasting glucose and HbA1c.[12,13] Fasting glucose is a direct measure of current glycemia. HbA1c is the proportion of hemoglobin bound to glucose, and measures average glycemia over 2-3 months, based on red blood cell turnover.[14] Fructosamine and glycated albumin are markers of glucose bound to serum proteins and estimate average glycemia over 2-4 weeks.[15] 1,5-AG is a monosaccharide mainly derived from the diet, and is normally almost completely reabsorbed by the kidney. In states of hyperglycemia (>180 mg/dL), glucose in the tubular lumen inhibits tubular reabsorption of 1,5-AG, and more 1,5-AG is excreted, resulting in lower serum 1,5-AG levels. Levels of 1,5-AG are inversely associated with average glycemia over the past 2-14 days.[16–20]

Higher levels of biomarkers of hyperglycemia have been associated with increased risk of microvascular and macrovascular complications.[21–26] However, prospective associations of nontraditional serum biomarkers of hyperglycemia with microvascular and macrovascular complications according to race have not been characterized, and could shed further light on the debate over the use of HbA1c. Whereas HbA1c is a measure of intracellular hyperglycemia and can be affected by non-glycemic factors, such as hemoglobin characteristics or alterations in red cell turnover, fructosamine, glycated albumin, and 1,5-AG are serum measures of extracellular hyperglycemia, and are therefore not affected by these non-glycemic factors. Therefore, comparing associations of each of these biomarkers in blacks and whites could provide insight into whether racial differences in levels of biomarkers may be attributed to glycemic or non-glycemic factors.

Our objective was to assess the associations of fasting glucose, HbA1c, and nontraditional serum markers of hyperglycemia (fructosamine, glycated albumin, and 1,5-AG) with incident CVD and ESRD in persons with and without diabetes, and to evaluate for differential associations between blacks and whites.

Methods

Setting and participants

We conducted a prospective cohort analysis of the Atherosclerosis Risk in Communities (ARIC) Study, a community-based cohort of 15,792 persons recruited in 1987-89 from

four field centers in the United States: Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland.[27] Follow-up visits 2 through 5 took place during 1990-92, 1993-95, 1996-98 and 2011-13, respectively. We restricted our study population to ARIC participants who attended visit 2 (N=14,348), since this was the earliest visit at which all biomarkers of hyperglycemia were measured. We excluded participants who did not have values for all biomarkers of hyperglycemia (n=1,159), or were missing key covariates (n=379). We further excluded a small number of non-black and non-white participants (n=38), as well as persons who were not fasting for ≥8 hours (n=396). Lastly, we excluded 1,273 persons with prevalent CVD at visit 2. For analyses of incident ESRD, we additionally excluded 3 persons with pre-existing ESRD based on linkage with the United States Renal Data System (USRDS). There were 11,103 and 11,100 participants included in our final study populations for analyses of incident CVD and incident ESRD, respectively.

Biomarkers of hyperglycemia

Fasting glucose was measured from serum in 1990-92 with the Coulter DACOS Analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) using a hexokinase method. HbA1c was measured from stored whole blood in 2003-04 and 2007-08 with the Tosoh A1c 2.2 Plus Glycohemoglobin Analyzer and Tosoh G7 Analyzer (Tosoh Bioscience, Inc., South San Francisco, CA, USA), respectively, using a high-performance liquid chromatography method, and was standardized to the Diabetes Control and Complications Trial assay.[28] Fructosamine, glycated albumin and 1,5-AG were measured from serum in 2012-13

using a Roche Modular P800 (Roche Diagnostics Corporation, Indianapolis, IN) analyzer. Fructosamine was measured using a colorimetric method (Roche Diagnostics Corporation, Indianapolis, IN, USA). Glycated albumin (Asahi Kasei Lucica GA-L, Tokyo, Japan) and 1,5-AG (GlycoMark, New York, NY) were measured using enzymatic methods. Glycated albumin was expressed as a percentage of total albumin, calculated using the following equation derived by the manufacturer: [(glycated albumin concentration in g/dL / serum albumin concentration in g/dL) / 1.14*100] + 2.9. The interassay coefficients of variation were 3% for fructosamine, 1.8% for glycated albumin, and 5% for 1,5-AG.

Covariates

We classified diagnosed diabetes based on a self-reported physician diagnosis of diabetes or self-reported use of glucose-lowering medication at visit 1 or visit 2. We defined prevalent CVD as self-reported history of coronary heart disease (CHD) or stroke at visit 1, prevalent heart failure at visit 1 (based on self-reported medication use or Gothenburg criteria score), or hospitalization for myocardial infarction (MI), fatal CHD, cardiac procedure, MI detected by electrocardiogram, stroke, or heart failure prior to visit 2.

The following covariates were self-reported by participants as responses to questionnaires at visit 2, unless otherwise specified: age, sex, education level (visit 1), alcohol consumption, smoking status, and physical activity (Baecke sport activity index at visit

1)[29]. Antihypertensive medication use was obtained via self-report or medication inventory. Cholesterol-lowering medication use was obtained via medication inventory.

Diastolic and systolic blood pressures were measured using a random zero sphygmomanometer, and recorded as the mean of the 2nd and 3rd readings. Body mass index (BMI) was calculated as measured weight (in kilograms) divided by measured height (in meters) squared. Total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides were measured using stored plasma with the Roche Cobas Bio (Roche Diagnostics, Indianapolis, IN). Total cholesterol and triglycerides were measured using an enzymatic method and HDL-c was measured using a precipitation method. LDL-c was calculated from measured total cholesterol, HDL-c, and triglycerides using the Friedewald equation. Creatinine was measured using a Jaffe method with the Coulter DACOS analyzer. We used the CKD-EPI equation to calculate estimated glomerular filtration rate (eGFR) using serum creatinine, age, sex, and race.[30]

Outcomes and follow-up

We used a composite CVD endpoint defined as a first CHD, stroke, or heart failure event, based on standard ARIC definitions: first occurrence of definite or probable hospitalized MI or death due to CHD;[31] definite or probable hospitalized stroke or death due to stroke;[32] or hospitalization or death due to heart failure, based on a 428 International

Classification of Diseases, 9th Revision (ICD-9) code or an ICD, 10th Revision (ICD-10) code of 150.[33] All cardiovascular events were ascertained via continuous surveillance of hospitalizations and death certificates, annual telephone follow-up with the participant or a proxy, and linkage to the National Death Index, through December 31, 2011. CHD and stroke events were adjudicated over the entire follow-up; and heart failure events were adjudicated beginning in 2005. We conducted secondary analyses with CHD, stroke, and heart failure as separate endpoints.

For analyses of incident ESRD, we identified treated cases through linkage with the USRDS national registry, through September 30, 2011, since that was the most current available linkage to the USRDS. The Centers for Medicare and Medicaid Services report any persons receiving renal replacement therapy to the USRDS within 45 days of initiation of treatment, which includes persons undergoing dialysis or a kidney transplant.

Statistical analysis

We calculated descriptive statistics for demographic and clinical characteristics, stratified by diagnosed diabetes status and race. In persons with and without diagnosed diabetes, we compared median levels of HbA1c, fructosamine, glycated albumin and 1,5-AG within clinical categories of fasting glucose separately in blacks and whites, and tested for differences using the Wilcoxon rank-sum test.

In persons without diagnosed diabetes, we used clinical cut-points recommended by the American Diabetes Association to categorize fasting glucose (<100, 100-125, ≥126 mg/dL) and HbA1c (<5.7, 5.7-6.4, ≥6.5%).[12] We categorized persons with diagnosed diabetes based on the clinically recommended cut-point of <7 vs ≥7% for HbA1c. Since clinical cut-points are not used for fasting glucose in persons with diabetes, and cut-points have not been established for fructosamine, glycated albumin, or 1,5-anhydroglucitol in persons with or without diabetes, we created cut-points at the percentiles that corresponded to those for accepted HbA1c cut-points in those with and without diagnosed diabetes.[12] Percentiles were determined using the entire study population (rather than identifying race-specific percentiles), in persons with and without diagnosed diabetes, separately. In persons without diagnosed diabetes, HbA1c values of 5.7% and 6.5% were at the 75th and 96.5th percentiles, respectively. In persons with diagnosed diabetes, the HbA1c value of 7% was at the 40th percentile. We therefore created cut-points at the 75th and 96.5th percentiles of each biomarker in persons without diagnosed diabetes (25th and 3.5th percentiles for 1,5-AG, because of inverse associations of 1,5-AG with glycemia), and at the 40th percentile of each biomarker in persons with diagnosed diabetes (60th percentile for 1,5-AG). These percentiles corresponded to the following cut-points: in persons without diagnosed diabetes, 239.9 and 268.8 mg/dL for fructosamine, 13.52 and 15.56% for glycated albumin, and 14.9 and 7.9 μg/mL for 1,5-AG; and in persons with diagnosed diabetes, 149 mg/dL for fasting glucose, 275.8 mg/dL for fructosamine, 16.47% for glycated albumin, and 9.2 μg/mL for 1,5-AG.

Using the cut-points described above, we created a 5-level variable for each biomarker based on diagnosed diabetes status and biomarker level, as follows: no diabetes (no diagnosed diabetes and biomarker level below the lower cut-point); no diabetes, intermediate levels (no diagnosed diabetes and biomarker level between the lower and upper cut-points); no diabetes, elevated levels (no diagnosed diabetes and biomarker level above the upper cut-point); diabetes (diagnosed diabetes and biomarker level below the cut-point); and diabetes, elevated levels (diagnosed diabetes and biomarker level above the cut-point). Categories of 1,5-AG were created in the opposite direction, to reflect inverse associations of 1,5-AG with glycemia.

To compare the absolute risk of CVD and ESRD in whites and blacks, we used Poisson regression to calculate age- and sex-adjusted race-specific incidence rates of each outcome separately in persons with and without diagnosed diabetes. We included an offset term of the natural log of person-years to account for differences in follow-up time. We used the Wald test to test differences in incidence rates comparing whites and blacks.

We used Cox proportional hazards regression models to assess associations of biomarkers of hyperglycemia with incident CVD and ESRD in whites and blacks. We created separate models for each biomarker and each outcome. Follow-up began at the visit 2 exam date (1990-92) and continued until the time of the event, last date of follow-up, or December 31, 2011 (September 30, 2011 for ESRD analyses), whichever occurred first. We adjusted for the following characteristics: age, sex, BMI, BMI-squared, LDL-c,

HDL-c, triglycerides, cholesterol-lowering medication use, systolic blood pressure, antihypertensive medication use, eGFR, family history of diabetes, education level, alcohol consumption, cigarette smoking status, and physical activity level. As a sensitivity analysis, we additionally adjusted for fasting glucose and HbA1c. To test for a linear trend in whites and blacks separately, we included the categorical biomarker variable as a continuous variable, and conducted a Wald test for the coefficient. To test for the interaction between biomarkers of hyperglycemia (categorized as above) and race (black and white), we used likelihood ratio tests to compare models with and without a 5-level interaction term. P-values <0.05 were considered statistically significant.

We verified the proportional hazards assumption was met using likelihood ratio tests ($P>0.05$ indicating no violation of the assumption).

All statistical analyses were conducted using Stata, version 13.0 (StataCorp, College Station, Texas, USA).

Results

Our study population included 10,375 participants without diagnosed diabetes (8,096 white and 2,279 black) and 728 participants with diagnosed diabetes (426 white and 302 black). Baseline characteristics varied between those with and without diagnosed diabetes,

as expected (**Table 1**). Furthermore, characteristics differed between white and black participants (**Table 1**). Even at similar levels of fasting glucose, blacks had higher levels of HbA1c, fructosamine, and glycated albumin (P<0.001 for all); and lower levels of 1,5-AG (P<0.07 for all), as compared to whites (**Table 2**). The magnitudes of these black-white differences were particularly large among persons with diagnosed diabetes (**Table 2**). Among persons without diagnosed diabetes, blacks had statistically significantly higher age- and sex-adjusted incidence rates of CVD and ESRD than whites (P<0.001 for both) (**Figure 1**). Among persons with diagnosed diabetes, incidence rates were higher and black-white differences remained (P=0.07 for CVD and P<0.001 for ESRD) (**Figure 1**).

Among 11,103 participants free of CVD at baseline, there were 2,495 incident cases of CVD over a median of 19.6 years of follow-up. Compared to persons with no diagnosed diabetes and biomarker levels in the lowest category of glycemia, those with no diabetes and levels of biomarkers indicating higher glycemia had greater risk of CVD for fasting glucose, HbA1c, fructosamine, and glycated albumin; we found similar results for those with diagnosed diabetes (**Figure 2 and Supplemental Table S1**). Levels of 1,5-AG were only statistically significant associated with CVD in persons with diagnosed diabetes. Magnitudes of association were greatest for HbA1c as compared to other biomarkers (**Figure 2 and Supplemental Table S1**). The associations followed a linear trend in both whites and blacks (P-trend<0.001 for all). These associations of biomarkers with incident CVD were similar in blacks and whites (P-values for interaction>0.30 for all biomarkers)

(**Figure 2 and Supplemental Table S1**). Patterns and magnitudes of associations of biomarkers of hyperglycemia with individual CVD outcomes (CHD, stroke, and heart failure) were similar to those of the composite CVD outcome, with no evidence of a race interaction (P-values for interaction>0.15 for all biomarkers and all individual outcomes) (**Supplemental Tables S2-S4**).

Among 11,100 participants who were free of CVD and ESRD at baseline, there were 170 incident cases of ESRD during a median of 19.9 years of follow-up. Patterns of associations of biomarkers of hyperglycemia with ESRD were similar to those for CVD, but the magnitudes of association with ESRD were substantially higher (**Figure 2 and Supplemental Table S5**). The associations followed a linear trend in both whites and blacks (P-trend<0.001 for all). Associations of biomarkers of hyperglycemia with incident ESRD were similar in blacks and whites (P-values for interaction>0.15 for all biomarkers) (**Figure 2 and Supplemental Table S5**).

Additional adjustment for continuous fasting glucose or HbA1c substantially attenuated the associations with outcomes, but inferences about racial comparisons of associations generally remained the same (**Supplemental Tables S1-S5**).

Discussion

Compared to whites, as seen in prior studies, blacks had higher levels of glycemia and higher absolute risk of CVD and ESRD. However, we show that the relative risks of these outcomes associated with all of the biomarkers of hyperglycemia were similar in blacks and whites with no evidence of an interaction by race. Thus, the prognostic utility of HbA1c and nontraditional serum biomarkers of hyperglycemia is similar in both blacks and whites.

Higher levels of fasting glucose, HbA1c, fructosamine, and glycated albumin were independently associated with incident CVD and ESRD in persons with and without diagnosed diabetes compared to persons without diagnosed diabetes and non-elevated levels of these glycemic markers. Lower levels of 1,5-AG were also associated with increased risk of incident CVD and ESRD, but only in persons with diagnosed diabetes, which may reflect the fact that serum concentrations of 1,5-AG only vary substantially (lowered by loss of glucose in the urine) in the setting of overt hyperglycemia. Adjustment for fasting glucose attenuated associations, but inferences generally remained the same, suggesting that HbA1c and nontraditional serum biomarkers of hyperglycemia provide information about microvascular and macrovascular risk above and beyond fasting glucose.

Our findings support previous studies that have shown similar associations of HbA1c with microvascular and macrovascular disease in blacks and whites,[34,35,24,25] and that have recommended using the same HbA1c diagnostic cut-points across races/ethnicities.[36,37]

Furthermore, our research supports the idea that racial differences in biomarkers of hyperglycemia may be due to real differences in glycemia (rather than to the differences in the behavior of the markers studied), perhaps due to differences in the presence or effects of glycemic factors such as diet and physical activity.[38] Even after controlling for fasting glucose concentrations, we observed here and previously that blacks had higher levels of glycemia compared to whites, as measured by higher HbA1c, fructosamine, glycated albumin, and lower 1,5-AG, indicating higher exposure to circulating glucose over intermediate and longer durations of time.[1] Fructosamine, glycated albumin, and 1,5-AG are completely independent of the red blood cell or hemoglobin. Therefore, the observation that these nontraditional serum markers of hyperglycemia exhibit a similar pattern of racial differences as HbA1c provides evidence that non-glycemic factors, such as hemoglobin glycation or red cell turnover, do not explain the observed racial disparities in levels of these biomarkers.[1] Prior population-based genetic studies also suggest that there are not important race-specific determinants of HbA1c that impact the clinical utility of this marker across ethnic groups.[39,40] Consistent with these findings suggesting that blacks have higher HbA1c because they indeed have higher levels of glucose, it is known that blacks do have higher absolute risks of diabetes and diabetes-related complications than whites.[41] We confirm these prior reports, and showed here that blacks had higher incidence rates of CVD and ESRD than whites. The use of new technology, such as continuous glucose monitoring, could provide additional valuable insight.

There were several limitations of this study. First, we used single measurements of biomarkers of hyperglycemia at baseline, which are less reliable than repeated measurements. Repeated measurements would have more closely resembled clinical guidelines, which recommend confirmatory testing. Second, it is unlikely that we were able to fully adjust for diet and physical activity, which are important to account for when assessing the association of hyperglycemia with CVD and ESRD. Third, we cannot rule out the possibility that differences in levels of biomarkers of hyperglycemia by race may be due to cultural differences by geography, rather than race alone. In the ARIC Study, race is closely aligned with geographic location, and black participants were recruited almost exclusively from two of the four field centers (Jackson, Mississippi and Forsyth County, North Carolina). Nonetheless, our study had several important strengths. This was one of the largest prospective studies to include HbA1c and nontraditional serum biomarkers of hyperglycemia in a predominately non-diabetes population, which is particularly important in regard to assessing the diagnostic utility of HbA1c. We also leveraged the large numbers of both black and white participants in the cohort to assess racial differences in hyperglycemia. Furthermore, the ARIC Study included rigorous follow-up of both CVD and ESRD over a median follow-up of nearly 20 years.

Characterizing associations of these biomarkers of hyperglycemia with hard clinical endpoints is of the utmost importance, since the goal of early diagnosis and improved disease management is prevention of microvascular and macrovascular complications in persons with and at risk for diabetes. Blacks may in fact have higher average circulating

glucose levels than whites, but biomarkers of hyperglycemia similarly reflect risk of clinical outcomes in blacks and whites. Indeed, our results suggest similar prognostic utility of HbA1c and nontraditional serum markers of hyperglycemia in black and white adults. Additional large, multi-ethnic prospective cohort studies that include persons with and without diabetes will advance our understanding of the utility of these biomarkers and can confirm that their predictive value does not vary across racial and ethnic minority groups.

Table 1. Characteristics of the study population by diabetes status and race, ARIC visit 2 (1990-92)

	No Diagnosed Diabetes			Diagnosed Diabetes		
	White (N=8,096)	Black (N=2,279)		White (N=426)	Black (N=302)	
	Mean (SD) or %	Mean (SD) or %	P-value*	Mean (SD) or %	Mean (SD) or %	P-value*
Age, years	57.3 (5.6)	56.2 (5.7)	<0.001	58.7 (5.7)	57.6 (5.7)	0.012
Male	43.9%	35.8%	<0.001	48.1%	29.5%	<0.001
<HS education	13.9%	35.0%	<0.001	24.2%	44.0%	<0.001
Obese (BMI ≥30 kg/m^2)	21.7%	41.5%	<0.001	47.4%	55.0%	0.045
Current smoking	20.5%	24.5%	<0.001	17.8%	21.2%	0.258
Current drinking	66.2%	36.7%	<0.001	48.4%	21.5%	<0.001
Family history of diabetes	21.9%	25.0%	0.002	41.8%	41.1%	0.845
Hypertension†	29.4%	51.4%	<0.001	51.4%	64.9%	<0.001
HDL-c, mg/dL	50.1 (16.6)	54.5 (17.0)	<0.001	42.1 (12.8)	49.1 (13.6)	<0.001
Total cholesterol, mg/dL	209.0 (37.4)	208.9 (40.0)	0.952	208.6 (39.2)	217.5 (45.6)	0.005
LDL-c, mg/dL	132.8 (35.6)	133.7 (38.3)	0.264	132.5 (35.3)	141.4 (42.7)	0.002
Triglycerides, mg/dL	130.7 (64.5)	103.5 (50.0)	<0.001	169.4 (75.3)	134.8 (68.3)	<0.001
Cholesterol-lowering medication use	5.5%	2.8%	<0.001	12.9%	5.0%	<0.001
Glucose-lowering meds‡	-	-	-	61.4%	76.5%	<0.001
eGFR<60 mL/min/1.73 m^2	1.1%	1.1%	0.936	2.6%	4.6%	0.134
Baecke sport index	2.5 (0.8)	2.2 (0.7)	<0.001	2.4 (0.8)	2.1 (0.6)	<0.001

Abbreviations: IQR, interquartile range; SD, standard deviation

*Two-sided P-values calculated using Student's t-test for continuous variables, and Chi Square tests for categorical variables.

†Hypertension defined as diastolic BP ≥90 mmHg or systolic BP≥140 mmHg or antihypertensive medication use

‡Among persons with diabetes, there are 13 black and 22 white participants missing a response to self-reported use of glucose-lowering medication.

Table 2. Baseline levels of biomarkers of hyperglycemia by diabetes status and race

Among persons without diagnosed diabetes

	White (N=8,096)	Black (N=2,279)	
	Median (25^{th}-75^{th} percentile)	Median (25^{th}-75^{th} percentile)	P-value*
Fasting glucose, mg/dL	101 (95-108)	104 (97-113)	<0.001
HbA1c, %	5.4 (5.1-5.6)	5.7 (5.4-6.0)	<0.001
Fructosamine, μmol/L	225 (214-237)	234 (220-250)	<0.001
Glycated albumin, %	12.5 (11.7-13.3)	13.3 (12.4-14.3)	<0.001
1,5-AG, μg/mL	18.9 (15.3-22.5)	17.3 (13.9-21.0)	<0.001
HbA1c, %			
FG <100 mg/dL	5.3 (5.1-5.4)	5.5 (5.2-5.7)	<0.001
FG 100-125 mg/dL	5.4 (5.2-5.7)	5.7 (5.4-6.0)	<0.001
FG ≥126 mg/dL	6.2 (5.8-6.8)	6.6 (6.2-7.2)	<0.001
Fructosamine, μmol/L			
FG <100 mg/dL	224 (212-234)	230 (217-242)	<0.001
FG 100-125 mg/dL	226 (214-237)	234 (220-249)	<0.001
FG ≥126 mg/dL	247 (229-268)	260 (236-285)	<0.001
Glycated albumin, %			
FG <100 mg/dL	12.4 (11.7-13.2)	13.1 (12.3-13.9)	<0.001
FG 100-125 mg/dL	12.4 (11.7-13.2)	13.3 (12.4-14.3)	<0.001
FG ≥126 mg/dL	14.1 (12.8-15.8)	15.2 (13.6-17.3)	<0.001
1,5-AG, μg/mL			
FG <100 mg/dL	18.6 (15.2-22.1)	17.3 (14.2-21.1)	<0.001
FG 100-125 mg/dL	19.4 (15.8-22.9)	17.7 (14.3-21.2)	<0.001
FG ≥126 mg/dL	15.5 (10.0-20.4)	14.6 (8.2-18.7)	0.055

Among persons with diagnosed diabetes

	White (N=426)	Black (N=302)	
	Median (25^{th}-75^{th} percentile)	Median (25^{th}-75^{th} percentile)	P-value*
Fasting glucose	158 (122-216)	194 (136-271)	<0.001
HbA1c, %	7.1 (5.9-8.6)	8.4 (6.7-10.6)	<0.001
Fructosamine, μmol/L	280 (241-360)	331 (267-423)	<0.001
Glycated albumin, %	16.6 (13.3-22.4)	21.7 (16.0-29.0)	<0.001
1,5-AG, μg/mL	7.7 (2.4-15.4)	4.0 (1.5-12.1)	<0.001
HbA1c			
FG <149 mg/dL	5.8 (5.4-6.5)	6.4 (5.9-7.1)	<0.001
FG ≥149 mg/dL	8.4 (7.4-9.8)	9.7 (8.0-11.4)	<0.001
Fructosamine			
FG <149 mg/dL	240 (221-260)	254 (237-281)	<0.001
FG ≥149 mg/dL	341 (290-404)	378 (316-465)	<0.001

Glycated albumin, %			
FG <149 mg/dL	13.1 (12.1-15.0)	15.0 (13.4-16.4)	<0.001
FG ≥149 mg/dL	21.1 (17.4-27.1)	25.0 (21.1-32.7)	<0.001
1,5-AG, μg/mL			
FG <149 mg/dL	15.1 (9.7-20.1)	13.3 (9.5-17.3)	0.048
FG ≥149 mg/dL	2.8 (1.4-7.1)	2.1 (1.2-5.3)	0.065

Abbreviations: FG, fasting glucose; 1,5-AG, 1,5-anhydroglucitol

*P-values calculated using the Wilcoxon rank-sum (Mann-Whitney) test

Figure 1. Age- and sex-adjusted incidence rates for incident cardiovascular disease and incident end-stage renal disease by race. Incidence rates are per 1,000 person-years. Error bars represent 95% confidence intervals for incidence rates. P-values were calculated using Wald tests to test for differences in incidence rates between whites and blacks. Abbreviations: CVD, cardiovascular disease; ESRD, end-stage renal disease.

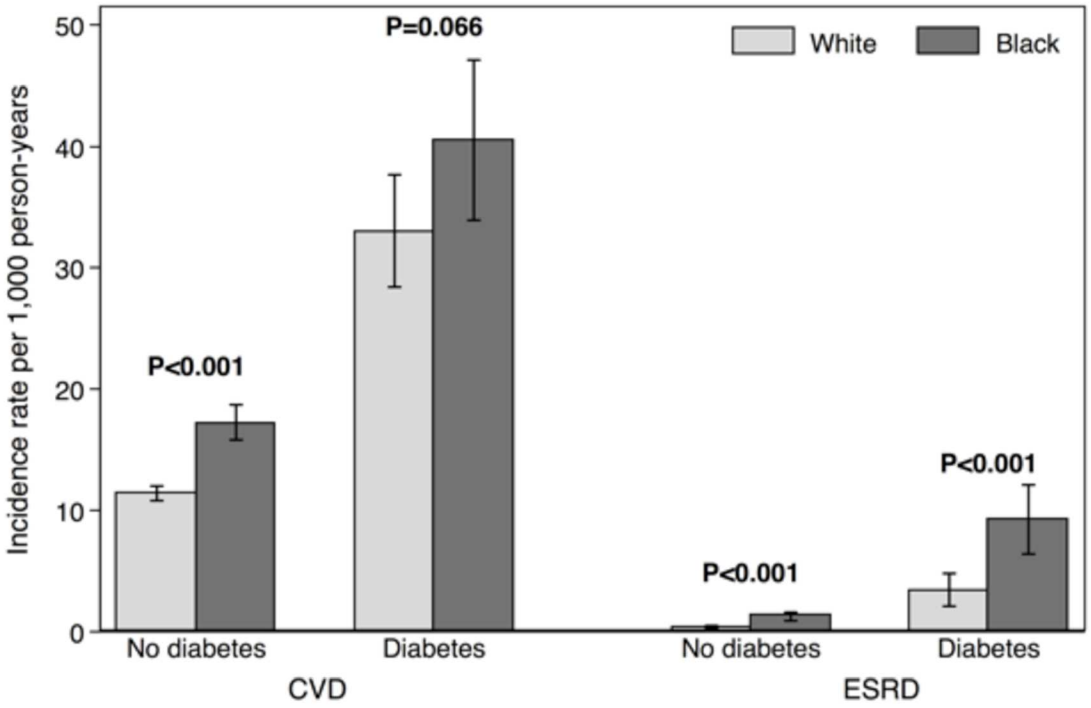


Figure 2. Adjusted associations of hyperglycemia with incident cardiovascular disease and end-stage renal disease by race. Hazard ratios were obtained using Cox proportional hazards regression models in white and black participants, separately. In models that included both white and black participants, P-values for interactions were calculated by conducting a likelihood ratio test to compare models with and without terms for the interaction between race and hyperglycemia. Models include adjustment for age, sex (male, female), BMI, BMI-squared, LDL-c, HDL-c, triglycerides, cholesterol-lowering medication use (yes, no), systolic blood pressure, antihypertensive medication use (yes, no), eGFR, family history of diabetes (yes, no), education level (less than high school, high school or some college, college or more), alcohol consumption (current, former, never), cigarette smoking status (current, former, never), and physical activity level. Categories of no diabetes; no diabetes, intermediate levels; no diabetes, elevated levels; diabetes; and diabetes, elevated levels were defined as follows for each biomarker:

Using fasting glucose: <100, 100-125, ≥126, <200, ≥200 mg/dL, respectively

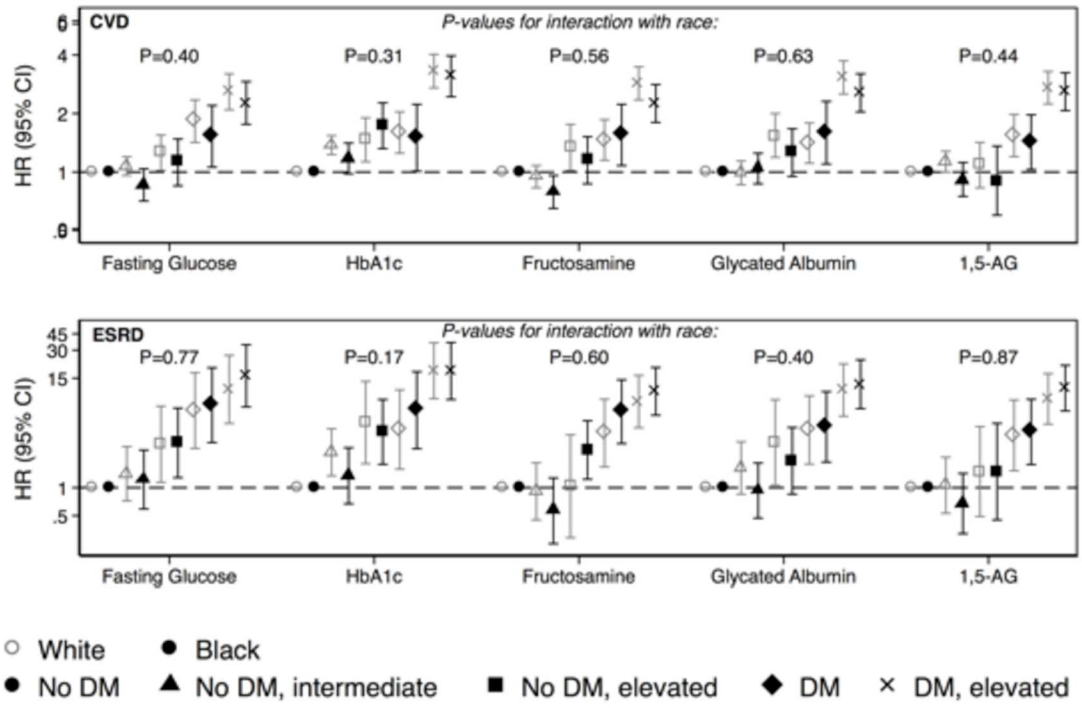
Using HbA1c: <5.7, 5.7-6.4, ≥6.5, <7.0, ≥7.0%, respectively

Using fructosamine: <239.9, 239.9-268.8, ≥268.9, <275.8, ≥275.8 mg/dL, respectively

Using glycated albumin: <13.52, 13.52-15.56, ≥15.57, <16.47, ≥16.47%, respectively

Using 1,5-AG: ≥15.0, 7.9-14.9, <7.9, >9.2, ≤9.2 μg/mL, respectively

Abbreviations: CVD, cardiovascular disease; DM, diabetes mellitus; ESRD, end-stage renal disease


CVD
P-values for interaction with race:
P=0.40
P=0.31
P=0.56
P=0.63
P=0.44
HR (95% CI)
Fasting Glucose
HbA1c
Fructosamine
Glycated Albumin
1,5-AG
ESRD
P-values for interaction with race:
P=0.77
P=0.17
P=0.60
P=0.40
P=0.87
HR (95% CI)
Fasting Glucose
HbA1c
Fructosamine
Glycated Albumin
1,5-AG
White
Black
No DM
No DM, intermediate
No DM, elevated
DM
DM, elevated

Chapter 4: Six-year change in high-sensitivity C-reactive protein and risk of diabetes, cardiovascular disease, and mortality

Am Heart J 2015; in press

Abstract

Introduction: Single measurements of elevated high-sensitivity C-reactive protein (hs-CRP) are associated with increased risk of diabetes, cardiovascular disease (CVD) and mortality. Large increases or sustained elevations in hs-CRP may be associated with even greater risk of these outcomes.

Objective: To characterize the association of six-year change in hs-CRP with incident diabetes, incident cardiovascular events (heart disease, stroke, and heart failure), and mortality.

Methods: We included 10,160 ARIC participants with hs-CRP measured at visits 2 (1990-92) and 4 (1996-98). Change in hs-CRP was categorized as sustained low/moderate (<3 mg/L at both visits); decreased (≥3 mg/L at visit 2 and <3 mg/L at visit 4); increased (<3 mg/L at visit 2 and ≥3 mg/L at visit 4); and sustained elevated (≥3 mg/L at both visits). Cox proportional hazards models were used to assess the association of 6-year change in hs-CRP with incident diabetes, cardiovascular events, and death during ~15 years following visit 4.

Results: Compared to persons with sustained low/moderate hs-CRP, those with increased or sustained elevated hs-CRP had an increased risk of incident diabetes (HRs [95% CIs]: 1.56 [1.38, 1.76] and 1.39 [1.25, 1.56], respectively), whereas those with decreased hs-

CRP did not. Persons with sustained elevated hs-CRP had an increased risk of coronary heart disease, ischemic stroke, heart failure and mortality (HRs [95% CIs]: 1.51 [1.23-1.85]; 1.70 [1.32-2.20]; 1.60 [1.35, 1.89]; 1.52 (1.37, 1.69), respectively) compared to those with sustained low/moderate hs-CRP. Associations for sustained elevated hs-CRP were greater than for those with increased hs-CRP over 6 years.

Conclusions: Large increases or sustained elevations in hs-CRP over a six-year period were associated with a subsequent increased risk of diabetes; and persons with sustained elevations in hs-CRP were at the highest risk of CVD and mortality. Two measurements of hs-CRP are better than one for characterizing risk and large increases are particularly prognostic.

Introduction

High-sensitivity C-reactive protein (hs-CRP) is a non-specific marker of inflammation that is commonly used for cardiovascular disease (CVD) risk stratification. Hs-CRP is an acute-phase reactant produced in the liver, and is secreted into the bloodstream in response to the presence of pro-inflammatory cytokines. Inflammation has been implicated in the development of insulin resistance, diabetes,[1,2] and atherosclerosis,[3–5] and high hs-CRP measured at a single time point has been widely studied and associated with CVD (including coronary heart disease [CHD], stroke, and heart failure),[6–12] incident diabetes[10,13–21] and all-cause mortality.[8,9] Furthermore, hs-CRP has been shown to improve cardiovascular risk prediction.[22] Randomized clinical trials have shown that the use of statins in individuals with elevated hs-CRP was associated with a reduction in hs-CRP and a decreased risk of vascular events.[23] This evidence forms the basis of various guidelines, including those from the American College of Cardiology/American Heart Association, the European Society of Cardiology, and the Canadian Cardiovascular Society, that recommend considering use of hs-CRP to inform treatment decisions, mainly for persons at intermediate risk.[24–27] Although hs-CRP may not necessarily be in the causal pathway,[28] these guidelines acknowledge its role as an established marker of future risk of CVD.

Although hs-CRP is a well-studied and well-known inflammatory biomarker, there are sparse data regarding its longitudinal associations with outcomes in the general population. It is unclear whether changes in hs-CRP or sustained elevations in hs-CRP

have added clinical value compared to a single measurement, although we would expect multiple measurements to lead to improved reliability and therefore result in stronger associations with outcomes. The objective of this study was to characterize the association of six-year change in hs-CRP (particularly, large increases) and sustained elevations, with incident diabetes, incident cardiovascular events (heart disease, stroke, and heart failure), and mortality during a maximum of 16 years of follow-up in a community-based sample.

Methods

Study population

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based cohort of 15,792 participants who were originally recruited from 1987 to 1989 from four field centers in the United States: Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland.[29] Participants were invited to return for four follow-up examinations during 1990-92, 1993-95, 1996-98 and 2011-13 (response rates were 93%, 86%, 80% and 65%, respectively). All procedures were approved by an institutional review board at each site and written informed consent was provided by all study participants.

The main analyses for this study were restricted to participants who had attended both visits 2 and 4 (1990-92 and 1996-98, respectively) and had hs-CRP measures available at

each of these visits. Because of small numbers, non-white and non-black participants were excluded, as well as black participants from either the Minneapolis or Washington County field centers. Additionally, participants were excluded if they were missing visit 2 or visit 4 covariates (**Figure 1**).

Measurement of high-sensitivity C-reactive protein

Visit 2 hs-CRP was measured during 2011-13 at the University of Minnesota (Minneapolis, MN) from serum stored at -70˚C using an immunoturbidimetric assay on the Roche Modular P chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Visit 4 hs-CRP was measured in 2010 at Baylor College of Medicine (Houston, TX) from plasma stored at -70˚C using a nephelometric method on the Siemens Dade Behring BN II analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). The coefficient of variation for visit 2 and visit 4 hs-CRP, after excluding outliers, was 7.0% and 6.5%, respectively. We conducted a laboratory calibration study to evaluate possible differences in the hs-CRP measurements between laboratories, specimen type, assay method, instrument and time of measurement, and found that the differences in hs-CRP were not large enough to warrant calibration.[30]

Outcome definitions

Cardiovascular events and all-cause mortality were ascertained via continuous surveillance of hospitalizations and death certificates, annual telephone follow-up with

the participant or a proxy, and linkage with the National Death Index. Incident CHD was defined as a first occurrence of either adjudicated hospitalization for definite/probable myocardial infarction or death due to CHD.[31] Fatal CHD was defined as the subset of incident CHD events that were confirmed to be definite fatal CHD events. Incident stroke was defined as a first occurrence of adjudicated hospitalization or death due to definite/probable ischemic stroke.[32] Incident heart failure was defined as a first occurrence of either hospitalization with a discharge code of 428 (428.0 to 428.9) in any position for diagnosis using the International Classification of Diseases, 9th Revision (ICD-9) or death due to heart failure based on a 428 ICD-9 code or an ICD, 10th Revision code of 150.[33] For analyses of incident CVD, we excluded participants with prevalent CVD at visit 4 (based on self-reported CVD history or events occurring up to and including the visit 4 date) (**Figure 1**).

Incident diabetes was defined as the first occurrence of self-reported physician diagnosis of diabetes or use of glucose-lowering medication, based on responses to annual telephone calls to all participants. Participants were administratively censored on the date of their last response to the annual telephone follow-up if they had not reported having diabetes up to and including that date. For analyses of incident diabetes, we excluded participants with prevalent diabetes at visit 4 (defined by self-reported physician diagnosis or glucose-lowering medication use) (**Figure 1**).

Additional covariates

The following variables were self-reported by participants: age, gender, race/ethnicity, years of education attained, cigarette smoking status, alcohol consumption, and physical activity level (as measured using the Baecke sport index[34]). Use of cholesterol-lowering and antihypertensive medications was obtained via self-report and an inventory of medications that were brought to each visit. Body mass index (BMI) was calculated from measured height and weight. Diastolic and systolic blood pressure was measured using a random zero sphygmomanometer, and was recorded as the mean of the 2nd and 3rd measurements at visit 2, and as the mean of 1st and 2nd measurements at visit 4 (since only two measurements were taken at this visit). Total cholesterol and high-density lipoprotein cholesterol (HDL-c) were measured at Baylor College of Medicine (Houston, TX) from plasma using the Roche Cobas Bio (Roche Diagnostics, Indianapolis, IN) at visit 2 and the Roche Hitachi 911 (Roche Diagnostics, Indianapolis, IN) at visit 4. Total cholesterol was measured using an enzymatic method and HDL-c was measured using a precipitation method.[30]

Statistical Analysis

First, we categorized hs-CRP at visits 2 and 4 as low/moderate (<3 mg/L) versus elevated (≥3 mg/L), based on established clinical cut-points.[5] Second, we created a four-level variable as follows: sustained low/moderate (hs-CRP <3 mg/L at both visits 2 and 4); decreased (≥3 mg/L at visit 2 and <3 mg/L at visit 4); increased (<3 mg/L at visit 2 and ≥3 mg/L at visit 4); and sustained elevated (≥3 mg/L at both visits 2 and 4).

We calculated the proportion of participants in each of the 4 categories of change in hs-CRP from visit 2 to visit 4. We compared demographic and clinical characteristics across categories of hs-CRP change. We used Cox proportional hazards regression models to assess the association of visit 2 hs-CRP, visit 4 hs-CRP, and hs-CRP change with each of the following incident outcomes, individually: diabetes, CHD, fatal CHD, ischemic stroke, heart failure and mortality (18 separate models). We modeled visit 2 hs-CRP and visit 4 hs-CRP as binary variables, and change in hs-CRP as a 4-level variable (as described above). We began follow-up at the date of the visit 4 examination and administratively censored participants on December 31, 2011. All models were adjusted for the following visit 4 covariates as continuous variables, unless otherwise specified: age, gender (male, female), race-center (Minneapolis whites, Jackson blacks, Washington County whites, Forsyth blacks and Forsyth whites), education level attained (<high school, high school or college, >high school; measured at visit 1), cigarette smoking (current, former, never), alcohol consumption (current, former, never), physical activity level (measured at visit 1), prevalent CVD (yes, no), prevalent diabetes (yes, no), use of cholesterol-lowering medication (yes, no), use of antihypertensive medication (yes, no), BMI, systolic blood pressure, total cholesterol, and HDL-c. The proportional hazards assumption was assessed using log-log plots of the survival function, and by testing the statistical significance of the interaction of hs-CRP with the natural log of time in each fully adjusted Cox model. The interaction was statistically significant for models of incident diabetes, so we conducted additional analyses for incident diabetes censoring participants at 5 years.

We conducted several sensitivity analyses. First, to assess whether visit 4 hs-CRP was independently associated with each of the outcomes above and beyond past hs-CRP level, we additionally adjusted analyses of visit 4 hs-CRP for visit 2 hs-CRP level, both modeled as binary variables. Second, to assess the effect of proximity of hs-CRP measurement to the timing of events, we conducted the analysis of visit 2 hs-CRP with each of the outcomes beginning follow-up from the date of the visit 2 examination, rather than that of the visit 4 examination. Third, we repeated the main analyses using a cut-point of 2 mg/L to define high levels of hs-CRP. Fourth, we repeated the main analyses excluding persons who had hs-CRP >10 mg/L at either visit 2 or visit 4, since levels in this range may indicate acute infection.[35] Fifth, we repeated the main analysis for incident diabetes additionally excluding persons with undiagnosed prevalent diabetes at visit 4 based on fasting glucose levels ≥126 mg/dL. Sixth, we conducted analyses of continuous change in hs-CRP from visit 2 to visit 4, by subtracting hs-CRP at visit 2 from hs-CRP at visit 4. To account for potential non-linear associations, we included 4 spline terms in the models, with knots at changes in hs-CRP of -3, 0, and 3 mg/L.

We used Stata version 13.0 (StataCorp, College Station, Texas) to conduct all statistical analyses. This work was supported by the NIH/NHLBI Cardiovascular Epidemiology training grant T32HL007024, and NIH/NIDDK grant R01DK089174. The ARIC Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper, and its final contents.

Results

The mean age of participants was approximately 57 years at visit 2 and 63 years at visit 4. Nearly half of the study population had sustained low/moderate hs-CRP and 29% had sustained elevated hs-CRP during the 6-year period (**Table 1**). Of the 6,385 persons with low/moderate hs-CRP at visit 2, 76% also had low/moderate hs-CRP at visit 4, 6 years later. Of the 3,775 persons with elevated hs-CRP at visit 2, 77% also had elevated hs-CRP at visit 4. Visit 2 and visit 4 hs-CRP were highly correlated (Spearman's correlation coefficient = 0.69, $P<0.0001$). Persons with sustained elevated hs-CRP were more likely to be black or female compared to those with sustained low/moderate hs-CRP (31% versus 16% and 71% vs 47%, respectively) (**Table 1**). Persons with increased or sustained elevated hs-CRP were more likely to be obese and to have prevalent hypertension, diabetes and CVD, compared to persons with sustained low/moderate hs-CRP (**Table 1**). Persons with hs-CRP that decreased were more likely to be taking cholesterol-lowering medications at visit 4 (**Table 1**).

The "increased" and "decreased" categories successfully identified participants who experienced substantially large changes in hs-CRP. In fact, persons whose hs-CRP level decreased below or increased above the clinical threshold of 3 mg/L over 6 years experienced greater changes in hs-CRP compared to those with either sustained low/moderate or elevated levels (median changes of -2.7 and 3.1 mg/L in persons with hs-CRP that decreased and increased, respectively; and median changes of 0.0 and 0.4 mg/L in persons with sustained low/moderate and elevated hs-CRP, respectively) (**Table**

1). Median duration of follow-up was 13 years for analyses of incident diabetes, and 14 years for analyses of incident CVD and all-cause mortality.

High hs-CRP (≥3 mg/L) measured at either visit 2 or visit 4 was statistically significantly associated with increased risk of incident diabetes, CHD, fatal CHD, stroke and heart failure, as well as all-cause mortality, with hazard ratios (HRs) for the CVD outcomes in the 1.3-2.1 range (**Table 2**). HRs for associations of high hs-CRP at visit 2 and visit 4 with incident diabetes were stronger when analyses were censored at 5 years (1.25 and 1.82, respectively) than when the entire follow-up was included.

Persons with increased or sustained elevated hs-CRP had an increased risk of incident diabetes compared to those with sustained low/moderate hs-CRP (HRs: 1.56 and 1.39, respectively; **Supplemental Figure S1, Figure 2 and Table 3**). HRs were stronger in analyses with shorter duration of follow-up (censored at 5 years) (2.06 and 1.79, respectively). Persons who had elevated hs-CRP at either one or both visits had an increased risk of incident CHD compared to those with sustained low/moderate hs-CRP (range of HRs: 1.3-1.5). Persons who had increased or sustained elevated hs-CRP had an increased risk of fatal CHD compared to those with sustained low/moderate hs-CRP, and magnitudes of association were greater than for non-fatal CHD (HRs were 2.0 and 2.2, respectively). Sustained elevated hs-CRP was associated with increased risk of ischemic stroke (HR was 1.7), whereas elevated hs-CRP at only one visit was not. Persons with increased or sustained elevated hs-CRP had a higher risk of incident heart failure

compared to those with sustained low/moderate hs-CRP (HRs were 1.4 and 1.6, respectively). Although not statistically significant, there was a suggestion of increased risk of heart failure for those with elevated hs-CRP at visit 2 only. Compared to persons with sustained low/moderate hs-CRP, persons with hs-CRP that decreased had increased risk of mortality, persons with hs-CRP that increased had slightly higher risk of mortality, and those with sustained elevated hs-CRP had the highest risk of mortality (range of HRs 1.2-1.5; **Figure 2 and Table 3**).

Results of sensitivity analyses supported our main findings. After adjusting for visit 2 hs-CRP, associations of visit 4 hs-CRP were similar for diabetes, and slightly attenuated for CVD and mortality (HRs for CVD and mortality ranged from 1.4-2.1 before adjustment and 1.25-2.0 after adjustment; **Supplemental Table S1**). Associations of visit 2 hs-CRP beginning follow-up at visit 2 were similar to those beginning follow-up at visit 4 (**Supplemental Table S1**). In analyses excluding persons with hs-CRP >10 mg/L at either visit, we observed similar results for associations of hs-CRP measured at a single time point and change in hs-CRP with each outcome (**Table 3 and Supplemental Table S2**). In analyses that defined elevated versus low/moderate hs-CRP using a cut-point of 2 mg/L, we observed a similar direction of association but generally diminished HRs (**Table 3 and Supplemental Table S3**). In analyses of incident diabetes that excluded persons with undiagnosed prevalent diabetes at visit 4, results were similar and only slightly attenuated (**Supplemental Table S4**). Lastly, we found that when analyzed continuously, the association of change in hs-CRP with outcomes was generally U-

shaped (**Supplemental Figure S2**). These continuous analyses support our main findings, that compared to persons with no or small changes, persons with large increases in hs-CRP had statistically significant increased risk of subsequent diabetes, CHD, heart failure and mortality; and there was evidence that those with large decreases also had an increased risk of CHD, heart failure and mortality (**Supplemental Figure S2**).

Discussion

We observed that hs-CRP measured at a single time point was associated with an approximately 40-50% increased risk of diabetes, cardiovascular events and death over nearly 15 years of follow-up. Furthermore, persons with sustained elevations in hs-CRP were at the highest relative risk of CVD and mortality. Large increases in and sustained elevations in hs-CRP that surpassed the 3 mg/L threshold were strongly associated with increased risk of future diabetes. Similarly, the more proximal measure of hs-CRP was a strong predictor of incident diabetes, regardless of hs-CRP measured six years earlier.

In the ARIC sample, 6-year increased hs-CRP and sustained elevated hs-CRP were associated with diabetes development. Obesity is an important cause of diabetes and elevated inflammatory markers. A previous analysis conducted in the ARIC Study and other studies suggest that inflammation may be on the causal pathway between obesity and diabetes.[1,36] Alternatively, the association of inflammation with diabetes may be

mediated by obesity, as reported by a previous study conducted in ARIC.[37] However, the mechanism(s) by which inflammation plays a role in the development of diabetes has yet to be fully characterized, although it has been suggested that inflammation is associated with and may even may intensify the effects of conditions such as endoplasmic reticulum stress and oxidative stress, which may lead to insulin resistance and ß-cell dysfunction.[1]

In contrast to our findings for diabetes, any elevation in hs-CRP, whether measured at visit 4 or six years earlier at visit 2, was associated with an increased risk of CHD, heart failure and mortality over nearly 15 years, and although some of the confidence intervals overlapped, there was a suggestion of even higher risk in persons with sustained elevated levels of inflammation. This supports prior evidence that chronically high levels of inflammation may either play an active role in the long-term development of atherosclerosis, or may be a marker of chronic endothelial insult. Interestingly, we only observed an increased risk of ischemic stroke in persons with sustained elevated levels of hs-CRP and not with a single elevated hs-CRP value, which may suggest a more long-term or chronic process involving inflammation in the development of ischemic stroke.

Previous papers have largely used a single measure of hs-CRP to measure inflammation and have not accounted for its inherently time-varying nature and short-term variability (intraclass correlation coefficients ranged from 0.6-0.8 using repeat measurements from a couple of weeks to a few years apart).[38–40] In fact, a joint statement from the Centers for Disease Control and Prevention and the American Heart Association previously

recommended using two measurements of hs-CRP about two weeks apart to reduce the within-person variability and increase stability of measurement values.[5] An analysis that corrected for regression dilution resulted in stronger associations of hs-CRP with clinical outcomes compared to using only a single measurement.[7] Therefore, we would expect stronger associations of hs-CRP with outcomes if combining multiple measurements.

As described previously, the categorical analysis of change in hs-CRP captured persons with large changes in hs-CRP that most likely reflect true biological changes in inflammation. Due to the high random variation in hs-CRP,[38–40] associations of continuous, small changes may not have substantial clinical significance. Indeed, our continuous analyses confirmed that small changes in hs-CRP were associated with small increases in risk, if any, and that large increases in hs-CRP were most strongly associated with future risk of events.

The few previous studies that have assessed the association of change in hs-CRP over several years with risk of diabetes, CVD and mortality have been inconclusive. Increases in hs-CRP and proximally measured elevated hs-CRP have been shown to be associated with increased risk of total mortality[41–43]. In the Cardiovascular Health All Stars Study of adults with a mean age of 85 years, increase in hs-CRP over 9 years was not associated with increased risk of CVD.[42] This study differed from ours in that there were fewer participants (N=597) and likely less power to detect moderate associations. The participants in CHS were also much older (mean age of 85 years) than the participants in

our study; different risk relationships might be expected in an older population with a higher prevalence of cardiovascular risk factors and co-morbidities. This previous study also assessed a doubling of hs-CRP rather than changes in clinical categories. In the Whitehall II Study of middle-aged adults, hs-CRP was higher over 15 years of follow-up in both persons who died of CVD and persons who developed diabetes compared to those who did not. There was a suggestion that among persons who died from CVD, past trajectories of hs-CRP had increased more steeply compared to those who were still alive; whereas among persons who developed diabetes, past trajectories of hs-CRP increased more slowly compared to those who did not.[44] Their conclusions that increases in hs-CRP were not associated with diabetes are different from what we report. In fact, the Whitehall investigators found that persons who developed diabetes had higher hs-CRP at baseline, but that past trajectories of hs-CRP in persons with and without diabetes actually converged over time. It should be noted that the Whitehall study included only persons who had complete follow-up data available (in contrast to traditional survival analysis methods involving censoring). It is plausible that some of the persons at highest risk of diabetes died from cardiovascular disease or other causes and were possibly more likely to be lost to follow-up and not included in the study, contributing to survival bias in these data and discrepancies between the findings from Whitehall and other studies.

There are several limitations that should be considered in the interpretation of our results. We only had measurements of hs-CRP at two time points, which cannot fully capture trajectories over time. However, requiring either elevation at both time points or

movement from one clinically relevant category to another, rather than small changes that could be due to random error (e.g., biological or analytical variability), strengthened our ability to place participants into appropriate categories, and was an attempt to minimize misclassification. As with all observational studies, we may not have been able to fully control for all potential biases and there remains a possibility of residual confounding. Strengths of our study included the large community-based sample with more than a decade of follow-up for important and rigorously assessed clinical outcomes.

In conclusion, for diabetes risk assessment, the most proximally measured value of hs-CRP may be more important than past measurements. We found that for cardiovascular outcomes and mortality, as expected, two measurements of hs-CRP are better than one for identification of persons at highest risk. Regardless of whether inflammation, and specifically hs-CRP, is in the causal pathway or is simply a marker of the pathogenesis of these outcomes, our results suggest that multiple measurements of hs-CRP may better indicate risk of disease development. Further study of repeated measurements of hs-CRP, in particular hs-CRP measured over a shorter time interval, would be especially useful in persons of intermediate risk, and could potentially inform risk classification and identification of high-risk participants for inclusion in randomized clinical trials or other research studies.

Table 1. Study population* characteristics by six-year change in and sustained levels of high-sensitivity C-reactive protein

	Sustained Low/Moderate (<3 mg/L at both visits) (N=4,859, 47.8%)		Decreased (≥3 to <3 mg/L) (N=869, 8.6%)		Increased (<3 to≥3 mg/L) (N=1,526, 15.0%)		Sustained Elevated (≥3 mg/L at both visits) (N=2,906, 28.6%)	
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %
	Visit 2 (1990-92)	Visit 4 (1996-98)	Visit 2 (1990-92)	Visit 4 (1996-98)	Visit 2 (1990-92)	Visit 4 (1996-98)	Visit 2 (1990-92)	Visit 4 (1996-98)
Age, years	56.6 (5.7)	62.6 (5.7)	57.6 (5.8)	63.6 (5.8)	56.5 (5.6)	62.6 (5.6)	56.7 (5.6)	62.7 (5.6)
Male	53.3%	--	46.3%	--	38.3%	--	28.6%	--
Black	16.0%	--	20.4%	--	18.9%	--	30.6%	--
Field center								
Minneapolis, MN	31.2%	--	28.9%	--	31.5%	--	22.9%	--
Jackson, MS	14.4%	--	17.8%	--	16.5%	--	27.3%	--
Washington County, MD	28.3%	--	30.0%	--	27.6%	--	25.7%	--
Forsyth, NC	26.1%	--	23.3%	--	24.4%	--	24.1%	--
Education								
<High school	15.1%	--	20.0%	--	17.0%	--	24.1%	--
High school or college	41.8%	--	42.7%	--	44.2%	--	43.1%	--
>College	43.1%	--	37.3%	--	38.8%	--	32.8%	--
Sport index	2.6 (0.81)	--	2.5 (0.76)	--	2.5 (0.80)	--	2.3 (0.74)	--
Alcohol consumption								
Current	62.6%	55.3%	57.6%	46.6%	61.5%	52.2%	51.2%	40.9%
Former	17.5%	26.6%	20.9%	32.9%	17.5%	28.4%	22.5%	33.9%
Never	19.9%	18.1%	21.5%	20.5%	21.0%	19.4%	26.3%	25.2%
Smoking status								
Current	15.8%	12.1%	20.6%	15.4%	22.2%	15.9%	23.4%	17.5%
Former	41.4%	45.7%	40.3%	45.2%	36.8%	43.1%	34.3%	40.0%
Never	42.8%	42.2%	39.1%	39.4%	41.0%	41.0%	42.3%	42.5%
Body mass index								
<25 kg/m^2	41.0%	33.0%	25.6%	24.4%	33.8%	23.7%	15.4%	13.7%
25-30 kg/m^2	42.5%	44.7%	41.0%	40.6%	42.6%	39.9%	36.1%	31.1%
≥30 kg/m^2	16.5%	22.3%	33.4%	35.0%	23.6%	36.4%	48.5%	55.2%

Hypertension†	25.0%	38.8%	40.0%	51.4%	30.8%	46.4%	44.9%	60.0%
HDL-c, mg/dL	50.6 (17.0)	50.7 (16.7)	46.9 (16.3)	48.8 (16.2)	51.4 (17.0)	49.8 (17.2)	49.3 (16.0)	49.0 (15.6)
Total cholesterol, mg/dL	207.7 (37.2)	199.6 (35.4)	211.5 (41.7)	201.9 (40.2)	209.9 (38.3)	202.6 (37.7)	211.5 (39.5)	202.3 (38.6)
Cholesterol-lowering medication	6.3%	14.4%	6.6%	20.5%	6.2%	11.8%	6.2%	14.1%
Prevalent diabetes	4.3%	6.9%	9.4%	15.8%	4.6%	8.5%	11.8%	18.8%
Prevalent CVD	6.3%	10.2%	11.3%	17.5%	7.4%	12.7%	12.5%	18.4%
hs-CRP, mg/L‡	1.1 (0.6-1.7)	1.1 (0.7-1.7)	4.4 (3.5-6.4)	1.9 (1.3-2.4)	1.9 (1.3-2.5)	4.9 (3.8-6.8)	6.4 (4.4-10.2)	7.1 (4.8-9.7)
Change in hs-CRP, mg/L‡	--	0.0 (-0.4, 0.4)	--	-2.7 (-4.9, -1.7)	--	3.1 (1.9, 5.3)	--	0.4 (-2.1, 2.7)

*The study population presented here is the population used for the analyses using all-cause mortality as the endpoint, N=10,160; all covariates presented are from visit 2, except for education level and the Baecke sport index, and field center, which was obtained at visit 1

†Hypertension was defined as diastolic ≥90 mmHg or systolic ≥140 mmHg or self-reported blood pressure-lowering medication

‡Median and interquartile range presented

Table 2. Association of hs-CRP measured at visit 2 (1990-92) or visit 4 (1996-98) with incident diabetes, incident cardiovascular events and all-cause mortality that occurred from 1996-98 through 2011

	Visit 2 hs-CRP		Visit 4 hs-CRP	
	Events/Total N (%)	HR (95% CI)	Events/Total N (%)	HR (95% CI)
Diabetes				
≥3 mg/L	890/2,976 (30%)	1.13 (1.03, 1.24)	1,122/3,617 (31%)	1.44 (1.31, 1.58)
<3 mg/L	1,211/5,772 (21%)	1 (Reference)	979/5,131 (19%)	1 (Reference)
CHD				
≥3 mg/L	276/3,086 (9%)	1.31 (1.11, 1.55)	321/3,702 (9%)	1.40 (1.18, 1.65)
<3 mg/L	361/5,697 (6%)	1 (Reference)	316/5,081 (6%)	1 (Reference)
Fatal CHD				
≥3 mg/L	69/3,086 (2%)	1.47 (1.04, 2.08)	88/3,702 (2%)	2.09 (1.46, 2.99)
<3 mg/L	77/5,697 (1%)	1 (Reference)	58/5,081 (1%)	1 (Reference)
Ischemic stroke				
≥3 mg/L	174/3,086 (6%)	1.50 (1.20, 1.87)	190/3,702 (5%)	1.43 (1.14, 1.79)
<3 mg/L	192/5,697 (3%)	1 (Reference)	176/5,081 (3%)	1 (Reference)
Heart failure				
≥3 mg/L	468/3,086 (15%)	1.35 (1.18, 1.56)	529/3,702 (14%)	1.46 (1.26, 1.68)
<3 mg/L	470/5,697 (8%)	1 (Reference)	409/5,081 (8%)	1 (Reference)
Mortality				
≥3 mg/L	1,100/3,775 (29%)	1.32 (1.21, 1.44)	1,227/4,432 (28%)	1.39 (1.27, 1.52)
<3 mg/L	1,283/6,385 (20%)	1 (Reference)	1,156/5,728 (20%)	1 (Reference)

Abbreviations: CHD, coronary heart disease; CI, confidence interval; HR, hazard ratio

Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

N=8,748 for diabetes analyses; N=8,783 for CVD analyses and N=10,160 for mortality analyses

Table 3. Association of six-year change in high-sensitivity C-reactive protein with incident diabetes, incident cardiovascular events and all-cause mortality

	Main analysis		Excluding persons with CRP>10 mg/L at either visit		Using a cut-point of 2 mg/L to define categories	
	Events/Total N (%)	HR (95% CI)	Events/Total N (%)	HR (95% CI)	Events/Total N (%)	HR (95% CI)
Diabetes						
Sustained elevated	730/2,264 (32%)	1.39 (1.25, 1.56)	480/1,488 (32%)	1.40 (1.24, 1.58)	1,089/3,469 (31%)	1.53 (1.37, 1.71)
Increased	392/1,353 (29%)	1.56 (1.38, 1.76)	362/1,249 (29%)	1.53 (1.34, 1.73)	316/1,270 (25%)	1.42 (1.23, 1.63)
Decreased	160/712 (22%)	1.07 (0.90, 1.27)	140/619 (23%)	1.05 (0.87, 1.25)	163/818 (20%)	1.02 (0.85, 1.21)
Sustained low/moderate	819/4,419 (19%)	1 (Reference)	819/4,419 (19%)	1 (Reference)	533/3,191 (17%)	1 (Reference)
CHD						
Sustained elevated	215/2,369 (9%)	1.51 (1.23,1.85)	220/2,643 (8%)	1.46 (1.16, 1.83)	305/3,548 (9%)	1.36 (1.11, 1.66)
Increased	106/1,333 (8%)	1.43 (1.14,1.81)	76/1,200 (6%)	1.31 (1.03, 1.68)	86/1,263 (7%)	1.17 (0.90, 1.51)
Decreased	61/717 (9%)	1.34 (1.01, 1.77)	58/772 (8%)	1.24 (0.91, 1.68)	66/832 (8%)	1.16 (0.87, 1.54)
Sustained low/moderate	255/4,364 (6%)	1 (Reference)	180/3,140 (6%)	1 (Reference)	180/3,140 (6%)	1 (Reference)
Fatal CHD						
Sustained elevated	60/2,369 (3%)	2.17 (1.43, 3.30)	62/2,643 (2%)	2.12 (1.34, 3.35)	85/3,548 (2%)	1.86 (1.22, 2.83)
Increased	28/1,333 (2%)	1.98 (1.24, 3.19)	13/1,200 (1%)	1.98 (1.21, 3.23)	14/1,263 (1%)	0.95 (0.51, 1.77)
Decreased	9/717 (1%)	1.02 (0.50, 2.08)	10/772 (1%)	0.84 (0.38, 1.87)	11/832 (1%)	0.89 (0.45, 1.77)
Sustained low/moderate	49/4,364 (1%)	1 (Reference)	36/3,140 (1%)	1 (Reference)	36/3,140 (1%)	1 (Reference)
Ischemic stroke						
Sustained elevated	143/2,369 (6%)	1.70 (1.32, 2.20)	86/1,536 (6%)	1.59 (1.19, 2.13)	194/3,548 (5%)	1.65 (1.26, 2.15)
Increased	47/1,333 (4%)	1.09 (0.78, 1.52)	41/1,230 (3%)	1.03 (0.72, 1.47)	48/1,263 (4%)	1.26 (0.88, 1.79)
Decreased	31/717 (4%)	1.13 (0.76, 1.67)	25/625 (4%)	1.02 (0.67, 1.57)	29/832 (3%)	0.95 (0.63, 1.45)
Sustained low/moderate	145/4,364 (3%)	1 (Reference)	145/4,364 (3%)	1 (Reference)	95/3,140 (3%)	1 (Reference)
Heart failure						
Sustained elevated	386/2,369 (16%)	1.60 (1.35, 1.89)	228/1,536 (15%)	1.54 (1.28, 1.85)	489/3,548 (14%)	1.37 (1.15, 1.63)
Increased	143/1,333 (11%)	1.38 (1.13, 1.68)	128/1,230 (10%)	1.34 (1.09, 1.65)	130/1,263 (10%)	1.37 (1.10, 1.71)
Decreased	82/717 (11%)	1.22 (0.96, 1.56)	68/625 (11%)	1.12 (0.86, 1.46)	100/832 (12%)	1.28 (1.01, 1.62)
Sustained low/moderate	327/4,364 (7%)	1 (Reference)	327/4,364 (7%)	1 (Reference)	219/3,140 (7%)	1 (Reference)
Mortality						
Sustained elevated	864/2,906 (30%)	1.52 (1.37, 1.69)	510/1,848 (28%)	1.42 (1.26, 1.60)	1,209/4,307 (28%)	1.40 (1.26, 1.56)
Increased	363/1,526 (24%)	1.34 (1.18, 1.52)	322/1,401 (23%)	1.31 (1.15, 1.49)	276/1,399 (20%)	1.08 (0.94, 1.24)

Decreased	236/869 (27%)	1.23 (1.06, 1.42)	196/757 (26%)	1.14 (0.98, 1.34)	253/982 (26%)	1.13 (0.97, 1.30)
Sustained low/moderate	920/4,859 (19%)	1 (Reference)	920/4,859 (19%)	1 (Reference)	645/3,472 (19%)	1 (Reference)

For main analyses: N=8,748 for diabetes analyses; N=8,783 for CVD analyses and N=10,160 for mortality analyses

For analyses excluding persons with hs-CRP >10 mg/L at either visit 2 or visit 4: N=7,775 for diabetes analyses, N=7,755 for CVD analyses, N=8,865 for mortality analyses

Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

Figure 1. Exclusion criteria for study population

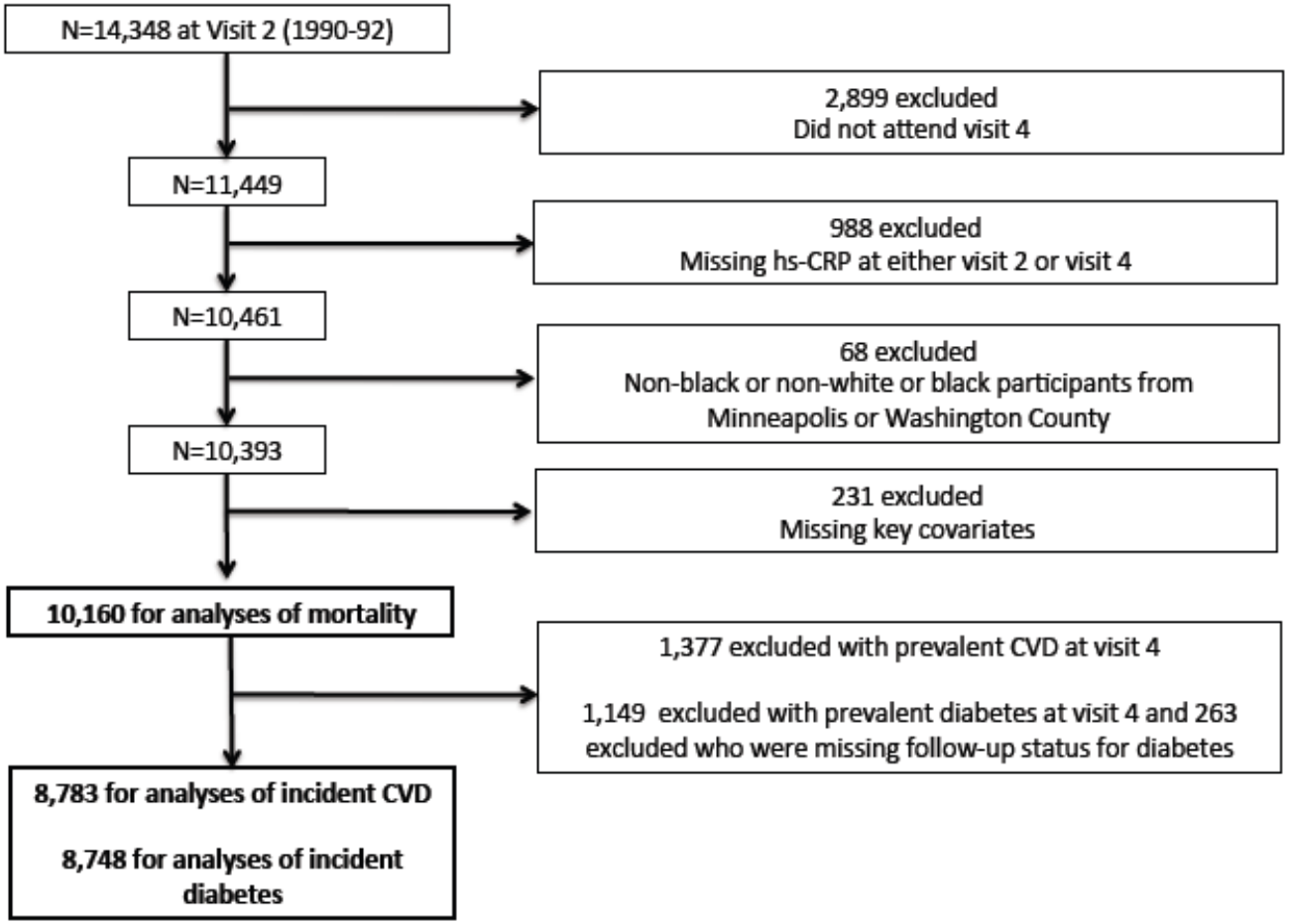
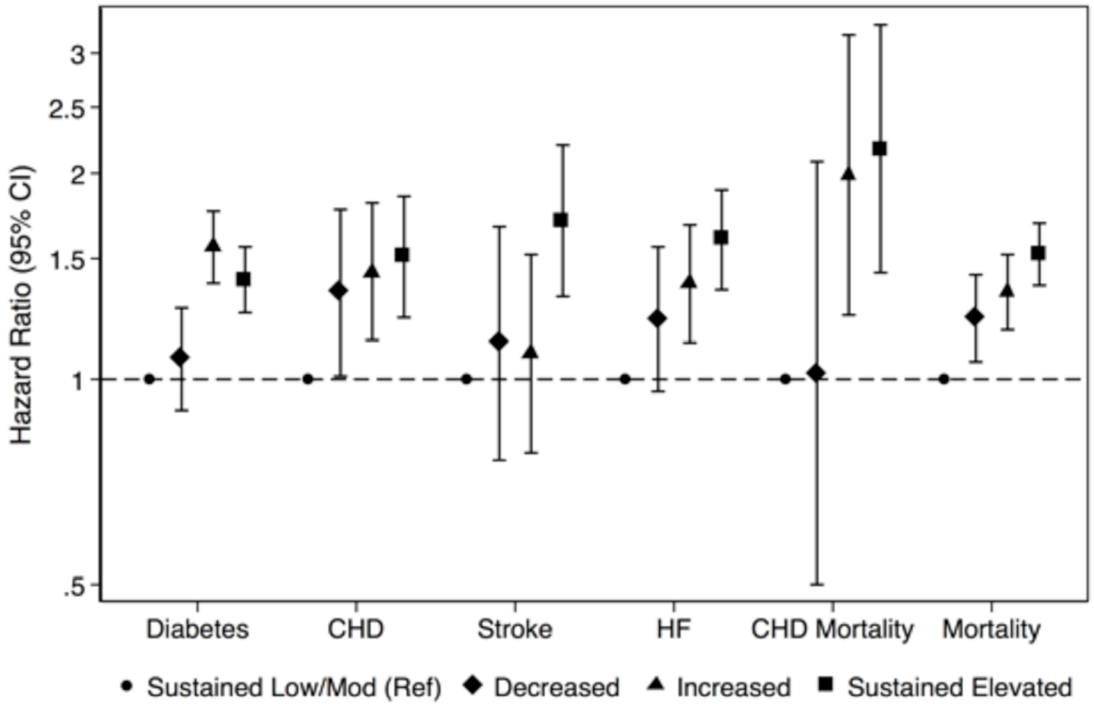


Figure 2. Association of six-year change or sustained elevation in high-sensitivity C-reactive protein with incident diabetes, incident cardiovascular events and all-cause mortality



Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

Chapter 5: Risk prediction of major complications in persons with diabetes: The Atherosclerosis Risk in Communities Study

Abstract

Objective: To develop a prediction equation for 10-year risk of major complications in persons with diabetes, using demographic and clinical information including a panel of traditional and nontraditional biomarkers. Whereas most prediction models predict risk of a single endpoint, we developed a model using a combined endpoint of any major complications.

Research design and methods: We included 654 persons in the ARIC Study with diagnosed diabetes (visit 2, 1990-92). We used a 3-stage approach to develop a model for combined prediction of any major complications (incident cardiovascular disease, chronic kidney disease, or lower extremity hospitalizations). Model 1 included demographic variables. Model 2 additionally included clinical variables. We then tested the addition of 13 biomarkers to model 2 to develop model 3 (biomarker model). We compared the three models using prediction and discrimination statistics.

Results: During a median of 9.8 years of follow-up there were 296 major complications. Each stage of model development improved risk prediction. The C-statistics of models 1 and 2 were 0.667 and 0.683, respectively (P=0.03 for difference). Of the 13 biomarkers considered, addition of HbA1c, beta-2 microglobulin, NT-proBNP, and high-sensitivity cardiac troponin T to model 2 substantially improved model discrimination (C-statistic =0.720, P<0.001 for difference from model without biomarkers).

Conclusions: Prediction models with a combined endpoint may have more clinical relevance than models that consider a single outcome. The addition of four biomarkers significantly improved the accuracy of 10-year risk prediction for any major complications in persons with diabetes. The use of these biomarkers for risk stratification in diabetes is promising.

Introduction

Diabetes is a major risk factor for chronic kidney disease (CKD) and end-stage renal disease (ESRD), lower extremity disease, coronary heart disease (CHD), stroke, heart failure, and mortality.[1–5] Indeed, diabetes is currently considered a "CHD risk equivalent",[6] which implies that all persons with diabetes have a risk of CHD similar to persons who have a prior history of CHD. However, recent evidence suggests that persons with diabetes may have varying degrees of risk depending on the presence and severity of other risk factors and co-morbidities. Understanding this heterogeneity in risk and identifying those persons most in need of aggressive cardiovascular risk management could help personalize and improve care for persons with diabetes. Recent evidence suggests that hemoglobin A1c (HbA1c) and other biomarkers not included in traditional cardiovascular risk equations may potentially improve risk prediction in diabetes.[7,8]

Risk prediction models for microvascular and macrovascular complications are of clinical interest. Risk scores for CVD developed in the general population have tended to underestimate risk when applied to persons with diabetes.[9,10] To improve predictive accuracy, several risk scores for CVD have been developed in populations of persons with diabetes.[11–18] The literature on prediction of microvascular complications in diabetes is more limited although several models have been developed to predict ESRD in persons with diabetes complicated by CKD,[19–21] as well as in persons with diabetes who do not have kidney disease.[22,23] Furthermore, most risk prediction scores have been developed in

largely white populations, which may impact their generalizability to the general population.[9]

There are several challenges in developing an accurate, clinically relevant risk score. Most risk scores have been developed to predict risk of either a single outcome or a single type of event (e.g., cardiovascular disease). Predicting risk of a combined endpoint, particularly combined microvascular and macrovascular events, has not been widely studied.[24] The clinical utility of a single risk score for multiple endpoints has broad application, and applying one single risk score to a patient rather than multiple risk scores is convenient and efficient. Furthermore, most existing risk scores have used traditional methods, such as Cox regression approaches, to account for loss to follow-up, but have not accounted for competing risks. Using a competing risks framework to develop a risk score can better estimate absolute risks, which are especially important to consider in clinical settings.[25,26]

We undertook this study to develop a risk prediction equation for 10-year risk of a combined endpoint of any major microvascular and macrovascular complications in white and black persons with diabetes, while accounting for competing risks. We used a 3-stage approach to develop risk prediction equations that incorporated demographic and clinical information and a panel of biomarkers of hyperglycemia, cardiac function, kidney function, liver function, and inflammation. We compared models at each stage of development.

Methods

Study population

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based cohort of 15,792 persons recruited from four field centers in the United States: Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland.[27] Visits 1 through 5 took place during 1987-89, 1990-92, 1993-95, 1996-98, and 2011-13, respectively. We used visit 2 (1990-1992) as the baseline exam in the present study as this was the first visit with relevant biomarker data available. Of the 14,348 participants who attended visit 2, there were 1,356 with diagnosed diabetes, defined as self-reported physician diagnosis of diabetes or self-reported use of glucose-lowering medication at either visit 1 or 2. After exclusion of participants who were missing covariate data (N=276), were fasting for fewer than 8 hours (N=172), were non-black or non-white (N=2), had prevalent CVD (N=227), had prevalent reduced kidney function (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2) (N=23), or had prevalent lower extremity amputation or peripheral vascular bypass (N=2), there were 654 participants eligible for our main analyses.

Covariates

All covariates were obtained at the visit 2 examination unless otherwise specified. The following variables were assessed during the participant interview: age (visit 1), sex (visit 1), education level (visit 1), alcohol consumption, smoking status, and physical activity

(Baecke sport activity index at visit 1)[28]. Antihypertensive, cholesterol-lowering, and glucose-lowering medication use was assessed via self-report and medication inventory. Recent diabetes was defined as having had diabetes at visit 2 but not at visit 1. Family history of CVD was defined as self-reported parental history of either stroke or CHD. Diastolic and systolic blood pressures were measured using a random zero sphygmomanometer, and recorded as the mean of the 2nd and 3rd readings. Body mass index (BMI) was calculated as measured weight (in kilograms) divided by measured height (in meters) squared.

Laboratory measurements

Total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglycerides were measured as part of the original ARIC study protocol on the Roche Cobas Bio (Roche Diagnostics, Indianapolis, IN). Total cholesterol and triglycerides were measured using an enzymatic method. HDL-c was measured using a precipitation method. Low-density lipoprotein cholesterol (LDL-c) was calculated from measured total cholesterol, HDL-c, and triglycerides using the Friedewald equation.[29] Serum glucose (hexokinase method) and creatinine (Jaffe method) were measured on a Coulter DACOS analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). HbA1c was measured from stored whole blood in 2003-04 and 2007-08 with the Tosoh A1c 2.2 Plus Glycohemoglobin Analyzer and Tosoh G7 Analyzer (Tosoh Bioscience, Inc., South San Francisco, CA, USA), respectively, using a high-performance liquid chromatography method, and was standardized to the Diabetes Control and Complications Trial assay.[30] The following

biomarkers were measured in 2012-2013 from stored serum samples on a Roche Modular P800 instrument (Roche Diagnostics Corporation, Indianapolis, IN) as part of an ARIC ancillary study: cystatin C was measured using the Gentian immunoassay (Gentian, Moss, Norway); beta-2 microglobulin (B2M) was measured using a latex agglutination method, alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transpeptidase (GGT) were measured using a kinetic rate method. Fructosamine was measured using a colorimetric method (Roche Diagnostics Corporation, Indianapolis, IN, USA). High-sensitivity C-reactive protein (hs-CRP) was measured using an immunoturbidimetric method. Glycated albumin (Asahi Kasei Lucica GA-L, Tokyo, Japan) and 1,5-AG (GlycoMark, New York, NY) were measured using enzymatic methods. Glycated albumin was expressed as a percentage of total albumin, calculated using the following equation from the manufacturer: [(glycated albumin concentration in g/dL / serum albumin concentration in g/dL) / 1.14*100] + 2.9. N-terminal probrain natriuretic peptide (NT-proBNP) and high-sensitivity cardiac troponin T (hs-cTnT) were also measured in 2011-13 from stored serum using a sandwich immunoassay on a Roche Elecsys autoanalyzer (Roche Diagnostics Corporation, Indianapolis, IN).

Incident Outcomes

We created a combined endpoint for the first occurrence of any major event (CHD, stroke, heart failure, CKD, lower extremity amputation, or peripheral vascular bypass) over a maximum of 10 years of follow-up. Outcomes were ascertained via continuous surveillance of all hospitalizations and death certificates, annual telephone follow-up with

participants or a proxy, and/or linkage to the National Death Index. CHD was adjudicated and defined as the first occurrence of a definite or probable hospitalized myocardial infarction, death due to CHD, or cardiac procedure.[31] Stroke was adjudicated and defined as the first occurrence of a definite or probable hospitalized stroke or death due to stroke.[32] Heart failure was defined as the first hospitalization or death due to heart failure, based on a 428 International Classification of Diseases, 9th Revision (ICD-9) code or an ICD, 10th Revision (ICD-10) code of 150.[33] Incident lower extremity amputation and peripheral vascular bypass events were identified from ICD-9-CM diagnostic and procedure codes using hospitalization data. Incident CKD was defined as eGFR<60 mL/min/1.73 m^2 and ≥25% decline in eGFR since visit 2, or hospitalization due to kidney disease, kidney transplant or dialysis, or death due to kidney disease.[34]

Persons who did not experience one of these events and did not die within 10 years of the date of their visit 2 examination were censored at 10 years following their visit 2 examination date. Persons who died from an event other than those listed above were considered to have experienced the competing risk of death. Persons who were lost to follow-up and did not experience the event of interest and did not die were censored at the time of last contact.

Statistical analysis

We calculated descriptive statistics of demographic and clinical characteristics in the study population overall, as well as stratified by having experienced any event, not having experienced any event, or having died from a competing risk.

We used a 3-stage approach to evaluate prediction models for 10-year risk of any major complication. We evaluated whether inclusion of clinical and traditional and/or nontraditional biomarkers improved prediction. Model 1 included demographic information (age, sex, race, education, smoking status, alcohol consumption, physical activity, family history of CVD, glucose-lowering medication use, antihypertensive medication use, cholesterol-lowering medication use, BMI, and whether onset of diabetes was recent); model 2 additionally included common clinical variables (LDL-c, HDL-c, triglycerides, and systolic blood pressure); and model 3 additionally tested the addition of 13 biomarkers of hyperglycemia (fasting glucose, HbA1c, fructosamine, glycated albumin, 1,5-AG), cardiac damage (hs-cTnT, NT-proBNP), kidney function (serum creatinine, cystatin C, B2M), liver function (AST, ALT, GGT), and inflammation (hs-CRP). We specified the variables for inclusion in models 1 and 2 a priori, based on prior knowledge, as well as several interactions to test in model 2: the interaction of sex with all variables, race with all variables, antihypertensive medication use with systolic blood pressure, and cholesterol-lowering medication use with LDL-c. The following interactions were considered statistically significant ($P<0.05$ using a Wald test) and were additionally included in the models: sex*triglycerides ($P=0.02$), sex*glucose-lowering medication (oral, insulin, or none) ($P=0.01$), race*triglycerides ($P=0.049$), and race*BMI

(P=0.03). To build the models, all continuous variables were centered. We natural log-transformed any variables that were not normally distributed. For biomarkers that had undetectable values, we imputed the values as one half of the lower limit of detection (1.5 ng/L for hs-cTnT, 2.5 pg/mL for NT-proBNP, and 2 U/L for ALT). We calculated eGFR using each creatinine and cystatin C, and used the inverse of B2M (1/B2M) in analyses, since these measures are better related to renal physiology.

We used a Fine and Gray model, a proportional hazards model for the subdistribution of a competing risk, to run models 1 and 2. We then evaluated whether the addition of the following 13 traditional and nontraditional biomarkers to model 2 improved prediction, also using a Fine and Gray approach: fasting glucose, HbA1c, fructosamine, glycated albumin, 1,5-AG, creatinine-based eGFR, cystatin C-based eGFR, the inverse of B2M, ln of hs-CRP, ln of AST, ln of ALT, ln of GGT, ln of NT-proBNP, and ln of hs-cTnT. We added each biomarker individually to model 2 and conducted two tests: 1) a Wald test of the coefficient and 2) a comparison of the change in the C-statistic from the full model before versus after addition of the biomarker. We then selected those biomarkers that had P<0.05 for both tests, and added them all to model 2 simultaneously. To determine which biomarkers to keep in the model, we then assessed the P-values of each of the biomarkers from the Wald test. We removed the biomarker with the highest P-value if it was above P=0.05, and re-ran the model. We continued this procedure until all biomarkers in the model had P<0.05, at which point we considered this the best and final model (model 3).

Model Comparison

We used the following measures of discrimination to assess incremental improvements in prediction between models 1 and 2, and between models 2 and 3: 1) the Harrell's C-statistic, which accounts for censoring in survival analysis; 2) the overall continuous net reclassification improvement (NRI) to quantify upward and downward reclassification, as well as the event and nonevent NRI separately, in order to determine the amount and direction of reclassification separately in people who did and did not experience an event; and 3) the relative integrated discrimination improvement (IDI) to assess the improvement in average sensitivity.[35–41] For the NRIs and relative IDI, we reported bias-corrected bootstrapped 95% confidence intervals (CIs).

To assess the calibration of each of the final models, we calculated the mean predicted risk within each decile of predicted risk. We also calculated the mean observed risk (the proportion of persons who experienced the event of interest) within each decile of predicted risk. We plotted the mean predicted risk on the X-axis against the mean observed risk on the Y-axis to visually assess their agreement. We excluded the 27 persons who had a competing risk event from this assessment of model calibration.

Results

Of the 654 persons with diagnosed diabetes followed for a maximum of 10 years, 296 had a major complication: 141 CVD (9 fatal and 132 non-fatal), 152 CKD (including 4 ESRD cases), and 3 lower extremity disease hospitalizations). There were 331 participants who did not experience any event of interest and 27 who died from an unrelated event. Compared to persons who did not experience any major complication during 10 years of follow-up, those who did were older and had a higher BMI, and a higher proportion were male, had hypercholesterolemia, had hypertension, or were on any glucose-lowering medication (**Table 1**).

The following four biomarkers were ultimately selected and included in model 3: HbA1c, 1/B2M, ln of NT-proBNP, and ln of hs-cTnT. We report the beta coefficients and corresponding subhazard ratios and P-values for each term included in the final models in **Table 2**. The baseline 10-year survival ($S_0(t)$, where t=10) for Models 1, 2, and 3 were 0.55, 0.65, and 0.60, respectively. The 10-year predicted risk for each participant for experiencing any major complication can then be calculated as:

$\hat{p} = 1 - S_0(t)^{exp(\sum_{i=1}^{n} \beta_i X_i - \sum_{i=1}^{n} \beta_i \bar{X}_i)}$, where ß=coefficient, X=value of the variable, $\bar{X}$=mean of the variable in the study population, and n=the number of all terms in the model.

Compared to Model 1, the improvement in the C-statistic for Model 2 was statistically significant (P=0.032), and improved further after the inclusion of HbA1c, 1/B2M, ln of

NT-proBNP, and ln of hs-cTnT in Model 3 (P<0.001) (**Table 3**). The improvements in the continuous NRI and relative IDI followed patterns similar to those observed for the C-statistic. Both persons who did and did not experience the event of interest were correctly re-classified in Models 2 and 3, and all improvements were statistically significant (**Table 3**). Furthermore, all models were rather well calibrated, as was demonstrated by the alignment of the observed and predicted risks along the line of equality (Y=X) (**Figure 1**).

Discussion

We successfully used a 3-stage approach for model development to construct a prediction model for 10-year risk of any major complication in middle-aged persons with diabetes in the community-based ARIC Study. Including HbA1c, a novel biomarker of kidney filtration (1/B2M), and two novel biomarkers of cardiac damage (NT-proBNP and hs-cTnT) significantly improved model performance. The final model including these biomarkers had overall good discrimination (C-statistic=0.720) and was well-calibrated. Furthermore, this model correctly re-classified a substantial number of persons who did and did not experience any major complication according to risk.

American Heart Association/American College of Cardiology guidelines recommend that all persons with diabetes between 40 and 75 years of age with LDL-c 70-189 mg/dL be treated with moderate intensity statin therapy for primary prevention of CVD. They also

recommend considering higher intensity statin therapy in those at high 10-year CVD risk (≥7.5% using the 10-year Pooled Cohort Equation for atherosclerotic CVD).[42] It is crucial to identify persons in whom aggressive treatment may be most appropriate. Our risk prediction equation for a combined endpoint could help identify those middle-aged individuals at highest risk for multiple major complications who might benefit most from a comprehensive risk reduction strategy. Nonetheless, aggressive glucose- and blood pressure-lowering strategies should be weighed against their potential harms in caring for persons with diabetes.

Standard approaches to survival analysis (i.e., Kaplan-Meier method and Cox proportional hazards regression) overestimate cumulative incidence when competing risks are present. These standard approaches may therefore affect the calibration of the risk prediction model, more so even than the discrimination.[25,26] Therefore, the Fine and Gray method that we used in this analysis may more accurately assess both discrimination and calibration. Accurate determination of absolute risk is vital for clinical prognosis and treatment decisions.

Whereas risk prediction equations developed in persons with diabetes may better predict risk than those developed in the general population,[43] many of these have performed poorly when applied to external populations of persons with diabetes.[44] For instance, the well-known United Kingdom Prospective Diabetes Study (UKPDS) risk engine, which is a risk prediction tool for CHD and stroke in persons with newly-diagnosed diabetes,[11,16]

has been shown to greatly overestimate risk (by up to 5 fold) in external populations.[45] Most risk scores have been developed in white European populations,[9] which may limit their generalizability. Having to use multiple risk scores to predict risk of diabetes complications is burdensome for practitioners,[46,47] whereas a risk prediction tool that comprehensively predicts risk of multiple diabetes complications may be convenient for clinical use. A recent paper developed a risk prediction model for multiple endpoints that included micro- and macrovascular complications in a Japanese population, and found that combining these outcomes improved classification of persons into low- and high-risk groups.[24] Few risk scores have comprehensively evaluated and compared traditional and nontraditional biomarkers of hyperglycemia, cardiac damage, kidney filtration, liver function, or inflammation.[9] The biomarkers evaluated here were selected because they are markers of physiological damage in the pathway to the clinical endpoints that we included, and they have been associated with increased risk of complications in persons with diabetes.[19–23,48–50] In fact, a risk score for CHD was previously developed in ARIC in persons with diabetes, but this model was never externally validated, nor did it evaluate HbA1c or the majority of the other biomarkers examined here.[14]

There are several limitations to note. The baseline for our study was in 1990-1992 and diagnostic and treatment practices for diabetes have changed since this time. Compared to current guidelines, diagnostic cut-points were higher in the early to mid-1990s, and therefore persons with diagnosed diabetes may have had more "severe" diabetes than those with diagnosed diabetes today.[51] Furthermore, rates of diabetes-related

complications, mainly CVD, have decreased in the past twenty years, which could be due to a variety of reasons, including improved care but also earlier detection of diabetes due to increased screening and lower diagnostic thresholds.[52] Urine was not collected at this examination and thus we were unable to include albuminuria as a potential biomarker in our study. Further, while we were able to distinguish between recently diagnosed diabetes (past 3 years) from longer duration diabetes, we may not have been able to fully adjust for the impact of diabetes duration, since information on age of diagnosis was not collected at the first or second ARIC examinations.

Strengths of our study included the rigorous measurement of clinical and biomarker data in a large number of middle-aged persons with diagnosed diabetes in the community. Few, if any, risk prediction models have evaluated such a comprehensive list of traditional and nontraditional variables. In particular, our results extend the current body of knowledge regarding the utility of these nontraditional biomarkers in both clinical and research settings. Long-term active surveillance of ARIC participants enabled us to develop prediction models for multiple major complications over 10 years, as well as capturing important endpoints over a meaningful period of time.

We demonstrated that the addition of traditional and nontraditional biomarkers to a model that included clinical and demographic information substantially improved the accuracy of a 10-year risk prediction equation for any major complication in persons with diabetes. Potential over- or undertreatment of cardiovascular risk factors in persons with diabetes is

of current interest, and we reported improved risk re-classification in both persons who did and did not experience an event. In particular, identifying persons with diabetes at highest risk may help inform those at greatest need for increased intensity of treatments for risk factors. Whereas further study of the use of these biomarkers in a clinical setting is necessary, the utility of these biomarkers for use in risk stratification is promising.

Table 1. Study population characteristics

	Overall (N=654)	Participants who did not experience an event (N=331)	Participants who experienced an event (N=296)	Participants who died from a non-event cause (N=27)
	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %	Mean (SD) or %
Age, years	58.1 (5.7)	57.3 (5.8)	58.9 (5.4)	60.3 (6.4)
Male	39.0%	32.3%	46.3%	40.7%
White	59.3%	58.9%	59.1%	66.7%
Education				
< HS	32.0%	30.2%	34.8%	22.2%
HS or college	40.0%	37.5%	43.6%	33.3%
> college	28.0%	32.3%	21.6%	44.4%
Current smoking	18.4%	17.8%	17.6%	33.3%
Current drinking	38.1%	37.8%	36.8%	55.6%
Physical activity	2.3 (0.7)	2.3 (0.7)	2.2 (0.7)	2.3 (0.9)
Family history of CVD*	56.7%	55.0%	57.1%	74.1%
Hypercholesterolemia†	29.8%	25.4%	35.1%	25.9%
BMI, kg/m^2	30.7 (5.8)	30.4 (6.1)	31.4 (5.3)	28.2 (6.1)
Hypertension‡	56.7%	47.7%	66.6%	59.3%
Glucose-lowering medication				
Insulin	19.4%	16.6%	22.6%	18.5%
Oral only	48.2%	42.6%	54.4%	48.2%
None	32.4%	40.8%	23.0%	33.3%
Recent onset of diabetes	30.1%	31.7%	27.0%	44.4%

*Family history of CVD defined as self-reported parental history of stroke or CHD

†Hypercholesterolemia was defined as total cholesterol ≥ 240 mg/dL or use of cholesterol-lowering medication.

‡Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication.

Table 2. Coefficients of prediction models for 10-year risk of major complications in persons with diabetes

	Model 1 (Demographics)			Model 2 (+ clinical variables)			Model 3 (+ biomarkers)		
	Beta	SHR (95% CI)	P-value	Beta	SHR (95% CI)	P-value	Beta	SHR (95% CI)	P-value
Age, per 1 year	0.02546	1.03 (1.00, 1.05)	0.018	0.02318	1.02 (1.00, 1.05)	0.031	-0.00404	1.00 (0.97, 1.02)	0.733
Male (vs. female)	0.50751	1.66 (1.03, 2.69)	0.039	0.88490	2.42 (1.19, 4.92)	0.014	1.07978	2.94 (1.42, 6.08)	0.004
White (vs. black)	-1.40805	0.24 (0.07, 0.84)	0.025	-0.97803	0.38 (0.10, 1.41)	0.147	-1.02520	0.36 (0.09, 1.40)	0.140
Education level (vs. <high school)									
High school or college	0.24573	1.28 (0.97, 1.69)	0.082	0.22601	1.25 (0.95, 1.66)	0.115	0.09019	1.09 (0.81, 1.47)	0.551
>College	-0.35003	0.70 (0.51, 0.98)	0.037	-0.39765	0.67 (0.48, 0.93)	0.018	-0.31334	0.73 (0.53, 1.01)	0.056
Cigarette smoking (vs. current)									
Former	0.07092	1.07 (0.77, 1.51)	0.682	0.07759	1.08 (0.76, 1.53)	0.662	0.13650	1.15 (0.79, 1.67)	0.477
Never	-0.08958	0.91 (0.64, 1.32)	0.630	-0.02587	0.97 (0.67, 1.41)	0.892	0.02343	1.02 (0.69, 1.51)	0.906
Alcohol (vs. current)									
Former	0.02047	1.02 (0.76, 1.37)	0.892	0.01905	1.02 (0.75, 1.38)	0.901	-0.08016	0.92 (0.68, 1.26)	0.615
Never	0.03490	1.04 (0.75, 1.42)	0.829	-0.03107	0.97 (0.70, 1.34)	0.849	-0.05882	0.94 (0.67, 1.32)	0.732
Physical activity	-0.13445	0.87 (0.74, 1.03)	0.112	-0.17582	0.84 (0.71, 0.99)	0.038	-0.09364	0.91 (0.77, 1.08)	0.285
Family history of CVD	-0.09562	0.91 (0.71, 1.16)	0.437	-0.10621	0.90 (0.71, 1.14)	0.386	-0.17333	0.84 (0.66, 1.08)	0.173
Glucose-lowering medication									

(vs. none)									
Oral only	0.36222	1.44 (0.97, 2.13)	0.071	0.25111	1.29 (0.87, 1.90)	0.209	0.16904	1.18 (0.78, 1.81)	0.433
Insulin	1.04865	2.85 (1.79, 4.54)	<0.001	1.02887	2.80 (1.75, 4.48)	<0.001	0.80246	2.23 (1.35, 3.68)	0.002
Antihypertensive medication	0.50648	1.66 (1.29, 2.14)	<0.001	0.47973	1.62 (1.25, 2.09)	<0.001	0.33799	1.40 (1.09, 1.81)	0.009
Cholesterol-lowering medication	0.29716	1.35 (0.96, 1.88)	0.083	0.22862	1.26 (0.89, 1.77)	0.187	0.32541	1.38 (0.96, 2.00)	0.083
BMI	-0.01076	0.99 (0.96, 1.02)	0.509	-0.01761	0.98 (0.95, 1.02)	0.316	-0.02500	0.98 (0.94, 1.01)	0.182
Recent diabetes	-0.08125	0.92 (0.71, 1.20)	0.549	-0.12491	0.88 (0.67, 1.16)	0.367	0.01084	0.99 (0.74, 1.33)	0.942
Male*Glucose-lowering medication									
Oral only	0.24972	1.28 (0.73, 2.25)	0.382	0.34687	1.41 (0.81, 2.46)	0.218	0.09634	1.10 (0.61, 2.00)	0.752
Insulin	-0.63781	0.53 (0.25, 1.10)	0.087	-0.69469	0.50 (0.24, 1.04)	0.064	-0.92049	0.40 (0.19, 0.85)	0.018
White*BMI	0.04564	1.05 (1.01, 1.09)	0.019	0.04896	1.05 (1.01, 1.09)	0.017	0.04647	1.05 (1.00, 1.09)	0.031
LDL-c		--		0.00511	1.01 (1.00, 1.01)	0.001	0.00398	1.00 (1.00, 1.01)	0.008
HDL-c		--		-0.00597	0.99 (0.98, 1.01)	0.319	-0.00092	1.00 (0.99, 1.01)	0.886
Triglycerides		--		0.00456	1.00 (1.00, 1.01)	0.004	0.00522	1.01 (1.00, 1.01)	0.001
Systolic BP		--		0.00570	1.01 (1.00, 1.01)	0.086	0.00387	1.00 (1.00, 1.01)	0.272
Male*Triglycerides		--		-0.00240	1.00 (0.99, 1.00)	0.112	-0.00245	1.00 (0.99, 1.00)	0.105
White*Triglycerides		--		-0.00378	1.00 (0.99, 1.00)	0.017	-0.00354	1.00 (0.99, 1.00)	0.029
HbA1c		--			--		0.12856	1.14 (1.07, 1.21)	<0.001

1/B2M	--	--	-2.35554	0.09 (0.03, 0.32)	<0.001
Ln of NT-proBNP	--	--	0.18337	1.20 (1.06, 1.37)	0.005
Ln of hs-cTnT	--	--	0.21787	1.24 (1.05, 1.47)	0.013

Baseline 10-year survival for Model 1 is 0.5462843, for Model 2 is 0.6508623, and for Model 3 is 0.6020032.

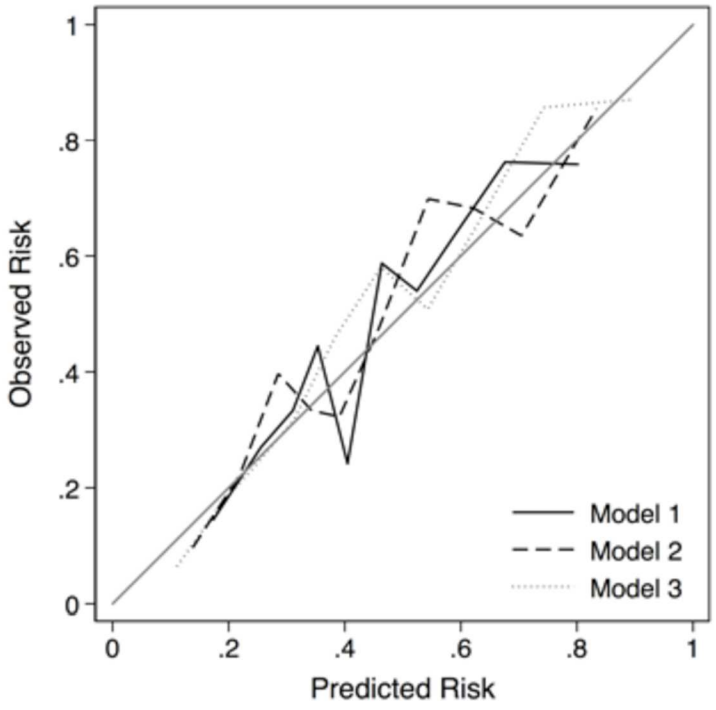
Table 3. Predictive statistics of risk prediction models

	C-statistic	Difference in C-statistic	P-value for difference	Continuous NRI*	Event NRI*	Non-event NRI*	Relative IDI*
Model 1	0.667 (0.64, 0.70)	--	--	--	--	--	--
Model 2 (vs. Model 1)	0.683 (0.65, 0.71)	0.015 (0.00, 0.03)	0.032	0.42 (0.31, 0.61)	0.18 (0.10, 0.26)	0.23 (0.16, 0.35)	0.28 (0.20, 0.35)
Model 3 (vs. Model 2)	0.720 (0.69, 0.75)	0.037 (0.02, 0.06)	<0.001	0.53 (0.47, 0.63)	0.17 (0.09, 0.27)	0.36 (0.29, 0.41)	0.29 (0.24, 0.38)

*Bias-corrected 95% CIs, obtained using a bootstrapping approach with 20 replications

Figure 1. Calibration of risk prediction models

The solid gray line is the line of equality (Y=X). The mean predicted risk within each decile of predicted risk is plotted on the X-axis. The observed risk (proportion of people who experienced the event of interest) within each decile of predicted risk is plotted on the Y-axis. The 27 persons who experienced the competing risk event were excluded from this analysis.



Conclusion

This dissertation focused on the epidemiology of nontraditional biomarkers of hyperglycemia. We examined various aspects of these biomarkers, including their variability, racial comparisons of their use as diagnostic and prognostic biomarkers in association with microvascular and macrovascular outcomes, and their potential utility for risk prediction of major complications in persons with diabetes.

Summary of Findings

In Chapter 1,[1] we conducted a review of the literature on nontraditional biomarkers of hyperglycemia. We found that there is a growing body of literature linking these biomarkers to increased risk of microvascular and macrovascular complications. In particular, fructosamine and glycated albumin have been shown to improve identification of persons with diabetes. Importantly, few prospective studies of fructosamine, glycated albumin, and 1,5-AG had been conducted with sufficient long-term follow-up to address important questions about their utility for diabetes diagnosis and management. Further, there have been no trials or intervention studies to examine whether the adoption of these biomarkers in the clinic can improve outcomes for patients with diabetes.

In Chapter 2, we quantified the short-term (~6 week) variability of nontraditional biomarkers of hyperglycemia in a subset of 200 ARIC participants. We then compared the variability of these biomarkers to that of traditional biomarkers (fasting glucose and HbA1c). We found that HbA1c and nontraditional biomarkers of hyperglycemia had

lower within-person variability than fasting glucose (9.6% and 5.3% in persons with and without diabetes, respectively). Of all 5 biomarkers, HbA1c had the lowest within-person variability (2.0% and 1.5% in persons with and without diabetes, respectively). The ICC and Spearman's rank correlation coefficient were lowest for fasting glucose and fructosamine, and highest for HbA1c and 1,5-AG. Understanding the within-person variability of any biomarker is important for its interpretation in the clinic and for research studies. Our findings suggest that fructosamine, glycated albumin, and 1,5-AG track well over a six-week time period, and confirm their use as intermediate-term biomarkers of hyperglycemia.

In Chapter 3, we compared in whites and blacks the associations of traditional and nontraditional biomarkers of hyperglycemia with incident CVD and ESRD. We aimed to address the current controversy regarding racial differences in hyperglycemia. We confirmed that levels of hyperglycemia were higher in blacks compared to whites, even among persons with similar levels of fasting glucose. We also confirmed that incidence rates of both CVD and ESRD were higher in blacks than whites. However, the relative associations of HbA1c and nontraditional biomarkers of hyperglycemia with CVD and ESRD were similar in whites and blacks (P-values for interaction with race were P=0.56 and P=0.60, respectively). Furthermore, the fact that we found no racial differences in associations of nontraditional biomarkers of hyperglycemia with these outcomes further suggests that racial discordance in hyperglycemia is likely due to glycemic factors, possibly including racial differences in diet and physical activity. Our findings suggest that HbA1c and nontraditional biomarkers of hyperglycemia have similar diagnostic and

prognostic value in whites and blacks and support current recommendations for uniform HbA1c cut-offs for diagnosis across racial/ethnic groups.

In Chapter 4,[2] we assessed the association of six-year changes in and sustained elevations in hs-CRP with incident diabetes, cardiovascular events, and mortality. We demonstrated that compared to persons with sustained low or moderate hs-CRP, those with larges increases or sustained elevations in measurements of hs-CRP six years apart were at an increased risk of diabetes (HRs [95% CIs] were 1.58 [1.38, 1.76] and 1.39 [1.25, 1.56], respectively). The more recently measured value of hs-CRP may most accurately assess future diabetes risk. Alternatively, persons with sustained elevated hs-CRP had the highest risk of incident CHD, stroke, heart failure, and mortality compared to those with sustained low or moderate hs-CRP (HRs were 1.51-1.70, all P-values<0.05). For cardiovascular outcomes and mortality, two measurements of hs-CRP better identified persons at highest risk than one measurement alone.

In Chapter 5, we developed a 10-year risk prediction equation for major complications in persons with diabetes; and we tested whether the addition of biomarkers of hyperglycemia, cardiac function, kidney function, liver function, and inflammation improved risk prediction. Our risk prediction model is unique in that it used a combined endpoint for major complications that included CVD, CKD, and lower extremity hospitalizations. We also accounted for the competing risk of death due to causes other than the events of interest. Of the 13 biomarkers that we tested, we found that the combined addition of four biomarkers (HbA1c, B2M, NT-proBNP, and hs-cTnT) improved prediction (c-statistic=0.679 vs. 0.716 after addition of biomarkers, P-value for

difference in c-statistics<0.001). Our results provide evidence that the combined use of these biomarkers may be a useful tool for researchers and clinicians. Improved risk stratification in epidemiologic studies could help better distinguish between persons at low versus high risk, and decrease misclassification. In a clinical setting, more accurate risk prediction could improve identification of persons at highest risk who are at greatest need for aggressive treatment of risk factors.

Implications and Future Directions

Diagnostic and Therapeutic Thresholds

There is much debate over the meaning of racial differences in hyperglycemia. It has been shown that among persons with and without diabetes, blacks have higher levels of hyperglycemia (as measured by higher HbA1c, fructosamine, and glycated albumin, and lower 1,5-AG) than whites, even at similar levels of fasting glucose.[3–8] However, the underlying reasons for these racial differences are not well studied. Previous studies have reported similar relative associations in whites and blacks of HbA1c with microvascular and macrovascular outcomes.[9,10] This dissertation expands upon those findings, and reports similar relative associations in whites and blacks of HbA1c and nontraditional biomarkers of hyperglycemia with CVD and ESRD. Fructosamine, glycated albumin, and 1,5-AG are extracellular glycated proteins that are unaffected by hemoglobin or erythrocyte characteristics or erythrocyte turnover. These findings, along with the observation that racial differences in levels of these biomarkers were similar to those seen with HbA1c, provide evidence to support the idea that racial differences in levels of

HbA1c are not the result of racial differences in non-glycemic factors. Conversely, they suggest that differences in biomarkers may be due to actual increases in chronic levels of circulating glucose in blacks, due to glycemic factors such as differences in diet or physical activity, which may lead to differences in non-fasting glucose levels. If this were the case, then the interpretation and prognostic value of HbA1c and nontraditional biomarkers of hyperglycemia are similar in whites and blacks. These results support the current ADA guidelines that recommend using the same cut-points in whites and blacks for diabetes diagnosis and monitoring. The use of newer technologies in glucose monitoring and devices could provide insight into the underlying cause of racial differences in levels of these biomarkers. For instance, future studies could use continuous glucose monitoring to compare patterns of circulating glucose levels in whites and blacks.

Research Implications

Compared to traditional biomarkers of hyperglycemia, nontraditional biomarkers may have certain advantages in both epidemiologic studies and randomized controlled trials (RCTs). Fasting glucose requires participants to arrive to the visit having fasted for at least 8 hours, whereas nontraditional biomarkers do not.[11] Measurement of HbA1c requires whole blood specimens, which may not be readily available in large cohort studies, whereas nontraditional biomarkers can be measured using serum or plasma.[1] Nontraditional biomarkers of hyperglycemia are associated with increased risk of incident diabetes and microvascular and macrovascular outcomes,[12–15] and could be used

as surrogate outcomes, since they are on the path to relevant clinical endpoints. Importantly, they measure average glycemia over a shorter duration of time than HbA1c,[1] and therefore may change in response to behavioral or therapeutic interventions more quickly than HbA1c. Certain study designs may actually necessitate short follow-up, due to prohibitive costs, intensive use of resources, or high participant burden. In particular, feeding trials, for which long duration of follow-up is not feasible, have examined the effect of various interventions on changes in these biomarkers as a way to detect potential effects over a short period of time.[16,17]

Biomarkers and Personalized Medicine

The addition of an individual biomarker to an existing CVD risk score typically only improves risk prediction minimally. Adding a combination of biomarkers may be a better and more efficient way to improve risk prediction to an extent that is meaningful in both clinical and research settings, especially if only one blood sample is available or the biomarkers reflect unique aspects of the underlying disease process. In order to maximize the predictive power of biomarkers, it is crucial that laboratory methods are standardized and measurement is reliable. Especially for new biomarkers, repeatability studies are necessary to assess reliability. We showed here that fructosamine, glycated albumin, and 1,5-AG had good reliability and low within-person variability, especially compared to fasting glucose. The reliability of these biomarkers is reassuring, especially for considering their clinical use, and helps explain why they may improve prediction above and beyond other biomarkers

We demonstrated that the addition of a combination of four biomarkers (HbA1c, B2M, NT-proBNP, and hs-cTnT) statistically significantly improved discrimination of a clinical risk equation for major complications in persons with diabetes. Improved risk prediction allows better identification of high-risk persons, who may then be targeted for more aggressive treatment to ameliorate their high-risk status, or could help better identify appropriate participants during the screening process for inclusion in RCTs.

Identifying panels of biomarkers that provide unique and complementary information to one another may contribute to the advancement of personalized medicine,[18] and enable us to further tailor treatment and counseling not only to a patient's current condition, but also to his or her risk of important clinical outcomes. Along with the expanding fields of metabolomics and transcriptomics, the use of biomarker panels could improve targeted and individualized approaches to early identification and prevention of diabetes and its complications.

Summary

This dissertation extends the current understanding of nontraditional biomarkers of hyperglycemia and demonstrates their potential utility for diagnosis and prognosis in diabetes. We showed that fructosamine, glycated albumin, and 1,5-AG had good reliability and low within-person variability over approximately six weeks. Compared to fasting glucose, these biomarkers had lower within-person variability, regardless of diabetes status, which is vital for accurate diagnosis and management in a clinical setting, and for minimizing misclassification in research studies. We also showed that similar to

HbA1c, there was no evidence for racial differences in the prognostic value of these biomarkers in association with important microvascular and macrovascular outcomes. Additionally, these biomarkers may have utility to improve risk prediction in persons with diabetes. This dissertation provides valuable prospective data of these biomarkers with important clinical outcomes, and suggests that fructosamine, glycated albumin, and 1,5-AG may have a role in both research and clinical settings as either alternatives or adjuncts to fasting glucose and HbA1c.

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References for Chapter 1

****Of major importance**
***Of importance**

Important References (within past 3 years)

**Nathan (2014) – This was an important study conducted in a subsample of participants from the Diabetes Control and Complications Trial (DCCT), to assess the association of short-term and intermediate glycemia on microvascular and macrovascular complications in persons with type 1 diabetes. Over a mean of 6.5 years of follow-up, this paper reported similar associations of HbA1c and glycated albumin with microvascular complications.

**Selvin (2014) – This has been the largest population-based study, with the longest follow-up, to assess prospective associations of fructosamine and glycated albumin with incident diabetes and microvascular outcomes in persons with and without diabetes. This study included more than 12,000 participants (nearly 1,000 with diabetes), who were followed for about 20 years.

**Freedman, 2011 – This was one of the first prospective studies to assess associations of glycated albumin with clinical outcomes in a population of persons with diabetes and ESRD. It included 444 participants with diabetes and included follow-up just over 2 years.

*Watanabe (2011) – This was a large population-based prospective study with long-term follow-up. It included approximately 2,000 persons (only about 30 people with diabetes) and assessed the association of 1,5-AG with incident cardiovascular events over about 11 years.

*Shafi (2013) – This was a large prospective cohort study of persons on hemodialysis over a median follow-up of 3.5 years, and describes associations of fructosamine and glycated albumin with clinical outcomes, which had not been previously described in this population.

*Sacks (2013) – This commentary discusses the current clinical utility of HbA1c, as well as its limitations. The author mentions fructosamine and glycated albumin as potential alternatives to HbA1c, however also notes the need for additional studies since there is a lack of data linking these markers to clinical outcomes from clinical trials and prospective studies, as well as no established clinical cut-points for use in persons with diabetes.

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Appendix A: Supplemental Material for Chapter 2

Supplemental Table S1. Characteristics of participants in the repeatability subsample compared to those in the entire cohort

	Repeatability Subsample (N=200)*	Entire Cohort (N=6,538)*
	Mean (SD) or %	**Mean (SD) or %**
Age, years	76.2 (4.9)	75.8 (5.3)
Male	38.5%	41.2%
Race		
White	71.0%	76.1%
Black	28.0%	23.6%
Asian	1.0%	0.2%
Native American	0.0%	0.1%
Field center		
Forsyth	24.0%	22.1%
Jackson	25.0%	21.7%
Minneapolis	25.5%	29.2%
Washington County	25.5%	27.1%
BMI, kg/m^2	28.9 (4.8)	28.7 (5.8)
Diabetes	36.5%	32.7%
Current smoking	5.3%	5.9%
Prevalent CVD	22.0%	20.5%
Hypertension†	72.1%	74.9%
eGFR<60 mL/min/1.73 m^2	27.8%	29.4%
Time between original and repeat visits, days	45.7 (17.0)	--

*In the repeatability subsample, 2 participants are missing BMI, 3 are missing hypertension, 2 are missing eGFR, and 12 are missing current smoking status. In the entire cohort, 269 participants are missing BMI, 93 are missing hypertension, 94 are missing eGFR, and 426 are missing current smoking status.
†Hypertension is defined as SBP >=140 or DBP >=90 or use of antihypertensive medication.

Supplemental Table S2. Total variability in biomarkers of hyperglycemia in older adults with and without diabetes, no exclusion of outliers, the Atherosclerosis Risk in Communities Study, 2011-13, N=174

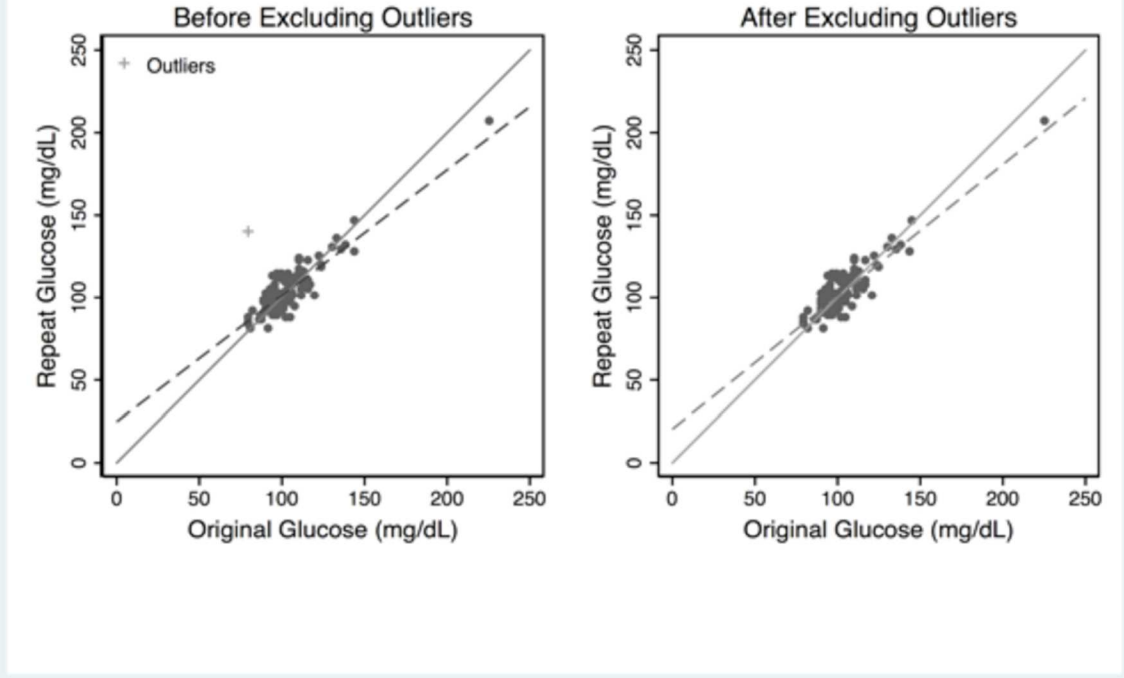
	Original exam Mean (SD)	Repeat exam Mean (SD)	Difference (Repeat-Original) Mean (SD)	CV_W (95% CI)†	ICC (95% CI)†	r (95% CI)	Index of Individuality (95% CI)†
No Diagnosed Diabetes (N=113)							
Fasting glucose, mg/dL	104.2 (17.1)	104.4 (15.7)	0.19 (9.6)	6.5% (4.4, 8.6)	0.82 (0.74, 0.92)	0.66 (0.54, 0.75)	0.46 (0.29, 0.63)
HbA1c, %	5.7 (0.4)	5.7 (0.5)	0.02 (0.3)	3.9% (1.4, 6.4)	0.75 (0.52, 0.97)	0.89 (0.85, 0.92)	0.58 (0.18, 0.98)
Fructosamine, µmol/L	240.5 (23.1)	237.9 (21.0)	-2.53 (12.0)	3.6% (3.1, 4.1)	0.85 (0.81, 0.88)	0.83 (0.76, 0.88)	0.43 (0.36, 0.49)
Glycated albumin, %	13.7 (1.5)	13.7 (1.6)	-0.06 (0.5)	2.7% (2.4, 3.1)	0.94 (0.93, 0.95)	0.91 (0.87, 0.94)	0.25 (0.21, 0.28)
1,5-AG, µg/mL	17.3 (6.2)	17.2 (6.3)	-0.15 (1.2)	4.8% (3.7, 5.9)	0.98 (0.98, 0.99)	0.98 (0.97, 0.99)	0.13 (0.10, 0.16)
Diagnosed Diabetes (N=61)							
Fasting glucose, mg/dL	135.8 (40.4)	136.7 (45.5)	0.89 (36.2)	18.6% (13.2, 24.1)	0.65 (0.51, 0.79)	0.76 (0.63, 0.85)	0.74 (0.51, 0.97)
HbA1c, %	6.7 (1.2)	6.7 (1.3)	0.04 (0.4)	4.6% (2.7, 6.5)	0.94 (0.90, 0.97)	0.94 (0.90, 0.96)	0.26 (0.15, 0.37)
Fructosamine, µmol/L	275.2 (49.4)	273.7 (50.5)	-1.57 (30.8)	7.9% (5.3, 10.4)	0.81 (0.72, 0.90)	0.77 (0.65, 0.86)	0.48 (0.32, 0.65)
Glycated albumin, %	16.6 (3.7)	16.8 (4.1)	0.2 (2.4)	10.1% (5.5, 14.7)	0.81 (0.69, 0.93)	0.87 (0.80, 0.92)	0.48 (0.25, 0.71)
1,5-AG, µg/mL	13.7 (7.2)	13.7 (7.2)	-0.04 (2.6)	13.6% (6.6, 20.5)	0.93 (0.88, 0.98)	0.93 (0.89, 0.96)	0.27 (0.13, 0.41)

Abbreviations: CI, confidence interval; CV_W, within-person coefficient of variation; ICC, intraclass correlation coefficient; r, Spearman's rank correlation coefficient; SD, standard deviation

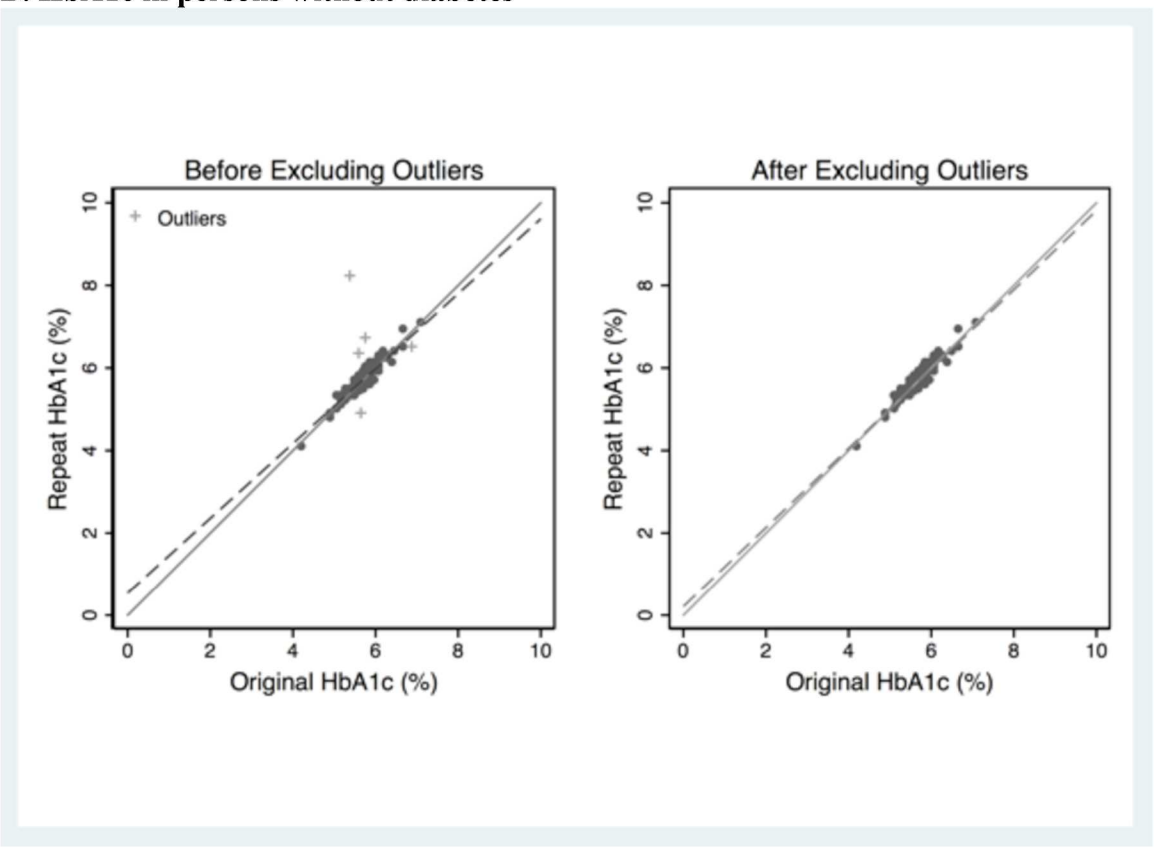
†95% CIs were bootstrapped using 200 replications

Supplemental Figure S1. Scatterplots of original versus repeat measurements before and after exclusion of outliers, in persons with and without diabetes. We used an iterative outlier removal approach to identify outlying data points that were likely due to error processes unrelated to usual variability. The crosses indicate data points that were identified as outliers and were excluded from main analyses. The solid line is the line of equality (Y=X). The dashed line is the regression line.

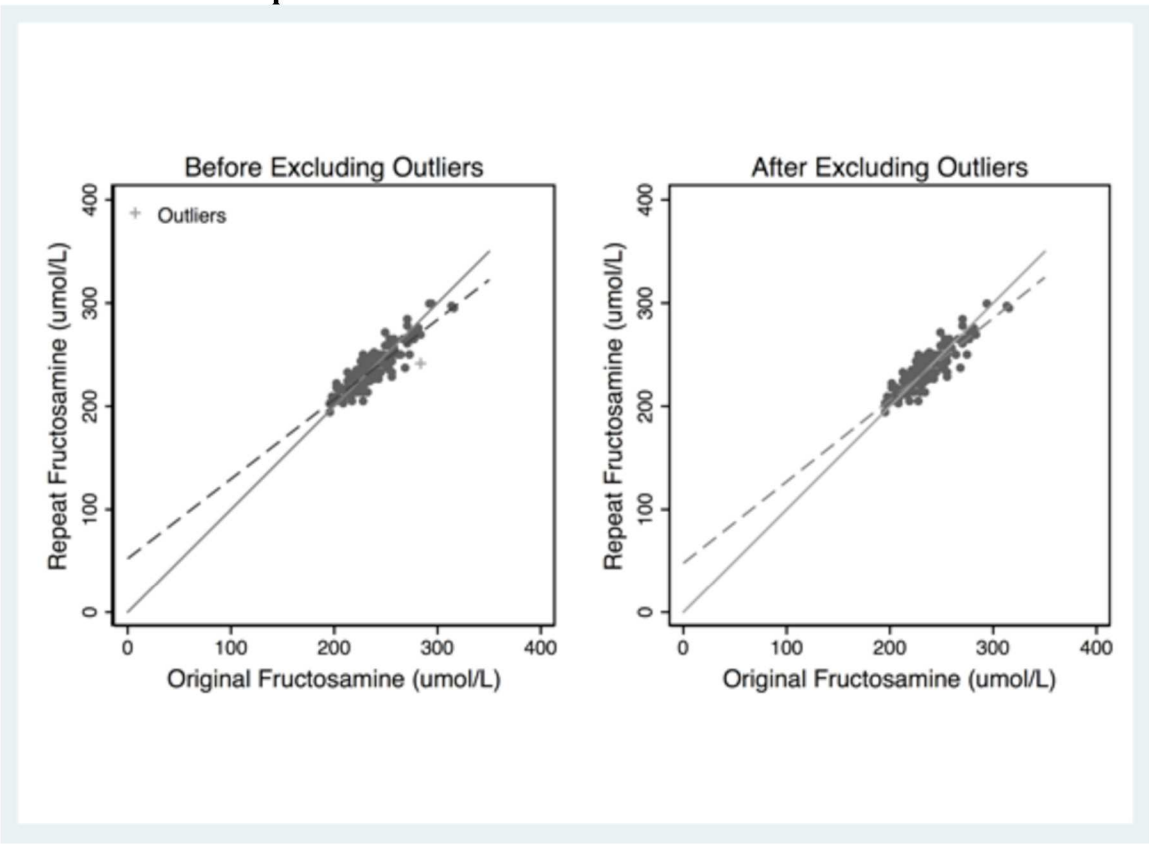
A. Fasting glucose in persons without diabetes



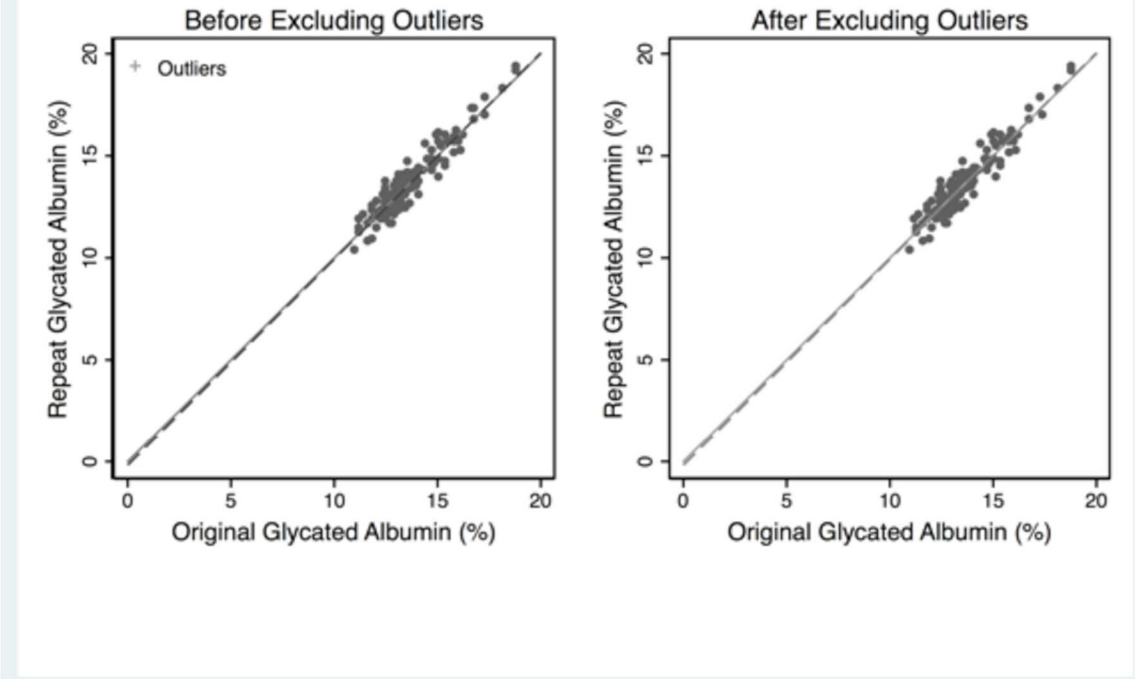
B. HbA1c in persons without diabetes



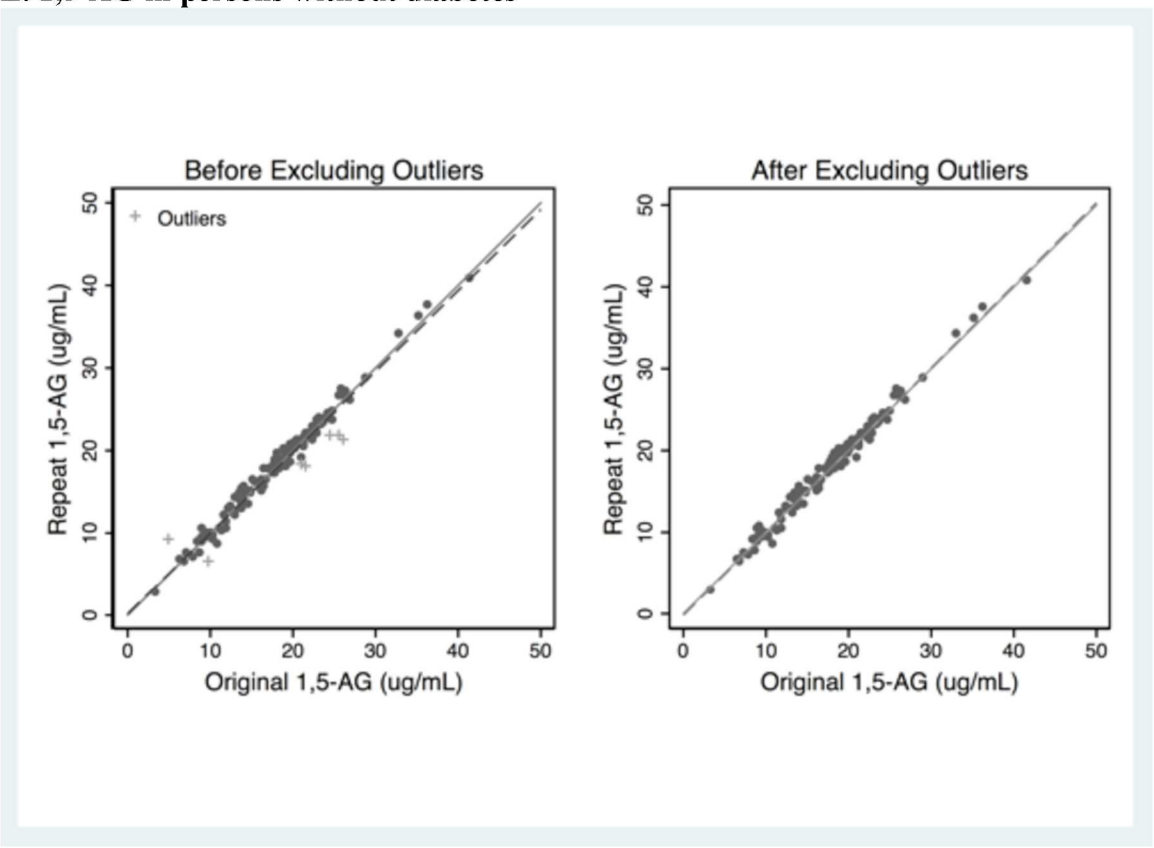
C. Fructosamine in persons without diabetes



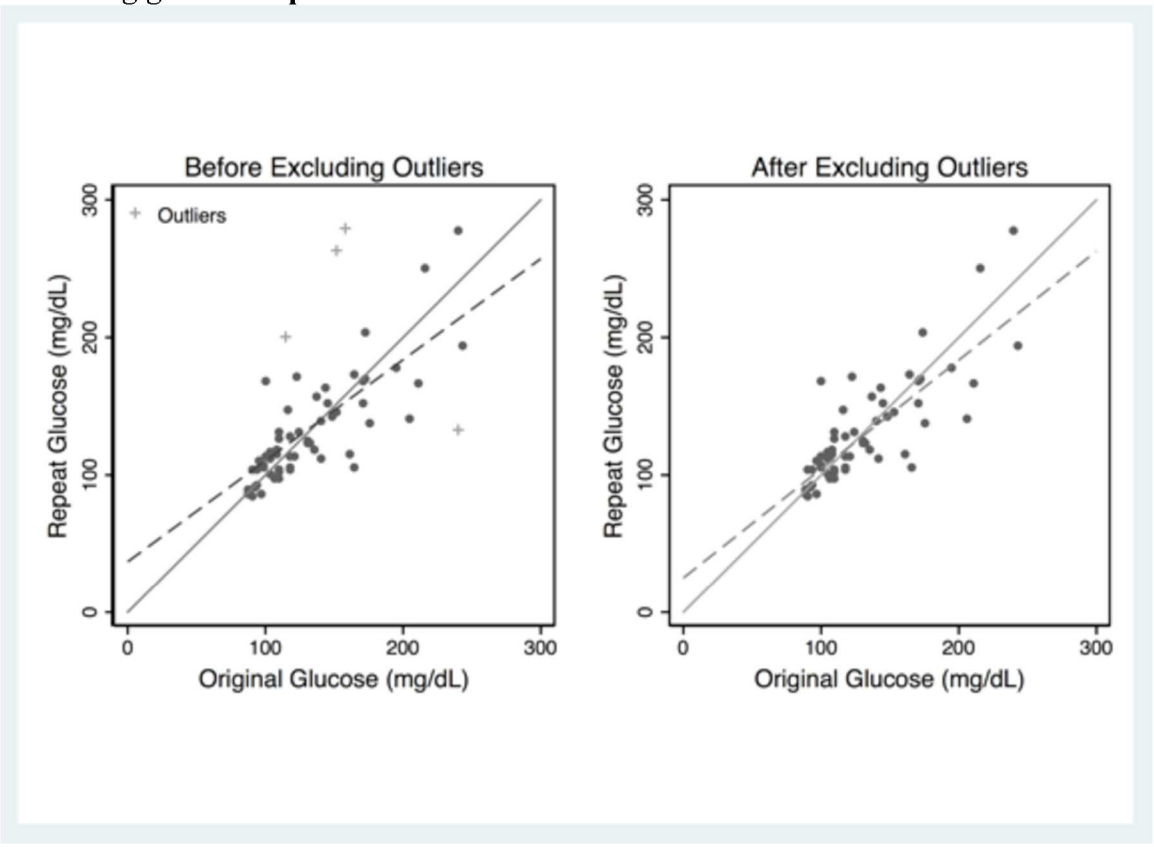
D. Glycated albumin in persons without diabetes



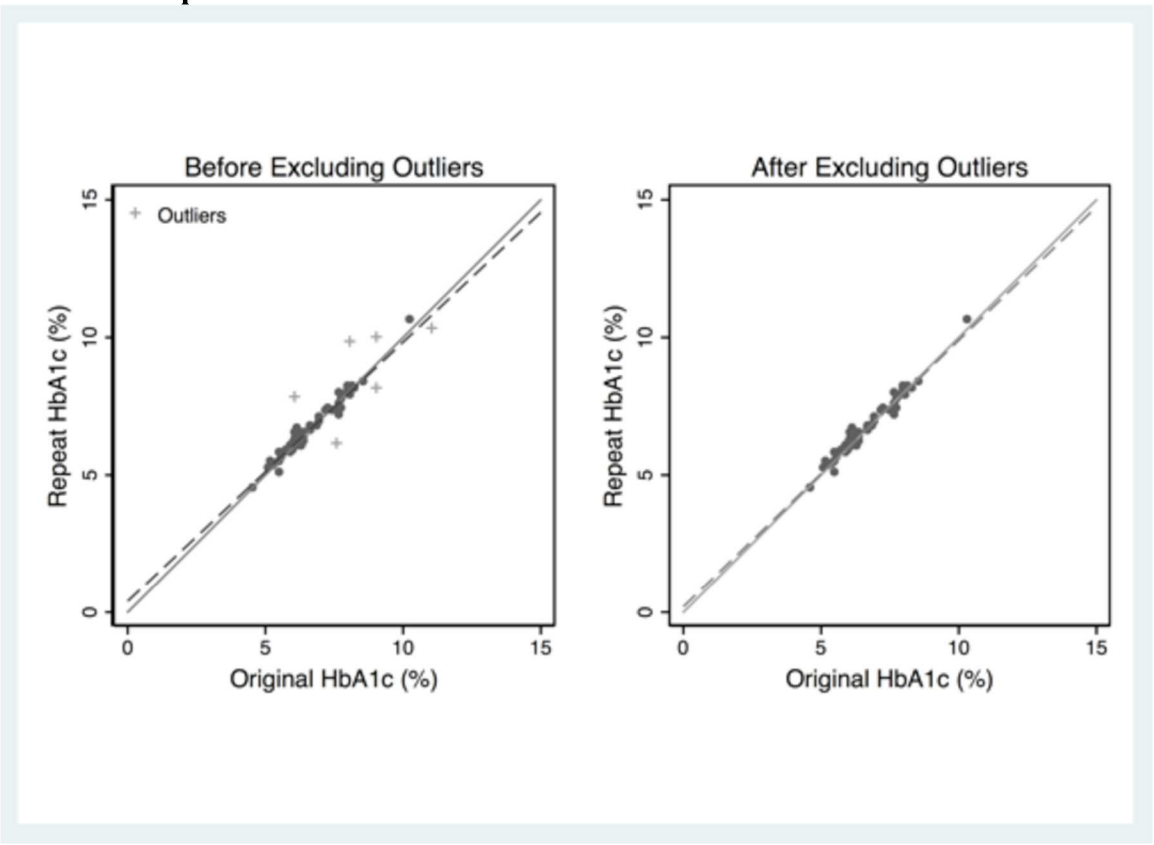
E. 1,5-AG in persons without diabetes



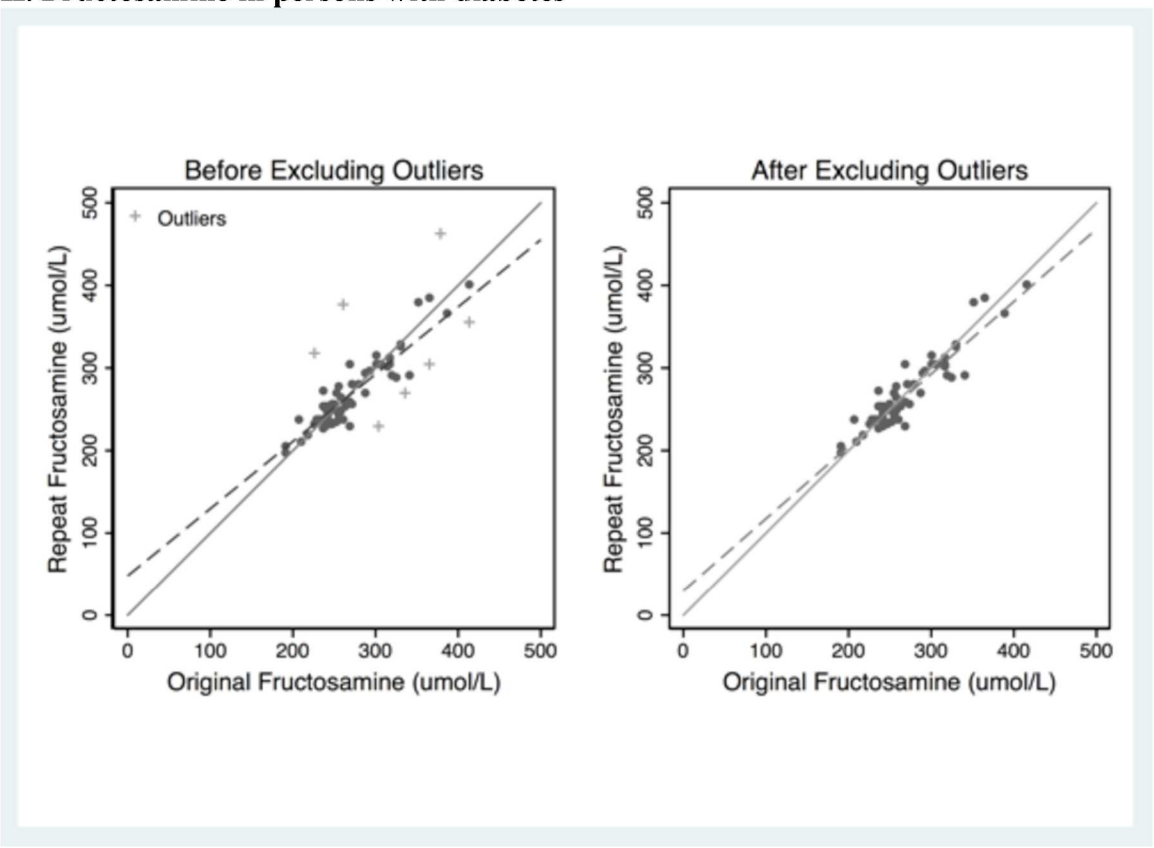
F. Fasting glucose in persons with diabetes



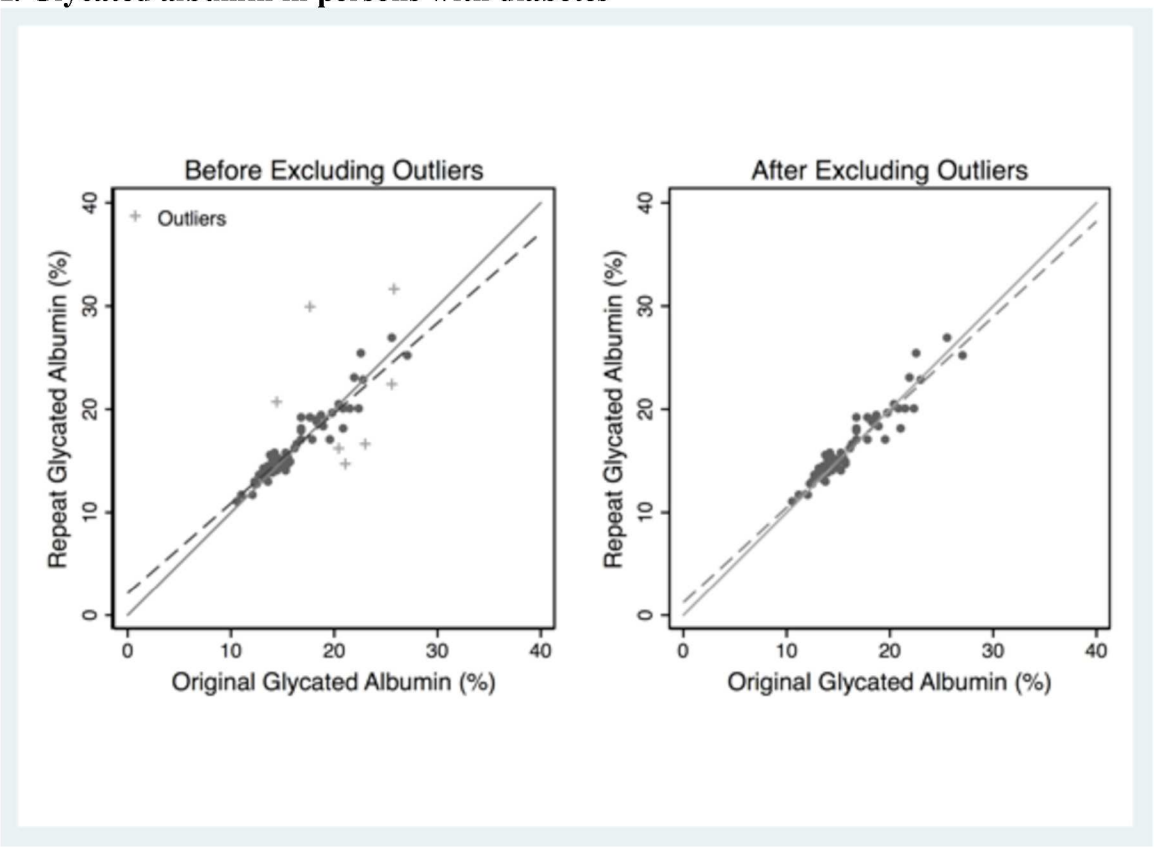
G. HbA1c in persons with diabetes



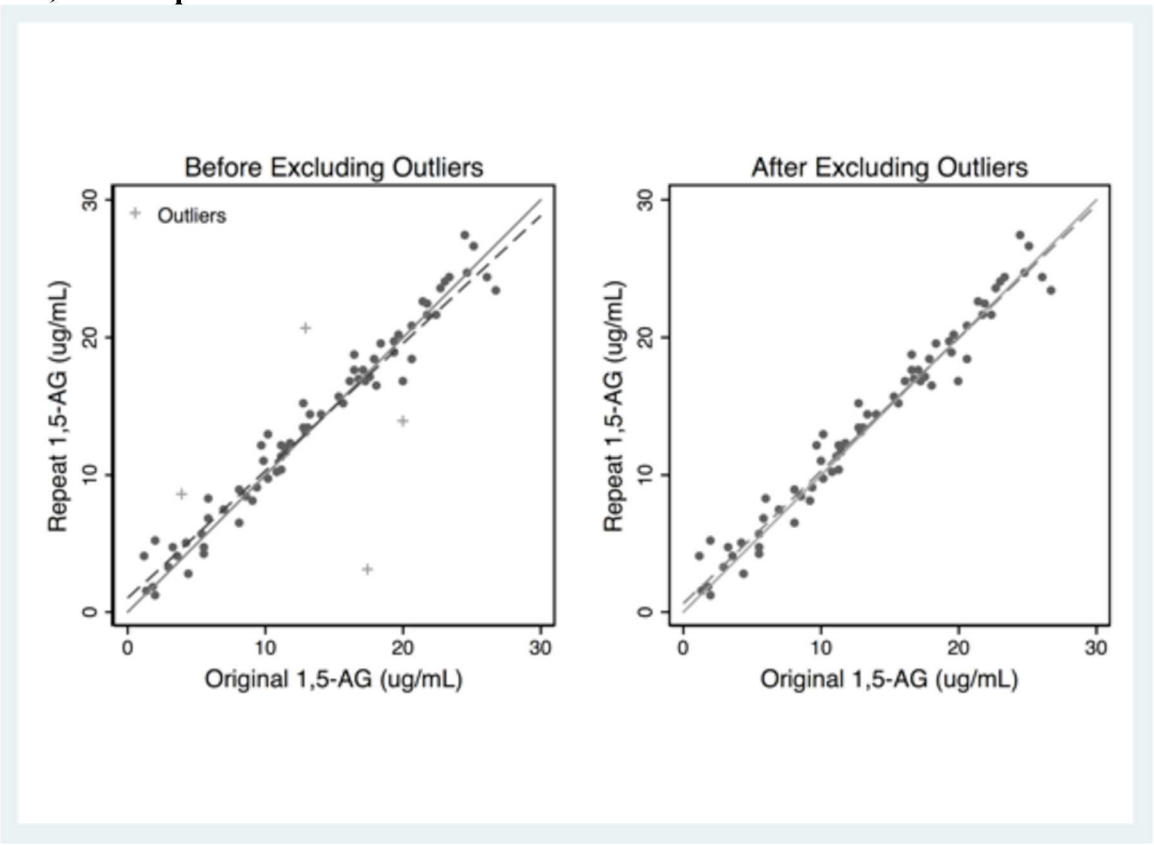
H. Fructosamine in persons with diabetes



I. Glycated albumin in persons with diabetes



J. 1,5-AG in persons with diabetes



Appendix B: Supplemental Material for Chapter 3

Supplemental Table S1. Associations of biomarkers of hyperglycemia with incident cardiovascular disease in black and white participants in ARIC

	Model 1		Model 2		Model 3		Model 4	
	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Fasting glucose								
No diabetes								
<100 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)			1 (Ref)	1 (Ref)
100-125 mg/dL	1.18 (1.05, 1.31)	0.95 (0.79, 1.14)	1.07 (0.96, 1.20)	0.87 (0.72, 1.05)			1.03 (0.92, 1.15)	0.84 (0.69, 1.01)
≥126 mg/dL	1.55 (1.26, 1.90)	1.38 (1.06, 1.81)	1.26 (1.03, 1.55)	1.17 (0.89, 1.55)		--	0.93 (0.74, 1.17)	0.92 (0.68, 1.24)
Diagnosed diabetes								
<149 mg/dL	2.24 (1.75, 2.87)	1.78 (1.24, 2.55)	1.84 (1.43, 2.36)	1.55 (1.07, 2.23)			1.55 (1.20, 2.00)	1.33 (0.92, 1.92)
≥149 mg/dL	3.31 (2.70, 4.07)	2.74 (2.16, 3.49)	2.61 (2.11, 3.22)	2.41 (1.87, 3.10)			1.23 (0.90, 1.67)	1.26 (0.86, 1.83)
HbA1c								
No diabetes								
<5.7 %	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)		
5.7-6.4%	1.63 (1.45, 1.82)	1.37 (1.14, 1.65)	1.37 (1.22, 1.54)	1.18 (0.98, 1.42)	1.36 (1.21, 1.53)	1.17 (0.97, 1.41)		
≥6.5%	1.90 (1.47, 2.45)	1.95 (1.49, 2.54)	1.49 (1.14, 1.93)	1.76 (1.34, 2.30)	1.42 (1.07, 1.90)	1.60 (1.20, 2.13)		--
Diagnosed diabetes								
<7%	1.96 (1.54, 2.50)	1.84 (1.25, 2.72)	1.60 (1.25, 2.05)	1.52 (1.02, 2.26)	1.57 (1.22, 2.02)	1.42 (0.95, 2.13)		
≥7%	4.05 (3.34, 4.91)	3.62 (2.87, 4.56)	3.34 (2.74, 4.08)	3.25 (2.55, 4.15)	3.05 (2.21, 4.22)	2.48 (1.75, 3.53)		
Fructosamine								
No diabetes								

<239.9 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
239.9-268.8 mg/dL	0.88 (0.77, 1.01)	0.77 (0.64, 0.93)	0.95 (0.83, 1.08)	0.79 (0.65, 0.96)	0.93 (0.81, 1.07)	0.77 (0.63, 0.93)	0.90 (0.79, 1.04)	0.75 (0.62, 0.91)
≥268.9 mg/dL	1.51 (1.16, 1.97)	1.34 (1.02, 1.76)	1.34 (1.02, 1.76)	1.13 (0.86, 1.50)	1.22 (0.91, 1.63)	0.95 (0.71, 1.28)	0.97 (0.72, 1.31)	0.88 (0.65, 1.19)
Diagnosed diabetes								
<275.8 mg/dL	1.72 (1.36, 2.18)	1.74 (1.22, 2.49)	1.47 (1.16, 1.87)	1.51 (1.05, 2.16)	1.40 (1.10, 1.79)	1.38 (0.96, 1.99)	1.30 (1.02, 1.65)	1.33 (0.93, 1.92)
≥275.8 mg/dL	3.24 (2.68, 3.92)	2.49 (2.01, 3.07)	2.87 (2.36, 3.50)	2.38 (1.90, 2.98)	2.28 (1.66, 3.13)	1.47 (1.04, 2.08)	1.48 (1.09, 2.01)	1.22 (0.86, 1.74)
Glycated albumin								
No diabetes								
<13.52%	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
13.52-15.56%	0.86 (0.75, 0.99)	0.95 (0.79, 1.14)	0.98 (0.86, 1.13)	1.03 (0.86, 1.23)	0.98 (0.85, 1.13)	1.01 (0.84, 1.21)	0.94 (0.82, 1.08)	0.98 (0.81, 1.17)
≥15.57%	1.69 (1.30, 2.18)	1.34 (1.01, 1.76)	1.55 (1.20, 2.01)	1.26 (0.95, 1.67)	1.48 (1.12, 1.96)	1.08 (0.80, 1.45)	1.15 (0.86, 1.54)	0.99 (0.73, 1.34)
Diagnosed diabetes								
<16.47%	1.67 (1.32, 2.11)	1.73 (1.20, 2.50)	1.42 (1.12, 1.80)	1.55 (1.07, 2.25)	1.38 (1.08, 1.77)	1.45 (1.00, 2.10)	1.27 (0.99, 1.62)	1.40 (0.97, 2.03)
≥16.47%	3.38 (2.80, 4.09)	2.70 (2.18, 3.34)	3.09 (2.54, 3.76)	2.67 (2.13, 3.35)	2.78 (2.03, 3.80)	1.74 (1.22, 2.47)	1.74 (1.28, 2.38)	1.44 (1.00, 2.06)
1,5-AG								
No diabetes								
≥15.0 μg/mL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
7.9-14.9 μg/mL	1.08 (0.95, 1.23)	0.81 (0.66, 0.98)	1.13 (1.00, 1.28)	0.93 (0.76, 1.13)	1.12 (0.99, 1.27)	0.92 (0.76, 1.13)	1.09 (0.97, 1.24)	0.93 (0.76, 1.13)
<7.9 μg/mL	1.10 (0.84, 1.43)	0.89 (0.60, 1.34)	1.09 (0.83, 1.42)	0.93 (0.62, 1.40)	1.00 (0.76, 1.31)	0.76 (0.49, 1.17)	0.84 (0.63, 1.11)	0.67 (0.43, 1.04)
Diagnosed diabetes								
>9.2 μg/mL	1.82 (1.42, 2.33)	1.57 (1.14, 2.16)	1.55 (1.21, 1.99)	1.42 (1.02, 1.96)	1.45 (1.13, 1.87)	1.27 (0.91, 1.77)	1.39 (1.08, 1.78)	1.23 (0.88, 1.72)
≤9.2 μg/mL	3.09 (2.56, 3.73)	2.69 (2.17, 3.33)	2.73 (2.25, 3.32)	2.75 (2.19, 3.44)	2.02 (1.51, 2.69)	1.73 (1.20, 2.50)	1.39 (1.05, 1.85)	1.37 (0.94, 2.01)

Model 1: Adjustment for age, gender (male, female), BMI, BMI-squared

Model 2: Model 1 + LDL-c, HDL-c, triglycerides, cholesterol-lowering medication use (yes, no), systolic blood pressure, antihypertensive medication use (yes, no), eGFR, family history of diabetes (yes, no), education level (less than high school, high school or some college, college or more), alcohol consumption (current, former, never), cigarette smoking status (current, former, never), physical activity level
Model 3: Model 2 + fasting glucose
Model 4: Model 2 + HbA1c

Supplemental Table S2. Associations of biomarkers of hyperglycemia with incident coronary heart disease in black and white participants in ARIC

	Model 1		Model 2		Model 3		Model 4	
	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Fasting glucose								
No diabetes								
<100 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)			1 (Ref)	1 (Ref)
100-125 mg/dL	1.37 (1.16, 1.63)	1.12 (0.82, 1.53)	1.22 (1.03, 1.45)	1.01 (0.74, 1.39)			1.17 (0.99, 1.39)	0.98 (0.72, 1.35)
≥126 mg/dL	1.60 (1.16, 2.21)	1.91 (1.25, 2.92)	1.25 (0.90, 1.74)	1.73 (1.12, 2.66)	--		0.92 (0.65, 1.32)	1.42 (0.89, 2.27)
Diagnosed diabetes								
<149 mg/dL	2.89 (2.03, 4.12)	2.38 (1.37, 4.16)	2.32 (1.62, 3.32)	2.07 (1.18, 3.63)			1.93 (1.34, 2.80)	1.82 (1.03, 3.23)
≥149 mg/dL	4.35 (3.25, 5.81)	3.40 (2.32, 5.00)	3.20 (2.37, 4.33)	3.06 (2.04, 4.59)			1.55 (1.00, 2.39)	1.79 (0.99, 3.25)
HbA1c								
No diabetes								
<5.7 %	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)		
5.7-6.4%	1.92 (1.62, 2.27)	1.66 (1.22, 2.25)	1.53 (1.29, 1.82)	1.40 (1.03, 1.90)	1.50 (1.26, 1.78)	1.39 (1.02, 1.89)		
≥6.5%	1.88 (1.25, 2.84)	2.79 (1.84, 4.24)	1.33 (0.88, 2.02)	2.44 (1.60, 3.72)	1.14 (0.72, 1.80)	2.27 (1.46, 3.53)	--	
Diagnosed diabetes								
<7%	2.53 (1.79, 3.56)	2.78 (1.56, 4.96)	2.07 (1.47, 2.94)	2.22 (1.24, 3.99)	1.93 (1.35, 2.76)	2.11 (1.17, 3.82)		
≥7%	4.84 (3.70, 6.33)	4.40 (3.04, 6.38)	3.61 (2.73, 4.78)	4.01 (2.70, 5.95)	2.66 (1.68, 4.20)	3.20 (1.83, 5.61)		
Fructosamine								
No diabetes								
<239.9 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
239.9-268.8	0.83	0.90	0.84	0.91	0.82	0.89	0.80	0.87

mg/dL	(0.68, 1.02)	(0.67, 1.21)	(0.68, 1.04)	(0.67, 1.23)	(0.66, 1.01)	(0.65, 1.20)	(0.65, 0.99)	(0.64, 1.18)
≥268.9 mg/dL	1.46 (0.97, 2.20)	1.26 (0.80, 1.98)	1.10 (0.72, 1.68)	1.00 (0.63, 1.58)	0.92 (0.59, 1.44)	0.85 (0.52, 1.37)	0.78 (0.50, 1.23)	0.78 (0.48, 1.28)
Diagnosed diabetes								
<275.8 mg/dL	1.96 (1.40, 2.76)	2.07 (1.19, 3.61)	1.70 (1.21, 2.40)	1.73 (0.99, 3.03)	1.56 (1.09, 2.21)	1.57 (0.89, 2.77)	1.49 (1.05, 2.11)	1.51 (0.85, 2.66)
≥275.8 mg/dL	3.75 (2.89, 4.86)	2.77 (2.00, 3.83)	2.97 (2.26, 3.91)	2.67 (1.88, 3.78)	1.97 (1.27, 3.06)	1.61 (0.93, 2.78)	1.49 (0.98, 2.27)	1.32 (0.76, 2.32)
Glycated albumin								
No diabetes								
<13.52%	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
13.52-15.56%	0.82 (0.66, 1.02)	1.02 (0.77, 1.37)	0.93 (0.75, 1.16)	1.13 (0.84, 1.52)	0.92 (0.74, 1.14)	1.11 (0.83, 1.50)	0.89 (0.71, 1.11)	1.08 (0.80, 1.45)
≥15.57%	1.59 (1.07, 2.38)	1.57 (1.03, 2.40)	1.31 (0.87, 1.97)	1.52 (0.99, 2.35)	1.17 (0.76, 1.81)	1.32 (0.84, 2.08)	0.96 (0.61, 1.51)	1.22 (0.77, 1.94)
Diagnosed diabetes								
<16.47%	1.87 (1.32, 2.64)	2.35 (1.37, 4.04)	1.59 (1.12, 2.25)	2.04 (1.18, 3.52)	1.50 (1.05, 2.14)	1.90 (1.09, 3.30)	1.41 (0.99, 2.01)	1.84 (1.06, 3.19)
≥16.47%	3.95 (3.05, 5.12)	2.93 (2.10, 4.09)	3.34 (2.55, 4.37)	3.00 (2.10, 4.29)	2.60 (1.68, 4.02)	1.99 (1.15, 3.43)	1.88 (1.22, 2.88)	1.65 (0.94, 2.90)
1,5-AG								
No diabetes								
≥15.0 μg/mL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
7.9-14.9 μg/mL	1.06 (0.87, 1.28)	0.75 (0.54, 1.04)	1.11 (0.91, 1.34)	0.83 (0.60, 1.15)	1.09 (0.90, 1.33)	0.83 (0.59, 1.15)	1.07 (0.88, 1.30)	0.83 (0.60, 1.15)
<7.9 μg/mL	0.93 (0.60, 1.44)	0.65 (0.31, 1.39)	0.87 (0.56, 1.34)	0.65 (0.30, 1.39)	0.77 (0.49, 1.21)	0.52 (0.24, 1.16)	0.66 (0.41, 1.05)	0.44 (0.19, 1.01)
Diagnosed diabetes								
>9.2 μg/mL	1.99 (1.39, 2.86)	1.66 (1.01, 2.74)	1.71 (1.19, 2.46)	1.50 (0.90, 2.49)	1.56 (1.08, 2.26)	1.33 (0.79, 2.24)	1.53 (1.06, 2.20)	1.29 (0.77, 2.16)
≤9.2 μg/mL	3.70 (2.86, 4.77)	2.83 (2.05, 3.92)	3.06 (2.34, 3.99)	2.85 (2.01, 4.03)	2.09 (1.39, 3.13)	1.72 (0.96, 3.09)	1.57 (1.05, 2.36)	1.29 (0.69, 2.42)

Model 1: Adjustment for age, gender (male, female), BMI, BMI-squared

Model 2: Model 1 + LDL-c, HDL-c, triglycerides, cholesterol-lowering medication use (yes, no), systolic blood pressure, antihypertensive medication use (yes, no), eGFR, family history of diabetes (yes, no), education level (less than high school, high school or some college, college or more), alcohol consumption (current, former, never), cigarette smoking status (current, former, never), physical activity level

Model 3: Model 2 + fasting glucose
Model 4: Model 2 + HbA1c

Supplemental Table S3. Associations of biomarkers of hyperglycemia with incident stroke in black and white participants in ARIC

	Model 1		Model 2		Model 3		Model 4	
	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Fasting glucose								
No diabetes								
<100 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)			1 (Ref)	1 (Ref)
100-125 mg/dL	1.12 (0.90, 1.40)	0.91 (0.64, 1.28)	1.03 (0.83, 1.28)	0.85 (0.60, 1.20)			0.97 (0.78, 1.21)	0.79 (0.56, 1.11)
≥126 mg/dL	1.88 (1.28, 2.76)	1.90 (1.22, 2.95)	1.57 (1.06, 2.32)	1.64 (1.05, 2.57)		--	1.07 (0.69, 1.66)	0.94 (0.57, 1.56)
Diagnosed diabetes								
<149 mg/dL	1.81 (1.07, 3.05)	1.29 (0.61, 2.72)	1.56 (0.92, 2.65)	1.19 (0.56, 2.51)			1.24 (0.72, 2.13)	0.83 (0.39, 1.78)
≥149 mg/dL	3.27 (2.19, 4.87)	2.80 (1.84, 4.28)	2.71 (1.79, 4.10)	2.45 (1.57, 3.83)			1.03 (0.56, 1.90)	0.59 (0.31, 1.12)
HbA1c								
No diabetes								
<5.7 %	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)		
5.7-6.4%	1.68 (1.34, 2.10)	1.58 (1.13, 2.22)	1.48 (1.18, 1.85)	1.39 (0.98, 1.96)	1.46 (1.16, 1.84)	1.36 (0.96, 1.92)		
≥6.5%	2.41 (1.49, 3.88)	3.32 (2.14, 5.14)	1.96 (1.21, 3.18)	3.14 (2.01, 4.91)	1.82 (1.06, 3.12)	2.65 (1.65, 4.25)	--	
Diagnosed diabetes								
<7%	1.78 (1.07, 2.97)	1.70 (0.77, 3.73)	1.56 (0.93, 2.61)	1.48 (0.67, 3.27)	1.51 (0.89, 2.55)	1.29 (0.57, 2.89)		
≥7%	3.82 (2.61, 5.57)	4.05 (2.67, 6.16)	3.33 (2.25, 4.93)	3.66 (2.35, 5.71)	2.84 (1.51, 5.35)	2.18 (1.16, 4.10)		
Fructosamine								
No diabetes								
<239.9 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
239.9-268.8	0.90	0.88	0.97	0.93	0.95	0.89	0.92	0.84

mg/dL	(0.70, 1.17)	(0.63, 1.24)	(0.74, 1.26)	(0.65, 1.31)	(0.73, 1.24)	(0.63, 1.26)	(0.70, 1.20)	(0.59, 1.18)
≥268.9 mg/dL	1.99 (1.25, 3.16)	2.03 (1.33, 3.11)	1.81 (1.12, 2.91)	1.84 (1.18, 2.85)	1.64 (0.98, 2.73)	1.47 (0.92, 2.36)	1.19 (0.70, 2.02)	1.06 (0.64, 1.75)
Diagnosed diabetes								
<275.8 mg/dL	1.48 (0.90, 2.46)	0.93 (0.38, 2.29)	1.32 (0.79, 2.20)	0.89 (0.36, 2.20)	1.25 (0.74, 2.11)	0.79 (0.32, 1.95)	1.11 (0.66, 1.87)	0.69 (0.28, 1.70)
≥275.8 mg/dL	3.10 (2.14, 4.49)	2.80 (1.95, 4.04)	2.90 (1.98, 4.27)	2.66 (1.80, 3.93)	2.26 (1.23, 4.14)	1.42 (0.78, 2.60)	1.19 (0.66, 2.16)	0.73 (0.40, 1.33)
Glycated albumin								
No diabetes								
<13.52%	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
13.52-15.56%	0.96 (0.74, 1.25)	1.20 (0.87, 1.65)	1.08 (0.83, 1.41)	1.36 (0.99, 1.89)	1.06 (0.81, 1.39)	1.32 (0.95, 1.83)	1.01 (0.77, 1.32)	1.23 (0.88, 1.70)
≥15.57%	1.74 (1.05, 2.89)	1.97 (1.26, 3.08)	1.60 (0.96, 2.65)	1.90 (1.20, 3.01)	1.40 (0.80, 2.42)	1.50 (0.91, 2.45)	0.94 (0.53, 1.68)	1.03 (0.61, 1.75)
Diagnosed diabetes								
<16.47%	1.57 (0.96, 2.57)	1.20 (0.52, 2.74)	1.40 (0.85, 2.30)	1.17 (0.51, 2.69)	1.31 (0.79, 2.17)	1.04 (0.45, 2.41)	1.15 (0.69, 1.91)	0.93 (0.40, 2.14)
≥16.47%	3.04 (2.09, 4.42)	3.07 (2.10, 4.49)	2.86 (1.94, 4.22)	3.03 (2.03, 4.53)	2.09 (1.13, 3.88)	1.54 (0.82, 2.88)	1.05 (0.57, 1.94)	0.75 (0.40, 1.40)
1,5-AG								
No diabetes								
≥15.0 μg/mL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
7.9-14.9 μg/mL	1.12 (0.88, 1.43)	0.73 (0.50, 1.05)	1.18 (0.92, 1.51)	0.83 (0.57, 1.21)	1.17 (0.91, 1.49)	0.82 (0.56, 1.20)	1.13 (0.89, 1.45)	0.82 (0.56, 1.19)
<7.9 μg/mL	1.77 (1.15, 2.73)	1.90 (1.13, 3.20)	1.78 (1.15, 2.75)	2.05 (1.21, 3.48)	1.63 (1.04, 2.57)	1.69 (0.95, 2.98)	1.29 (0.80, 2.08)	1.06 (0.56, 1.99)
Diagnosed diabetes								
>9.2 μg/mL	1.59 (0.94, 2.68)	0.74 (0.33, 1.68)	1.45 (0.86, 2.45)	0.69 (0.30, 1.57)	1.35 (0.79, 2.30)	0.60 (0.26, 1.39)	1.25 (0.73, 2.12)	0.52 (0.23, 1.20)
≤9.2 μg/mL	3.05 (2.11, 4.40)	2.98 (2.08, 4.28)	2.77 (1.89, 4.05)	2.98 (2.03, 4.36)	2.04 (1.17, 3.53)	1.82 (0.99, 3.36)	1.20 (0.69, 2.09)	0.83 (0.44, 1.58)

Model 1: Adjustment for age, gender (male, female), BMI, BMI-squared

Model 2: Model 1 + LDL-c, HDL-c, triglycerides, cholesterol-lowering medication use (yes, no), systolic blood pressure, antihypertensive medication use (yes, no), eGFR, family history of diabetes (yes, no), education level (less than high school, high school or some college, college or more), alcohol consumption (current, former, never), cigarette smoking status (current, former, never), physical activity level

Model 3: Model 2 + fasting glucose
Model 4: Model 2 + HbA1c

Supplemental Table S4. Associations of biomarkers of hyperglycemia with incident heart failure in black and white participants in ARIC

	Model 1		Model 2		Model 3		Model 4	
	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)	White (N=8,522)	Black (N=2,581)
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Fasting glucose								
No diabetes								
<100 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)			1 (Ref)	1 (Ref)
100-125 mg/dL	1.13 (0.97, 1.31)	0.95 (0.75, 1.22)	1.03 (0.89, 1.20)	0.88 (0.69, 1.12)			0.99 (0.85, 1.14)	0.85 (0.66, 1.08)
≥126 mg/dL	1.47 (1.13, 1.92)	1.15 (0.81, 1.65)	1.22 (0.93, 1.59)	0.98 (0.68, 1.41)		--	0.87 (0.65, 1.17)	0.79 (0.54, 1.17)
Diagnosed diabetes								
<149 mg/dL	2.03 (1.47, 2.80)	2.48 (1.65, 3.74)	1.59 (1.15, 2.21)	2.20 (1.45, 3.33)			1.33 (0.95, 1.85)	1.93 (1.27, 2.95)
≥149 mg/dL	3.83 (3.00, 4.89)	3.58 (2.69, 4.76)	3.09 (2.40, 3.97)	3.16 (2.33, 4.29)			1.39 (0.96, 2.01)	1.83 (1.16, 2.89)
HbA1c								
No diabetes								
<5.7 %	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)		
5.7-6.4%	1.48 (1.28, 1.72)	1.31 (1.03, 1.66)	1.27 (1.09, 1.48)	1.13 (0.89, 1.43)	1.25 (1.07, 1.46)	1.11 (0.87, 1.41)		
≥6.5%	2.11 (1.55, 2.87)	1.54 (1.08, 2.19)	1.72 (1.26, 2.35)	1.35 (0.94, 1.94)	1.52 (1.07, 2.14)	1.18 (0.81, 1.72)	--	
Diagnosed diabetes								
<7%	1.76 (1.28, 2.43)	2.57 (1.66, 3.96)	1.40 (1.01, 1.93)	2.17 (1.40, 3.37)	1.32 (0.95, 1.83)	1.98 (1.26, 3.10)		
≥7%	4.81 (3.83, 6.03)	4.55 (3.46, 5.98)	4.05 (3.21, 5.13)	4.07 (3.03, 5.46)	3.14 (2.14, 4.62)	2.76 (1.81, 4.20)		
Fructosamine								
No diabetes								
<239.9 mg/dL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
239.9-268.8	0.94	0.77	1.04	0.78	1.00	0.75	0.98	0.75

mg/dL	(0.79, 1.12)	(0.60, 0.99)	(0.87, 1.24)	(0.60, 1.01)	(0.84, 1.20)	(0.58, 0.97)	(0.82, 1.18)	(0.58, 0.96)
≥268.9 mg/dL	1.29 (0.89, 1.87)	1.42 (1.01, 2.00)	1.21 (0.83, 1.76)	1.16 (0.82, 1.65)	0.98 (0.65, 1.46)	0.94 (0.65, 1.36)	0.83 (0.55, 1.26)	0.95 (0.65, 1.38)
Diagnosed diabetes								
<275.8 mg/dL	1.62 (1.20, 2.19)	2.59 (1.74, 3.85)	1.38 (1.02, 1.87)	2.35 (1.57, 3.50)	1.25 (0.91, 1.70)	2.15 (1.44, 3.22)	1.20 (0.88, 1.63)	2.15 (1.44, 3.22)
≥275.8 mg/dL	3.90 (3.12, 4.87)	3.32 (2.59, 4.24)	3.48 (2.76, 4.39)	3.12 (2.39, 4.07)	2.18 (1.49, 3.19)	1.82 (1.21, 2.74)	1.69 (1.17, 2.44)	1.87 (1.24, 2.84)
Glycated albumin								
No diabetes								
<13.52%	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
13.52-15.56%	0.88 (0.73, 1.06)	0.93 (0.74, 1.18)	1.00 (0.83, 1.20)	0.97 (0.76, 1.23)	0.97 (0.81, 1.18)	0.94 (0.74, 1.19)	0.95 (0.79, 1.14)	0.93 (0.73, 1.17)
≥15.57%	1.66 (1.20, 2.31)	1.31 (0.93, 1.87)	1.58 (1.14, 2.20)	1.16 (0.81, 1.66)	1.36 (0.95, 1.94)	0.95 (0.65, 1.39)	1.14 (0.78, 1.65)	0.95 (0.65, 1.40)
Diagnosed diabetes								
<16.47%	1.61 (1.19, 2.17)	2.58 (1.73, 3.85)	1.34 (0.99, 1.81)	2.33 (1.56, 3.50)	1.24 (0.91, 1.69)	2.16 (1.44, 3.25)	1.18 (0.87, 1.61)	2.16 (1.43, 3.25)
≥16.47%	3.99 (3.19, 4.98)	3.53 (2.74, 4.54)	3.68 (2.93, 4.64)	3.43 (2.62, 4.49)	2.65 (1.81, 3.86)	2.03 (1.34, 3.09)	2.00 (1.38, 2.92)	2.08 (1.35, 3.21)
1,5-AG								
No diabetes								
≥15.0 μg/mL	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
7.9-14.9 μg/mL	1.10 (0.94, 1.30)	0.81 (0.63, 1.05)	1.15 (0.97, 1.36)	0.92 (0.72, 1.19)	1.13 (0.96, 1.33)	0.92 (0.71, 1.19)	1.11 (0.94, 1.31)	0.92 (0.71, 1.19)
<7.9 μg/mL	0.99 (0.69, 1.43)	0.71 (0.40, 1.28)	1.02 (0.70, 1.47)	0.76 (0.42, 1.36)	0.87 (0.59, 1.27)	0.60 (0.33, 1.11)	0.76 (0.51, 1.12)	0.61 (0.33, 1.13)
Diagnosed diabetes								
>9.2 μg/mL	1.67 (1.21, 2.30)	2.20 (1.53, 3.15)	1.37 (0.99, 1.89)	2.01 (1.40, 2.90)	1.22 (0.88, 1.70)	1.80 (1.24, 2.61)	1.20 (0.87, 1.67)	1.84 (1.27, 2.67)
≤9.2 μg/mL	3.70 (2.97, 4.61)	3.56 (2.78, 4.56)	3.34 (2.66, 4.19)	3.68 (2.82, 4.79)	2.07 (1.47, 2.92)	2.21 (1.43, 3.42)	1.65 (1.17, 2.33)	2.30 (1.46, 3.64)

Model 1: Adjustment for age, gender (male, female), BMI, BMI-squared

Model 2: Model 1 + LDL-c, HDL-c, triglycerides, cholesterol-lowering medication use (yes, no), systolic blood pressure, antihypertensive medication use (yes, no), eGFR, family history of diabetes (yes, no), education level (less than high school, high school or some college, college or more), alcohol consumption (current, former, never), cigarette smoking status (current, former, never), physical activity level

Model 3: Model 2 + fasting glucose
Model 4: Model 2 + HbA1c

Supplemental Table S5. Associations of biomarkers of hyperglycemia with incident end-stage renal disease in black and white participants in ARIC

	Model 1		Model 2		Model 3		Model 4	
	White (N=8,521)	Black (N=2,579)	White (N=8,521)	Black (N=2,579)	White (N=8,521)	Black (N=2,579)	White (N=8,521)	Black (N=2,579)
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Fasting glucose, mg/dL								
No diabetes								
<100	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)			1 (Ref)	1 (Ref)
100-125	1.80 (0.9, 3.5)	1.21 (0.6, 2.5)	1.41 (0.7, 2.8)	1.22 (0.6, 2.5)			1.28 (0.7, 2.5)	1.15 (0.6, 2.4)
≥126	4.03 (1.6, 10.1)	2.79 (1.2, 6.5)	2.93 (1.1, 7.5)	3.03 (1.3, 7.2)		--	1.53 (0.6, 4.2)	2.30 (0.9, 5.7)
Diagnosed diabetes								
<149	8.90 (3.6, 21.9)	7.00 (2.9, 17.1)	6.74 (2.6, 17.2)	7.69 (3.1, 19.4)			4.22 (1.6, 11.2)	6.46 (2.5, 16.5)
≥149	15.67 (7.2, 34.0)	12.52 (6.2, 25.2)	11.37 (4.9, 26.4)	15.91 (7.4, 34.4)			2.01 (0.7, 6.3)	7.18 (2.5, 20.4)
HbA1c, %								
No diabetes								
<5.7	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)		
5.7-6.4	3.16 (1.8, 5.6)	1.35 (0.7, 2.7)	2.40 (1.3, 4.3)	1.34 (0.7, 2.7)	2.32 (1.3, 4.2)	1.30 (0.7, 2.6)		
≥6.5	5.62 (2.1, 15.1)	3.47 (1.6, 7.6)	5.01 (1.8, 13.9)	3.96 (1.8, 8.8)	3.86 (1.3, 11.5)	3.32 (1.5, 7.6)	--	
Diagnosed diabetes								
<7	5.05 (1.9, 13.3)	6.20 (2.5, 15.4)	4.2 (1.6, 11.3)	6.79 (2.6, 17.6)	3.63 (1.3, 10.0)	6.10 (2.3, 15.9)		
≥7	20.99 (11.3, 39.1)	13.70 (7.3, 25.6)	18.04 (9.0, 36.0)	17.91 (8.9, 36.2)	10.88 (3.8, 30.8)	10.22 (4.2, 25.1)		
Fructosamine, mg/dL								
No diabetes								
<239.9	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
239.9-268.8	0.94	0.61	0.91	0.57	0.84	0.53	0.83	0.52

	(0.5, 1.9)	(0.3, 1.4)	(0.5, 1.9)	(0.3, 1.3)	(0.4, 1.7)	(0.2, 1.2)	(0.4, 1.7)	(0.2, 1.2)
≥268.9	2.17 (0.7, 7.0)	3.41 (1.7, 6.9)	1.04 (0.3, 3.7)	2.54 (1.2, 5.2)	0.79 (0.2, 2.8)	1.95 (0.9, 4.1)	0.57 (0.2, 2.1)	1.84 (0.9, 4.0)
Diagnosed diabetes								
<275.8	3.99 (1.8, 9.1)	6.26 (2.9, 13.3)	3.86 (1.7, 8.9)	6.55 (3.0, 14.4)	3.05 (1.3, 7.2)	5.92 (2.7, 13.0)	2.80 (1.2, 6.6)	5.77 (2.6, 12.8)
≥275.8	11.10 (6.3, 19.7)	9.29 (5.6, 15.5)	8.42 (4.4, 16.1)	10.82 (6.0, 19.5)	2.67 (0.9, 7.6)	4.94 (2.2, 11.3)	1.29 (0.5, 3.6)	4.43 (1.8, 10.8)
Glycated albumin, %								
No diabetes								
<13.52	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
13.52-15.56	1.55 (0.8, 2.9)	0.84 (0.4, 1.6)	1.63 (0.9, 3.1)	0.93 (0.5, 1.8)	1.53 (0.8, 2.9)	0.91 (0.5, 1.8)	1.45 (0.8, 2.8)	0.88 (0.5, 1.7)
≥15.57	3.14 (1.1, 8.9)	2.19 (1.0, 4.9)	3.06 (1.1, 8.8)	1.95 (0.9, 4.4)	2.21 (0.7, 6.7)	1.59 (0.7, 3.7)	1.35 (0.4, 4.3)	1.53 (0.7, 3.6)
Diagnosed diabetes								
<16.47	4.42 (1.9, 10.1)	4.49 (1.9, 10.4)	4.17 (1.8, 9.7)	4.50 (1.9, 10.8)	3.37 (1.4, 8.0)	4.12 (1.7, 9.9)	2.98 (1.3, 7.1)	4.08 (1.7, 9.8)
≥16.47	12.83 (7.2, 22.9)	9.91 (5.9, 16.6)	11.19 (5.9, 21.4)	12.92 (7.1, 23.6)	5.07 (1.9, 13.9)	6.96 (3.0, 16.1)	2.22 (0.8, 6.1)	6.36 (2.5, 16.2)
1,5-AG, µg/mL								
No diabetes								
≥15.0	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
7.9-14.9	1.03 (0.5, 2.1)	0.63 (0.3, 1.3)	1.06 (0.5, 2.1)	0.67 (0.3, 1.4)	1.02 (0.5, 2.1)	0.67 (0.3, 1.4)	0.98 (0.5, 2.0)	0.66 (0.3, 1.4)
<7.9	2.16 (0.8, 6.1)	1.26 (0.4, 4.1)	1.49 (0.5, 4.5)	1.48 (0.5, 4.9)	1.20 (0.4, 3.7)	1.18 (0.4, 4.0)	0.89 (0.3, 2.8)	1.16 (0.3, 4.0)
Diagnosed diabetes								
>9.2	4.17 (1.8, 9.9)	3.63 (1.7, 7.8)	3.65 (1.5, 8.8)	3.97 (1.8, 8.9)	2.87 (1.2, 7.0)	3.57 (1.6, 8.1)	2.77 (1.1, 6.7)	3.54 (1.6, 8.0)
≤9.2	10.61 (6.0, 18.9)	9.38 (5.8, 15.2)	8.99 (4.8, 16.8)	11.74 (6.7, 20.6)	3.67 (1.4, 9.4)	6.24 (2.7, 14.2)	1.75 (0.7, 4.5)	5.80 (2.3, 14.6)

Model 1: Adjustment for age, gender (male, female), BMI, BMI-squared

Model 2: Model 1 + LDL-c, HDL-c, triglycerides, cholesterol-lowering medication use (yes, no), systolic blood pressure, antihypertensive medication use (yes, no), eGFR, family history of diabetes (yes, no), education level (less than high school, high school or some college, college or more), alcohol consumption (current, former, never), cigarette smoking status (current, former, never), physical activity level

Model 3: Model 2 + fasting glucose
Model 4: Model 2 + HbA1c

Appendix C: Supplemental Material for Chapter 4

Supplemental Table S1. Association of hs-CRP measured at visit 2 (1990-92) and visit 4 (1996-98) with incident diabetes, incident cardiovascular events and all-cause mortality, with different follow-up and additional adjustment

	Visit 2 hs-CRP (beginning follow-up at Visit 2)		Visit 4 hs-CRP (additionally adjusting for visit 2 hs-CRP)	
	Events/Total N (%)	**HR (95% CI)**	**Events/Total N (%)**	**HR (95% CI)**
Diabetes				
≥3 mg/L	1,471/4,210 (35%)	1.21 (1.12, 1.31)	1,122/3,617 (31%)	1.47 (1.33, 1.63)
<3 mg/L	1,677/7,224 (23%)	1 (Reference)	979/5,131 (19%)	1 (Reference)
CHD				
≥3 mg/L	557/4,417 (13%)	1.27 (1.12, 1.44)	321/3,702 (9%)	1.31 (1.08, 1.58)
<3 mg/L	615/7,262 (8%)	1 (Reference)	316/5,081 (6%)	1 (Reference)
Fatal CHD				
≥3 mg/L	184/4,417 (4%)	1.46 (1.15, 1.84)	88/3,702 (2%)	2.03 (1.37, 3.02)
<3 mg/L	150/7,262 (2%)	1 (Reference)	58/5,081 (1%)	1 (Reference)
Ischemic stroke				
≥3 mg/L	321/4,417 (7%)	1.39 (1.17, 1.65)	190/3,702 (5%)	1.25 (0.97, 1.60)
<3 mg/L	316/7,262 (4%)	1 (Reference)	176/5,081 (3%)	1 (Reference)
Heart failure				
≥3 mg/L	877/4,417 (20%)	1.38 (1.24, 1.54)	529/3,702 (14%)	1.35 (1.15, 1.58)
<3 mg/L	747/7,262 (10%)	1 (Reference)	409/5,081 (8%)	1 (Reference)
Mortality				
≥3 mg/L	1,935/5,185 (37%)	1.35 (1.26, 1.45)	1,227/4,432 (28%)	1.30 (1.18, 1.43)
<3 mg/L	1,938/7,882 (25%)	1 (Reference)	1,156/5,728 (20%)	1 (Reference)

For analyses starting follow-up at visit 2: N=11,434 for diabetes analyses, N=11,679 for CVD analyses, N=13,067 for mortality analyses

For analyses of visit 4 hs-CRP (starting follow-up at visit 4), the following number of participants were included in analyses: N=8,748 for diabetes analyses, N=8,783 for CVD analyses and N=10,160 for mortality analyses

Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). For analyses of visit 2 hs-CRP, all covariates were visit 2 values, except for physical activity and education, which were measured at visit 1. For analyses of visit 4 hs-CRP, all covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

Supplemental Table S2. Association of hs-CRP measured at visit 2 (1990-92) and visit 4 (1996-98) with incident diabetes, incident cardiovascular events and all-cause mortality, excluding persons with hs-CRP >10 mg/L

	Visit 2 hs-CRP		Visit 4 hs-CRP	
	Events/Total N (%)	HR (95% CI)	Events/Total N (%)	HR (95% CI)
Diabetes				
≥3 mg/L	971/3,376 (29%)	1.13 (1.02, 1.25)	1,115/3,824 (29%)	1.45 (1.31, 1.60)
<3 mg/L	830/4,399 (19%)	1 (Reference)	686/3,951 (17%)	1 (Reference)
CHD				
≥3 mg/L	188/2,161 (9%)	1.28 (1.06, 1.55)	228/2,766 (8%)	1.34 (1.12, 1.61)
<3 mg/L	346/5,594 (6%)	1 (Reference)	306/4,989 (6%)	1 (Reference)
Fatal CHD				
≥3 mg/L	46/2,161 (2%)	1.36 (0.92, 2.00)	65/2,766 (2%)	2.11 (1.44, 3.10)
<3 mg/L	75/5,594 (1%)	1 (Reference)	56/4,989 (1%)	1 (Reference)
Ischemic stroke				
≥3 mg/L	111/2,161 (5%)	1.39 (1.08, 1.78)	127/2,766 (5%)	1.33 (1.04, 1.70)
<3 mg/L	186/5,594 (3%)	1 (Reference)	170/4,989 (3%)	1 (Reference)
Heart failure				
≥3 mg/L	296/2,161 (14%)	1.28 (1.10, 1.50)	356/2,766 (13%)	1.42 (1.22, 1.66)
<3 mg/L	455/5,594 (8%)	1 (Reference)	395/4,989 (8%)	1 (Reference)
Mortality				
≥3 mg/L	1,045/4,064 (26%)	1.23 (1.11, 1.36)	1,074/4,481 (24%)	1.34 (1.21, 1.47)
<3 mg/L	903/4,801 (19%)	1 (Reference)	874/4,384 (20%)	1 (Reference)

N=7,775 for analyses of diabetes, N=7,755 for analyses of CVD, N=8,865 for mortality analyses

Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

Supplemental Table S3. Association of hs-CRP measured at visit 2 (1990-92) and visit 4 (1996-98) with incident diabetes, incident cardiovascular events and all-cause mortality, using a cutoff of 2 mg/L

	Visit 2 hs-CRP		Visit 4 hs-CRP	
	Events/Total N (%)	HR (95% CI)	Events/Total N (%)	HR (95% CI)
Diabetes				
≥2 mg/L	1,252/4,287 (29%)	1.25 (1.14,1.37)	1,405/4,739 (30%)	1.49 (1.35, 1.64)
<2 mg/L	849/4,461 (19%)	1 (Reference)	606/4,009 (17%)	1 (Reference)
CHD				
≥2 mg/L	371/4,380 (8%)	1.24 (1.05, 1.47)	391/4,811 (8%)	1.25 (1.05, 1.48)
<2 mg/L	266/4,403 (6%)	1 (Reference)	246/3,972 (6%)	1 (Reference)
Fatal CHD				
≥2 mg/L	96/4,380 (2%)	1.64 (1.14, 2.36)	99/4,811 (2%)	1.64 (1.13, 2.37)
<2 mg/L	50/4,403 (1%)	1 (Reference)	47/3,972 (1%)	1 (Reference)
Ischemic stroke				
≥2 mg/L	223/4,380 (5%)	1.37 (1.09, 1.71)	242/4,811 (5%)	1.55 (1.23, 1.95)
<2 mg/L	143/4,403 (3%)	1 (Reference)	124/3,972 (3%)	1 (Reference)
Heart failure				
≥2 mg/L	589/4,380 (13%)	1.20 (1.04, 1.39)	619/4,811 (13%)	1.27 (1.10, 1.47)
<2 mg/L	349/4,403 (8%)	1 (Reference)	319/3,972 (8%)	1 (Reference)
Mortality				
≥2 mg/L	1,462/5,289 (28%)	1.30 (1.19, 1.42)	1,485/5,706 (26%)	1.27 (1.16, 1.38)
<2 mg/L	921/4,871 (19%)	1 (Reference)	898/4,454 (20%)	1 (Reference)

N=8,748 for diabetes analyses; N=8,783 for CVD analyses and N=10,160 for mortality analyses

Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

Supplemental Table S4. Association of hs-CRP measured at visit 2 (1990-92) and visit 4 (1996-98) and six-year change in hs-CRP with incident diabetes, excluding persons with prevalent undiagnosed diabetes at visit 4

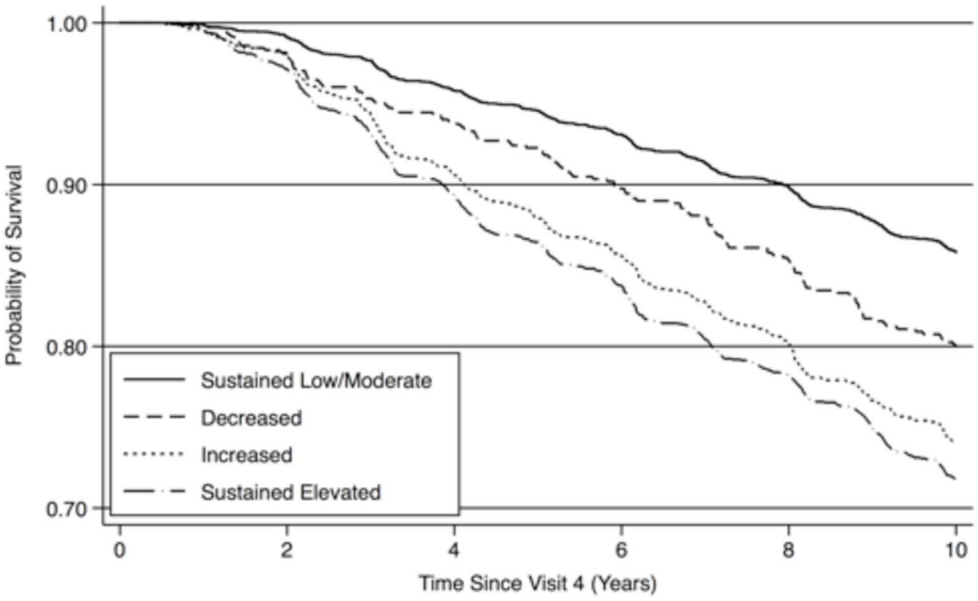
	Events/Total N (%)	HR (95% CI)
Visit 2 hs-CRP		
≥3 mg/L	682/2,699 (25%)	1.10 (0.98, 1.22)
<3 mg/L	979/5,442 (18%)	1 (Reference)
Visit 4 hs-CRP		
≥3 mg/L	859/3,278 (26%)	1.37 (1.23, 1.52)
<3 mg/L	802/4,863 (16%)	1 (Reference)
Six-year change in hs-CRP		
Sustained elevated	558/2,036 (27%)	1.32 (1.16, 1.50)
Increased	301/1,242 (24%)	1.46 (1.27, 1.67)
Decreased	124/663 (19%)	1.01 (0.83, 1.22)
Sustained low/moderate	678/4,200 (16%)	1 (Reference)

N=8,141

Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL cholesterol, total cholesterol, body mass index, prevalent CVD. All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1.

Supplemental Figure S1. Kaplan-Meier graphs of incident diabetes, incident cardiovascular events, and all-cause mortality by change in high-sensitivity C-reactive protein. We used Kaplan-Meier graphs to compare the survival function of each endpoint across categories of six-year change in hs-CRP. Graphs are truncated at 10 years of follow-up.

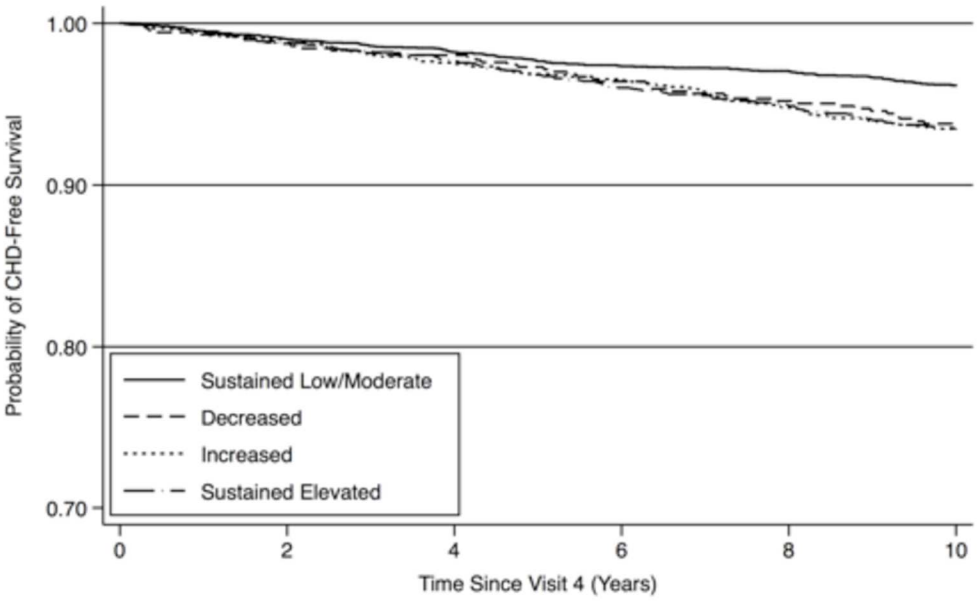
A. Incident diabetes



Number at risk

Sustained Low/Moderate	4419	4359	4131	3910	3651	3351
Decreased	712	692	648	600	550	487
Increased	1353	1319	1191	1102	988	876
Sustained Elevated	2264	2182	1963	1764	1561	1350

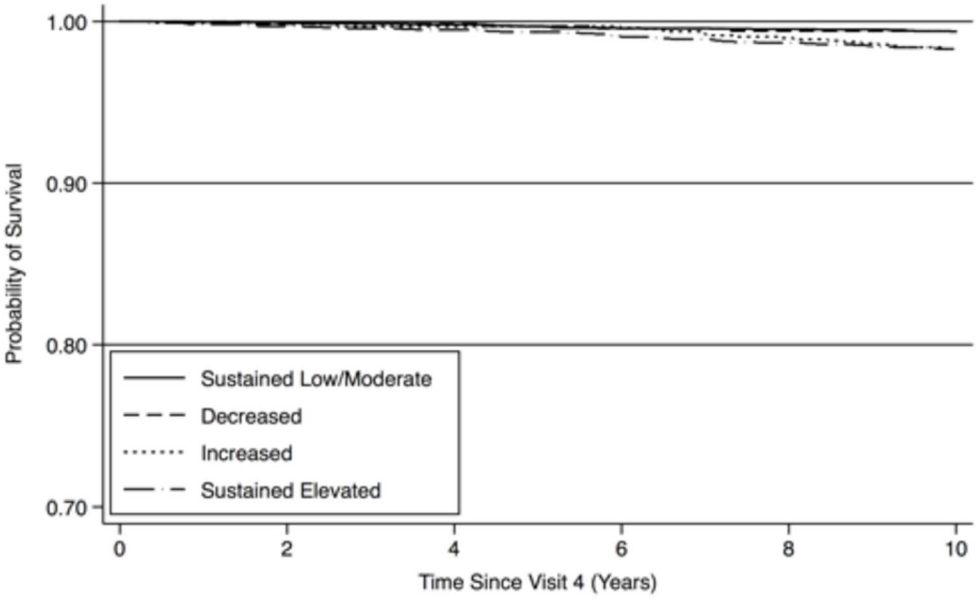
B. Incident coronary heart disease



Number at risk

Sustained Low/Moderate	4364	4288	4184	4071	3961	3808
Decreased	717	698	678	643	622	582
Increased	1333	1303	1254	1225	1173	1112
Sustained Elevated	2369	2315	2252	2150	2060	1948

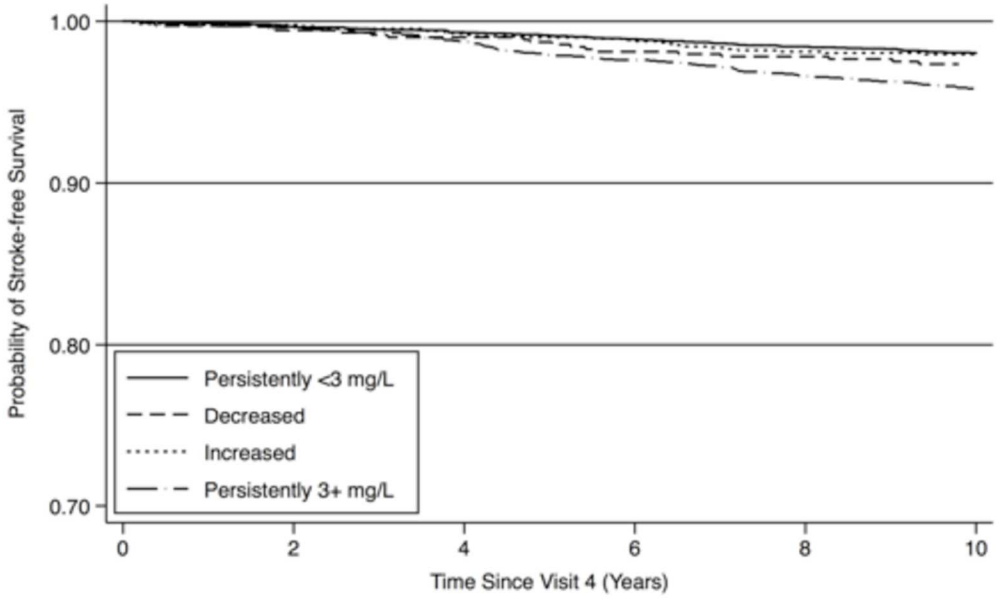
C. Fatal coronary heart disease



Number at risk

Sustained Low/Moderate	4364	4327	4249	4161	4059	3937
Decreased	717	706	691	665	649	616
Increased	1333	1314	1281	1263	1223	1168
Sustained Elevated	2369	2333	2291	2211	2133	2033

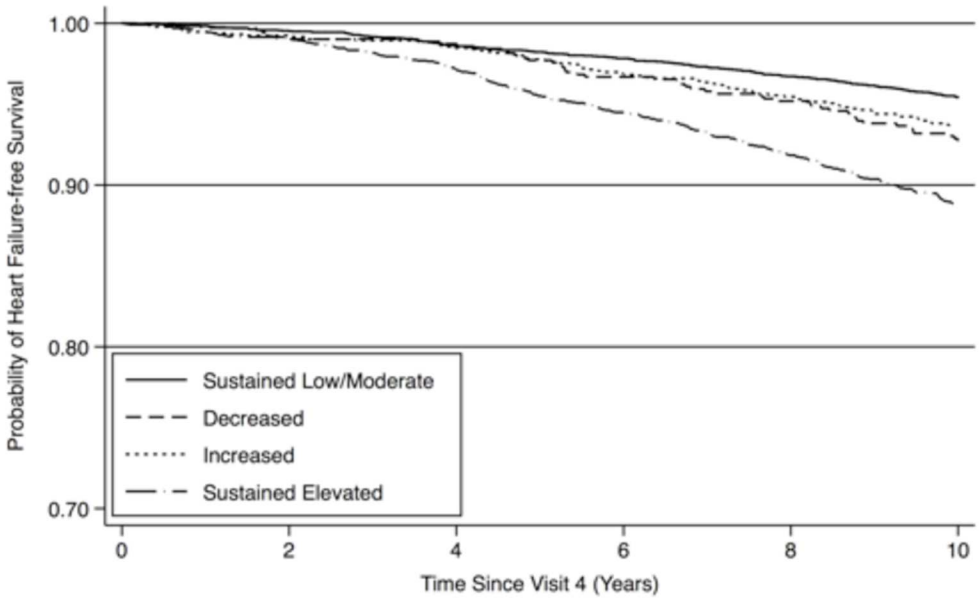
D. Incident ischemic stroke



Number at risk

Remains Low/Medium	4364	4312	4223	4123	4009	3874
High to Low/Medium	717	702	684	656	638	602
Low/Medium to High	1333	1312	1273	1251	1205	1149
Remains High	2369	2325	2267	2171	2084	1973

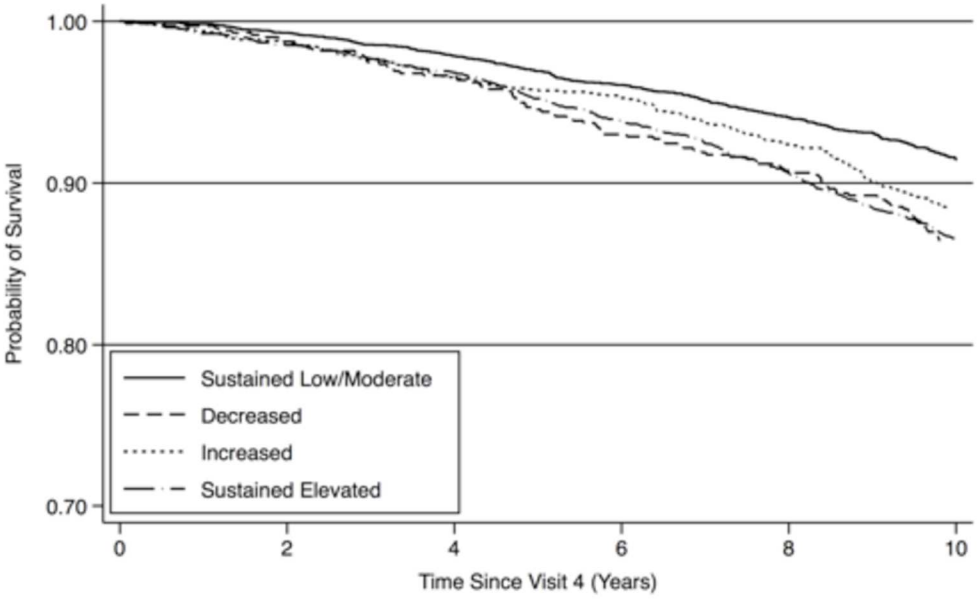
E. Incident heart failure



Number at risk

Sustained Low/Moderate	4364	4309	4206	4099	3972	3815
Decreased	717	702	686	653	629	588
Increased	1333	1306	1264	1227	1176	1116
Sustained Elevated	2369	2315	2239	2123	2016	1887

F. All-cause mortality

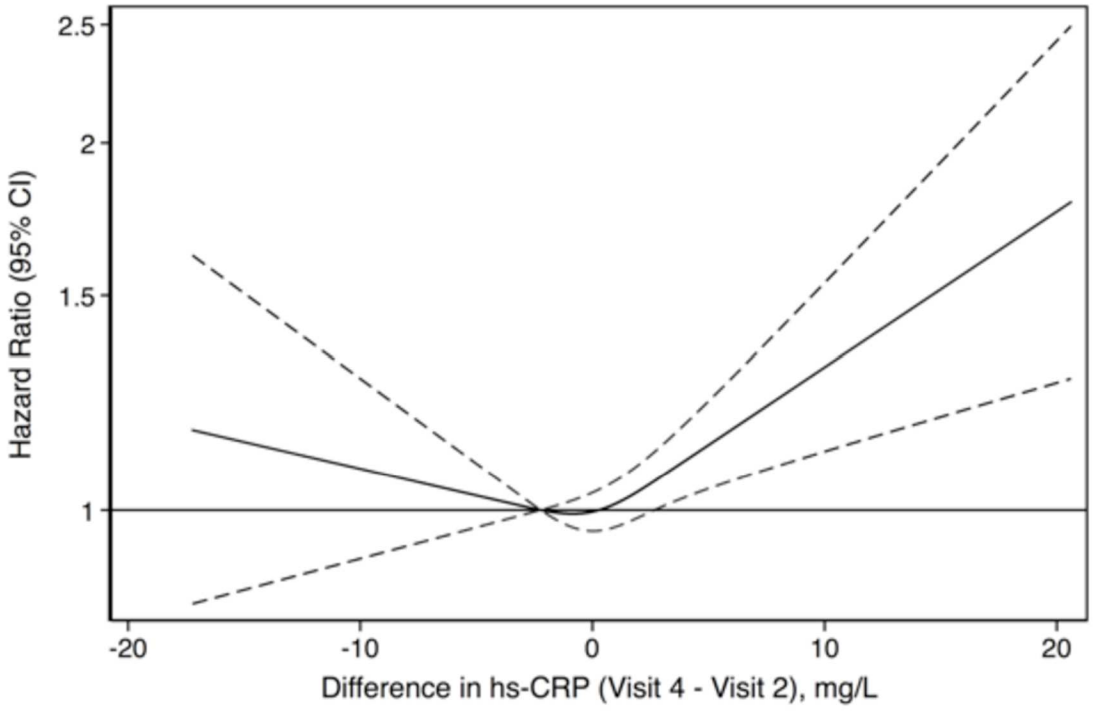


Number at risk

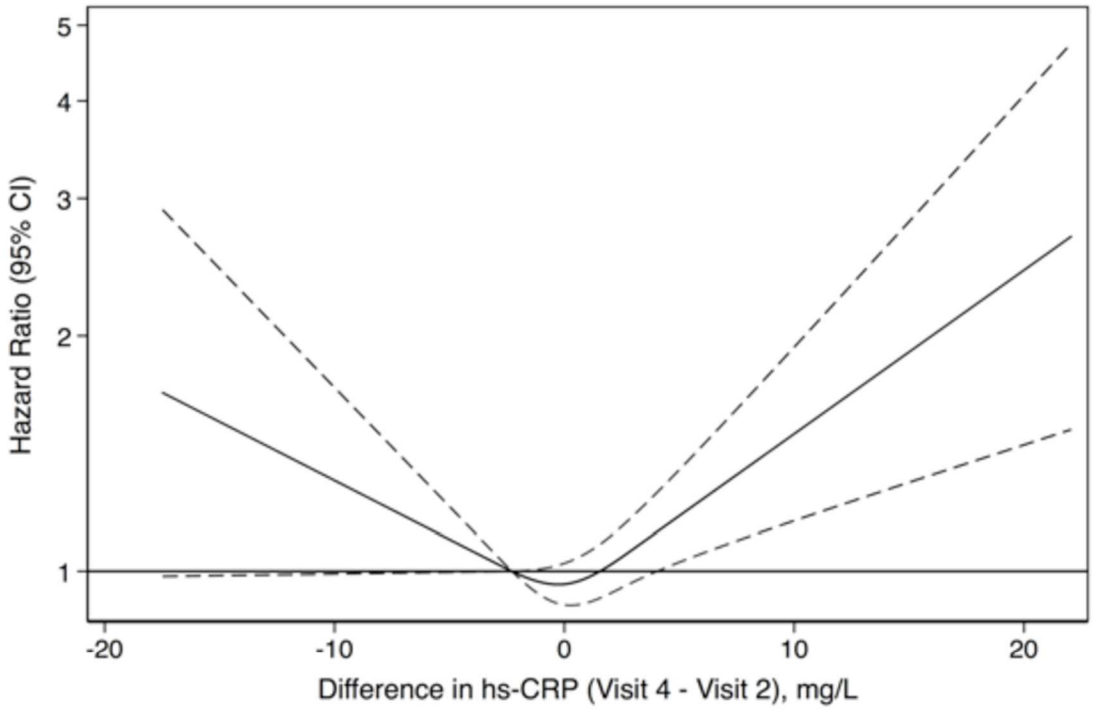
Sustained Low/Moderate	4364	4327	4249	4161	4059	3937
Decreased	717	706	691	665	649	616
Increased	1333	1314	1281	1263	1223	1168
Sustained Elevated	2369	2333	2291	2211	2133	2033

Supplemental Figure S2. Restricted cubic splines of the association of the difference in hs-CRP with risk of diabetes, cardiovascular disease, and mortality. We present restricted cubic splines with knots at -3, 0, and 3 mg/L, centered at the 10th percentile. Cox proportional hazards models were adjusted for the following covariates: age, gender, race-center, education level, alcohol consumption, smoking status, physical activity (Baecke sport activity index), systolic blood pressure, blood pressure-lowering medication, cholesterol-lowering medication, HDL-cholesterol, total cholesterol, body mass index, prevalent diabetes (for analyses of non-diabetes outcomes), prevalent CVD (for analyses of non-CVD outcomes). All covariates were visit 4 values, except for physical activity and education, which were measured at visit 1. All variables were centered at the mean. Dashed lines indicate the 95% confidence limits. Graphs are truncated at the 1st and 99th percentiles of the distribution of the difference in hs-CRP.

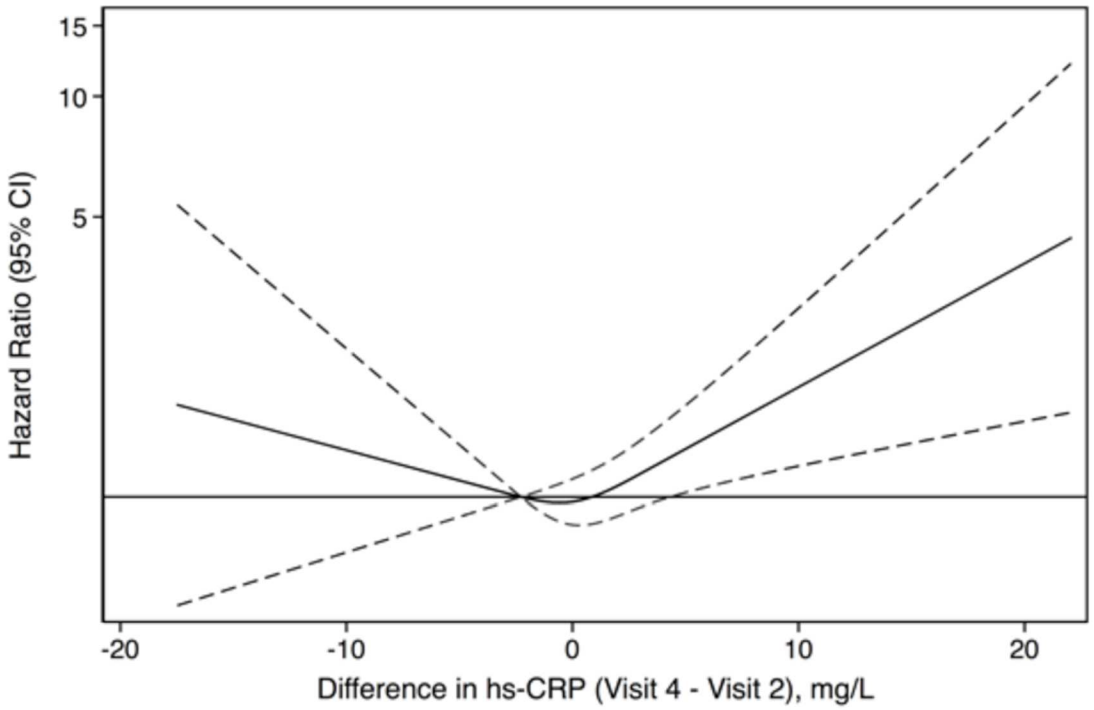
A. Diabetes



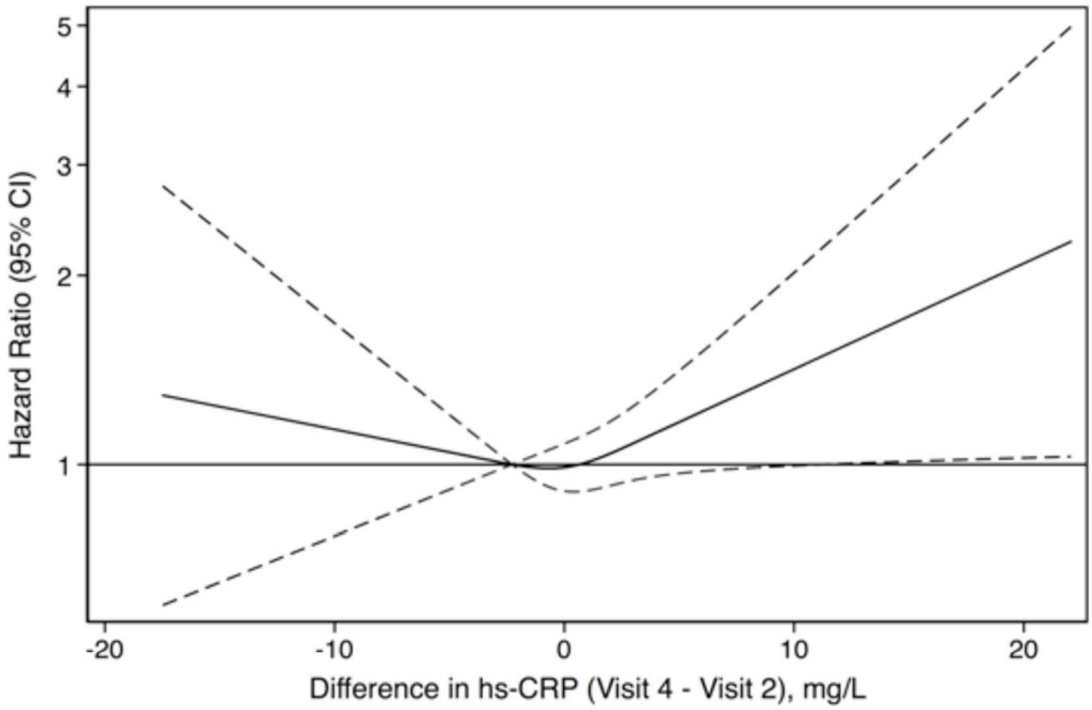
B. CHD



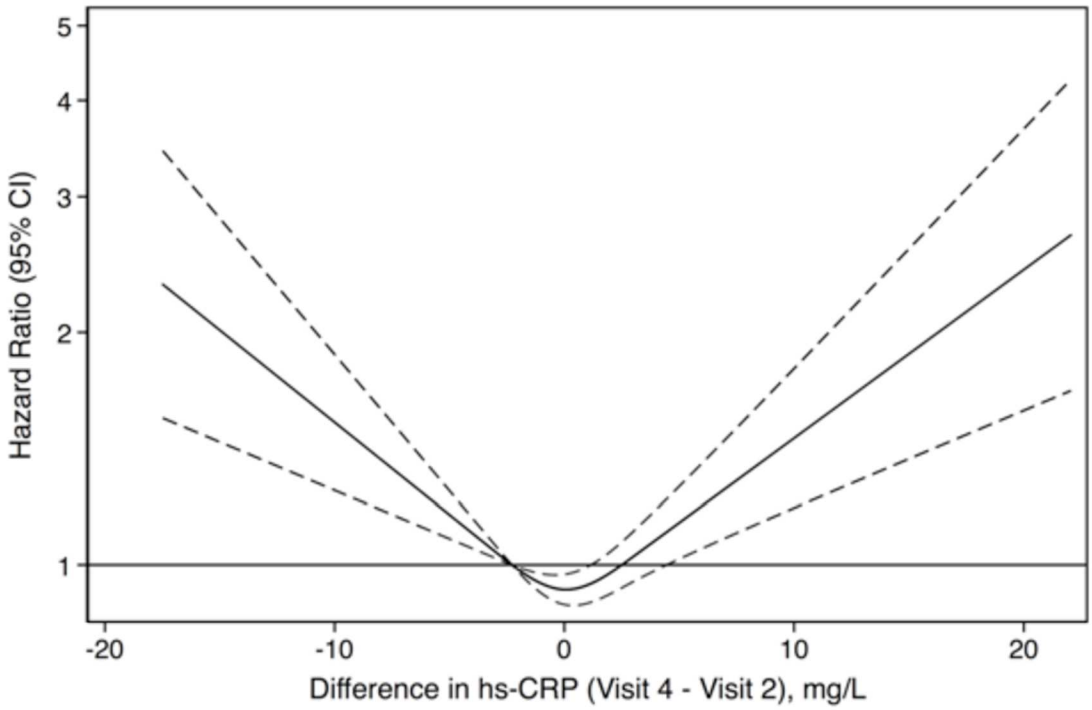
C. Fatal CHD



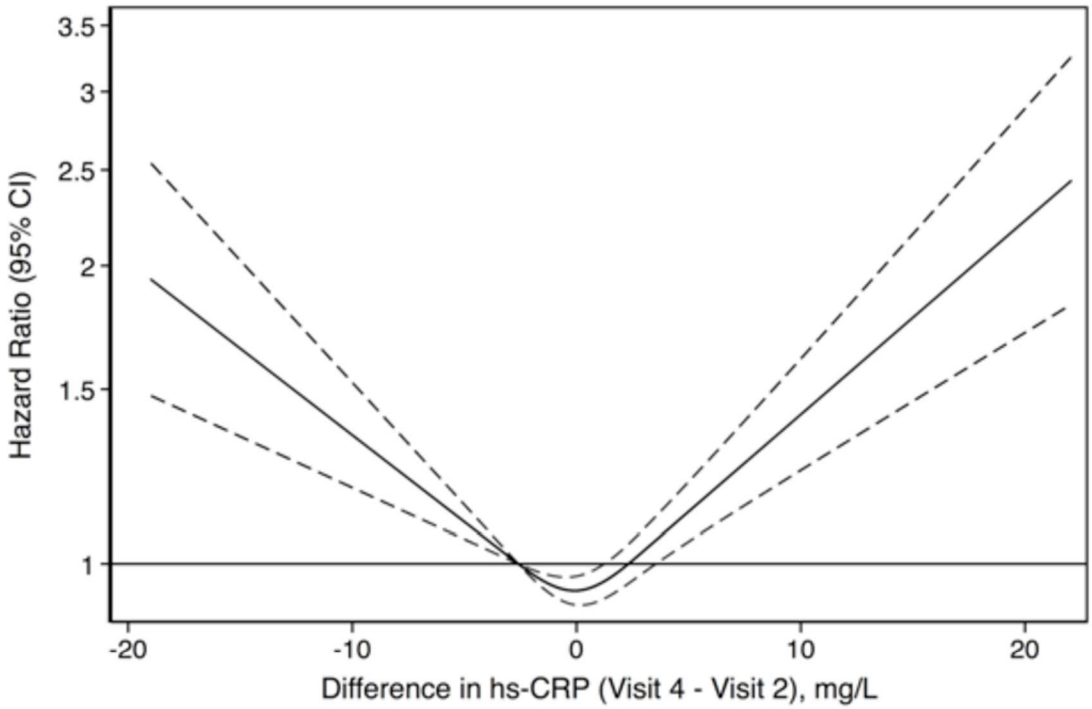
D. Ischemic Stroke



E. Heart failure



F. Mortality


3.5
3
2.5
2
1.5
1
Hazard Ratio (95% CI)
-20
-10
0
10
20
Difference in hs-CRP (Visit 4 - Visit 2), mg/L

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EDUCATION

2012-2015 (expected) Doctor of Philosophy (PhD), Epidemiology
Area of concentration: Cardiovascular epidemiology
Johns Hopkins Bloomberg School of Public Health
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Thesis: The Epidemiology of Nontraditional Biomarkers of Hyperglycemia and their Prognostic Value
Advisor: Elizabeth Selvin, PhD, MPH

2008-2010 Master of Public Health (MPH), Epidemiology
Columbia University Mailman School of Public Health
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Thesis: Seasonality of Tuberculosis in New York City, 1990-2007
Advisor: Rachel Gordon, MD, MPH

2000-2004 Bachelor of Arts (BA), Biology (program of study in nutrition) and American Studies
Cornell University, College of Arts and Sciences
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PROFESSIONAL EXPERIENCE

2010-2012 Biostatistician
Department of Epidemiology and Population Health
Albert Einstein College of Medicine of Yeshiva University
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2009-2010 Surveillance Intern
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New York City Department of Health and Mental Hygiene
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2008-2009 Research/Administrative Assistant
Department of Epidemiology
Columbia University Mailman School of Public Health
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2007-2008	Healthcare Senior Account Executive Ricochet Public Relations New York, NY
2005-2006	Electronic Medical Records Implementation Project Analyst ENT and Allergy Associates, LLP New York, NY

TEACHING EXPERIENCE

2013-Present	Ingenuity Program at Baltimore Polytechnic Institute Baltimore Polytechnic Institute-Johns Hopkins University, Baltimore, MD *Role: Mentor to high school student*
2013-Present	PH340.871: Welch Center Research Seminar Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 8 terms *Role: Course Coordinator (Enrollment: 25 students)* *Course Instructor: Elizabeth Selvin*
2013	PH340.751: Epidemiologic Methods I Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 1 term *Role: Lead Teaching Assistant (Enrollment: 220 students)* *Course Instructors: Stephen Gange, Elizabeth Selvin, Catherine Sutcliffe*
2011-2012	HSD 269: Fundamentals of Biostatistics for Health Professionals Undergraduate class Lehman College, Bronx, NY 3 semesters *Role: Adjunct Lecturer (Enrollment: 25 students)*
2011-2012	HEA 600: Biostatistics Masters level class Lehman College, Bronx, NY 2 semesters *Role: Adjunct Lecturer (Enrollment: 25 students)*

2011	PUB 6100: Fundamentals of Epidemiology in Public Health Masters level class Albert Einstein College of Medicine, Bronx, NY 1 summer term *Role: Teaching Assistant (Enrollment: ~20 students)* *Course Instructor: Paul Marantz*
2009-2010	P6103: Introduction to Biostatistics Columbia University Mailman School of Public Health, New York, NY 2 semesters *Role: Teaching Assistant (Enrollment: ~200 students)* *Course Instructors: Martina Pavlicova, Sara Lopez-Pintado*
2007-2008	Math Pre-GED Tutor (Volunteer) Literacy Partners, Inc, New York, NY
2004-2005	Middle School Science Teacher M.S. 385, Brooklyn, NY

PROFESSIONAL ACTIVITIES

MEMBERSHIPS

2015-Present	Society for Epidemiologic Research
2014-Present	American Diabetes Association
2013-Present	American Heart Association

EDITORIAL ACTIVITIES

Reviewer for the Following Journals:

Diabetes Care
Diabetic Medicine
Endocrine Practice
JAMA
JAMA Internal Medicine
Journal of Clinical Endocrinology & Metabolism
Journal of Diabetes

HONORS AND AWARDS

2014	Epidemiology and Prevention Early Career Travel Grant, American Heart Association
2010	Student Conference Travel Fund Award, Columbia University Mailman School of Public Health

PEER-REVIEWED PUBLICATIONS

1. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, Xue X, **Parrinello CM**, Hunt P, Deeks SG, Hodis HN. T cell activation predicts carotid artery stiffness among HIV-infected women. *Atherosclerosis*. 2011 Jul;217(1):207-13. PMID:21492857, PMCID:PMC3139014.

2. **Parrinello CM**, Crossa A, Harris TG. Seasonality of Tuberculosis in New York City: 1990-2007. *Int J Tuberc Lung Dis*. 2012 Jan;16(1):32-7. PMID: 22236842.

3. **Parrinello CM**, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, Xue X, Hunt PW, Deeks SG, Hodis HN, Kaplan RC. Cytomegalovirus immunoglobulin G antibody is associated with subclinical carotid artery disease among HIV-infected women. *J Infect Dis*. 2012 Jun 15;205(12):1788-96. PMID: 22492856, PMCID:PMC3415890.

4. Kaplan RC, Landay AL, Hodis HN, Gange SJ, Norris PJ, Young M, Anastos K, Tien PC, Xue X, Lazar J, **Parrinello CM**, Benning L, Tracy RP. Potential cardiovascular disease risk markers among HIV-infected women initiating antiretroviral treatment. *J Acquir Immune Defic Syndr*. 2012 Aug 1;60(4):359-68. PMID:22592585, PMCID:PMC3400505.

5. **Parrinello CM**, Landay AL, Hodis HN, Gange SJ, Norris PJ, Young M, Anastos K, Tien PC, Xue, X, Lazar J, Benning L, Tracy RP, Kaplan RC. Association of lipid levels with HIV infection, treatment, inflammatory biomarkers and subclinical atherosclerosis in the Women's Interagency HIV Study. *Atherosclerosis*. 2012 Dec;225(2):408-11. PMID:23089369, PMCID:PMC3696584.

6. Herron AJ, Mariani JJ, Pavlicova M, **Parrinello CM**, Bold KW, Levin FR, Nunes EV, Sullivan MA, Raby WN, Bisaga A. Assessment of riboflavin as a tracer substance: Comparison of a qualitative to a quantitative method of riboflavin measurement. *Drug Alcohol Depend*. 2013 Feb;128(1-2):77-82. PMID:22921475, PMCID: PMC3556739.

7. Kuniholm MH, **Parrinello CM**, Anastos K, Augenbraun M, Plankey M, Nowicki M, Peters M, Golub ET, Lurain N, Landay AL, Strickler HD, Kaplan RC. Hepatitis C is

associated with cytomegalovirus IgG antibody levels in HIV-infected women. *PLoS ONE*. 2013 Apr;8(4): e61973. PMID:23613990, PMCID:PMC3629158.

8. **Parrinello CM**, Landay AL, Hodis HN, Gange SJ, Norris PJ, Young M, Anastos K, Tien PC, Xue X, Lazar J, Benning L, Tracy RP, Kaplan RC. Treatment-related changes in serum lipids and inflammation: clinical relevance remains unclear. Analyses from the Women's Interagency HIV Study. *AIDS*. 2013 Jun;27(9):1516-9. PMID:23435295, PMCID:PMC3909663.

9. Selvin E, **Parrinello CM**, Sacks DB, Coresh J. Trends in prevalence and control of diabetes in the U.S., 1988-1994 and 1999-2010. *Ann Intern Med*. 2014 Apr;160(8):517-25. PMID:24733192.

10. Kaplan RC, Avilés-Santa ML, **Parrinello CM**, Hanna DB, Jung M, Castañeda SF, Hankinson AL, Isasi CR, Birnbaum-Weitzman O, Kim RS, Daviglus ML, Talavera GA, Schneiderman S, Cai J. Body mass index, sex and cardiovascular disease risk factors among Hispanic / Latino adults: Hispanic Community Health Study / Study of Latinos. *J Am Heart Assoc.* 2014 Jul;3(4). PMID:25008353.

11. **Parrinello CM** and Selvin E. Beyond HbA1c and glucose: the role of nontraditional glycemic markers in diabetes diagnosis, prognosis and management. *Curr Diab Rep*. 2014 Nov;14(11):548. PMID:25249070, PMCID:PMC4214073.

12. Isasi CR, **Parrinello CM**, Jung MM, Carnethon MR, Birnbaum-Weitzman O, Espinoza RA, Penedo FJ, Perreira KM, Schneiderman N, Sotres-Alvarez D, Van Horn L, Gallo LC. Psychosocial stress is associated with obesity and diet quality in Hispanic/Latino adults. *Ann Epidemiol.* 2015 Feb;25(2):84-9. PMID:25487969, PMCID:PMC4306634.

13. Jung M, **Parrinello CM**, Xue X, Mack WJ, Anastos K, Lazar JM, Selzer RH, Shircore AM, Plankey M, Tien P, Cohen M, Gange SJ, Hodis HN, Kaplan RC. Echolucency of the carotid artery intima-media complex and intima-media thickness have different cardiovascular risk factor relationships: the Women's Interagency HIV Study. *J Am Heart Assoc*. 2015 Feb;4(2). PMID:25699995, PMCID: PMC4345869.

14. **Parrinello CM**, Isasi CR, Xue X, Bandiera FC, Cai J, Ji M, Lee DJ, Navas-Nacher EL, Perreira KM, Salgado H, Kaplan RC. Risk of cigarette smoking initiation during adolescence among US-born and non-US-born Hispanics/Latinos: The Hispanic Community Health Study / Study of Latinos. *Am J Public Health*. 2015 Jun;105(6):1230-6. PMID:25322293, PMCID: PMC4431078.

15. Al Rifai M, Schneider ALC, Alonso A, Maruthur N, **Parrinello CM**, Astor BC, Hoogeveen RC, Soliman E, Chen LY, Ballantyne CM, Halushka MK, Selvin E. sRAGE, inflammation, and risk of atrial fibrillation: Results from the Atherosclerosis Risk in

Communities (ARIC) Study. *J Diabetes Complications*. 2015 Mar;29(2):180-5. PMID:25499973, PMCID: PMC4333077.

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17. **Parrinello CM**, Rastegar I, Godino JG, Miedema MD, Matsushita K, Selvin E. Prevalence of and racial disparities in risk factor control in older adults with diabetes: The Atherosclerosis Risk in Communities Study. *Diabetes Care*. 2015 Jul; 38(7):1290-8. PMID:25852205, PMCID: PMC4477331.

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LETTERS

Selvin E and **Parrinello CM**. Age-related differences in glycaemic control in diabetes. *Diabetologia*. 2013 Dec;56(12):2549-51. PMID:24092493, PMCID:PMC3842214.

PUBLICATIONS IN PROGRESS

Parrinello CM, Sharrett AR, Maruthur NM, Bergenstal R, Grams ME, Coresh J, Selvin E. Racial differences in levels and prognostic value of biomarkers of hyperglycemia. [under review]

Parrinello CM, Lutsey PL, Couper D, Eckfeldt JH, Steffes MW, Coresh J, Selvin E. Short-term total variability in biomarkers of hyperglycemia in older adults. [under review]

Isasi CR, Jung M, **Parrinello CM**, Kaplan RC, Kim RS, Crespo NC, Gonzales P, Gouskova NA, Penedo FJ, Perreira KM, Perrino T, Sotres-Alvarez D, Van Horn L, Gallo LC. Childhood economic hardship is associated with adult height but not with adult adiposity among Hispanics/Latinos. The HCHS/SOL Socio-Cultural Ancillary Study. [under internal HCHS/SOL review]

INVITED PRESENTATIONS

Clinical biomarkers of type 2 diabetes. Partnering Toward Discovery: Conversations on Research and Medicine. December 2013, Johns Hopkins University, Baltimore, MD. [Also as a podcast for Johns Hopkins Health Media]

The art of reading a scientific article. Current Topics in Epidemiologic Research. September 26, 2014. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

ABSTRACT PRESENTATIONS

*Presenter
†Mentored by Ms. Parrinello

Oral presentations

1. **Parrinello CM**, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, Xue X, Hunt PW, Deeks SG, Hodis HN, Kaplan RC*. Cytomegalovirus IgG antibody is associated with subclinical carotid artery disease among HIV-infected women. *International Workshop on HIV & Aging, October 27-28, 2011, Baltimore, Maryland, USA.*

2. **Parrinello, CM***, Lutsey PL, Ballantyne CM, Folsom AR, Pankow JS, Selvin E. Six-year change in C-reactive protein levels and risk of incident diabetes, cardiovascular events and mortality. *American Heart Association Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2014 Scientific Sessions, March 18-21, 2014, San Francisco, CA, USA.*

3. **Parrinello CM***, Matsushita K, Woodward M, Steffes MW, Coresh J, Selvin E. Risk prediction of major complications in persons with diabetes. *Accepted for presentation at the American Diabetes Association's 75th Scientific Sessions, June 5-9, 2015, Boston, MA, USA.*

Poster presentations

1. **Parrinello CM***, Crossa A, Harris TG. Seasonality of Tuberculosis in New York City: 1990-2007. *Society for Epidemiologic Research 43rd Annual Meeting, June 23-26, 2010, Seattle, Washington.*

2. Herron,AJ*, Mariani JJ, Pavlicova M, **Parrinello CM**, Williams K, Bisaga A. Assessment of Riboflavin as a Tracer Substance: Comparison of a Qualitative to a Quantitative Method of Riboflavin Measurement. *American Academy of Addiction*

Psychiatry (AAAP) 21st Annual Meeting & Symposium, December 2-5, 2010, Boca Raton, Florida, USA.

3. **Parrinello CM***, Landay AL, Hodis HN, Gange SJ, Norris PJ, Anastos K, Tien PC, Xue X, Tracy RP, Kaplan RC. Potential atherogenic biomarkers among HIV-infected women initiating antiretroviral treatment. *CROI 2012, March 5-8, 2012, Seattle, Washington, USA.*

4. Kaplan RC, **Parrinello CM**, Hodis HN, Gange SJ, Young M, Anastos K, Tien PC, Lazar J, Landay AL, Desai S*. Impact of HAART initiation on immune regulation in aging HIV-infected women – Women's Interagency HIV Study. *CROI 2012, March 5-8, 2012, Seattle, Washington.*

5. Raiszadeh F, Kuniholm MH, **Parrinello CM**, French A, Golub E, Karim R, Lazar J, Plankey M, Tien PC, Peters M, Anastos K, Kaplan RC*. Subclinical Atherosclerosis is Associated with FIB-4 among Women Co-infected with HIV and Hepatitis C: Results from the Women's Interagency HIV Study (WIHS). *International Workshop on HIV Observational Databases, March 29-31, 2012, Athens, Greece.*

6. **Parrinello CM**, Landay AL, Gange SJ, Norris PJ, Anastos K, Young M, Tien PC, Xue X, Lazar J, Tracy RP, Hodis HN, Kaplan RC*. Circulating inflammation and coagulation biomarkers predict subclinical atherosclerosis in HIV-infected women. *International Workshop on HIV Observational Databases, March 29-31, 2012, Athens, Greece.*

7. Kaplan RC*, Avilés-Santa ML, **Parrinello CM**, Castañeda SF, Hankinson AL, Isasi CR, Birnbaum-Weitzman O, Kim RS, Daviglus ML, Talavera GA, Schneiderman S, Cai J. Severe obesity is associated with dramatic increase in cardiovascular disease risk factor prevalence: Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *American Heart Association Cardiovascular Disease, Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2013 Scientific Sessions, March 19-22, 2013, New Orleans, LA, USA.*

8. **Parrinello CM*,** Isasi CR, Xue X, Bandiera FC, Cai J, Ji M, Lee DJ, Navas-Nacher EL, Perreira KM, Salgado H, Kaplan RC. Risk of smoking initiation among US-born and US immigrant Hispanic adolescents: Results from the Hispanic Community Health Study / Study of Latinos. *American Heart Association Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2013 Scientific Sessions, March 19-22, 2013, New Orleans, LA, USA.*

9. **Parrinello CM***, Grams ME, Couper D, Ballantyne CM, Hoogeveen RC, Eckfeldt JH, Selvin E, Coresh J. Calibration of analytes over twenty-five years in the Atherosclerosis Risk in Communities Study. *American Heart Association Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism 2014 Scientific Sessions, March 18-21, 2014, San Francisco, CA, USA.*

10. **Parrinello CM***, McEvoy JW, Woodward M, Folsom AR, Ballantyne CM, Coresh J, Selvin E. High-sensitivity cardiac troponin T and NT-proBNP in cardiovascular risk prediction in persons with diabetes. *American Diabetes Association's 74th Scientific Sessions, June 13-17, 2014, San Francisco, CA, USA.*

11. **Parrinello CM***, Sharrett AR, Klein R, Bergenstal R, Coresh J, Selvin E. Racial Differences in Hyperglycemia: A Comparison of Traditional and Alternative Glycemic Markers with Retinopathy. *American Diabetes Association's 74th Scientific Sessions, June 13-17, 2014, San Francisco, CA, USA.*

12. Rawlings AM*, Sharrett AR, Maruthur NM, **Parrinello CM**, Rebholz CM, Steffes MW, Selvin E. Glycemic excursions and cognitive function in older adults with diabetes. *Accepted for presentation at the American Heart Association Epidemiology and Prevention/Lifestyle and Cardiometabolic Health 2015 Scientific Sessions, March 3-6, 2015, Baltimore, MD, USA.*

13. Rawlings AM*, Sharrett AR, Knopman D, **Parrinello CM**, Palta P, Wruck L, Bandeen-Roche K, Gottesman RF, Albert M, Coresh J, Mosley T, Selvin E. Prevalence of cognitive dysfunction among older adults with diabetes. *Accepted for presentation at the American Heart Association Epidemiology and Prevention/Lifestyle and Cardiometabolic Health 2015 Scientific Sessions, March 3-6, 2015, Baltimore, MD, USA.*

14. **Parrinello CM**, Rastegar I*†, Godino JG, Miedema MD, Matsushita K, Selvin E. Racial Disparities in Risk Factor Control in Older Adults with Diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Accepted for presentation at the American Heart Association Epidemiology and Prevention/Lifestyle and Cardiometabolic Health 2015 Scientific Sessions, March 3-6, 2015, Baltimore, MD, USA.*

15. **Parrinello CM***, Maruthur NM, Sharrett AR, Klein R, Bergenstal R, Grams ME, Coresh J, Selvin E. Racial differences in hyperglycemia: Comparative prognostic value of traditional and nontraditional glycemic markers. *Accepted for presentation at the American Heart Association Epidemiology and Prevention/Lifestyle and Cardiometabolic Health 2015 Scientific Sessions, March 3-6, 2015, Baltimore, MD, USA.*

16. Isasi CR*, Jung M, **Parrinello CM**, Kaplan RC, Kim RS, Crespo NC, Gonzales P, Gouskova NA, Penedo FJ, Perreira K, Perrino T, Sotres-Alvarez D, Van Horn L, Gallo LC. Is childhood economic hardship associated with adult height and adiposity among Hispanics/Latinos living in the US? Results from the HCHS/SOL Socio-Cultural Study. *Submitted for presentation at the Society for Epidemiologic Research's 48th Annual Meeting, June 16-19, 2015, Denver, CO, USA.*

17. Selvin E, **Parrinello CM***, Bergenstal RM. Trends in diabetes control and insulin use in the US, 1988-2010. *Accepted for presentation at the American Diabetes Association's 75th Scientific Sessions, June 5-9, 2015, Boston, MA, USA.*

18. **Parrinello CM***, Lutsey PL, Couper D, Eckfeldt JH, Coresh J, Selvin E. Short-term total variability in nontraditional biomarkers of hyperglycemia in older adults. *Accepted for presentation at the American Diabetes Association's 75th Scientific Sessions, June 5-9, 2015, Boston, MA, USA.*

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