HEMOGLOBIN A1C AND PREDICTION OF CARDIOVASCULAR DISEASE AND ALL-CAUSE MORTALITY

BY

AMIR AZEEM

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Approved By

Lynne E. Wagenknecht, DrPH, Chair
David M. Herrington, MD, MHS, Advisor
Alain G. Bortoni, MD, MPH
Fang-Chi Hsu, PhD
Gregory L. Burke, MD, MSc
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Amir Azeem
“True knowledge exists in knowing that you know nothing.” Socrates

“Without knowledge action is useless and knowledge without action is futile.” Abu Bakr
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ABBREVIATIONS

ABI, ankle-brachial index
ACCC, American College of Cardiology Foundation
ADA, American Diabetes Association
ADAG, A1C-derived average glucose
AGE, advanced glycation end products
AHA, American Heart Association
ANOVA, Analysis of variance
ARIC, Atherosclerosis Risk in Communities
AUC, area under the curve
BMI, body mass index
CAC, coronary artery calcium
CABG, coronary artery bypass graft
CHD, coronary heart disease
CHDRA, coronary heart disease risk assessment algorithm
CI, confidence interval
CVD, cardiovascular disease
DBP, diastolic blood pressure
DCCT, Diabetes Control and Complications Trial
EGP, early glycation end products
EPIC-Norfolk, European Prospective Investigation into Cancer in Norfolk
FBG, fasting blood glucose
FMD, flow-mediated dilation
FRS, Framingham Risk Score
HbA1c, hemoglobin A1c
Hb-AGE, hemoglobin-AGE
HDL, high density cholesterol
HPLC, high-performance liquid chromatography
HR, hazard ratio
hs-CRP, high sensitivity c-reactive protein
HTN, hypertension
IFCC, International Federation of Clinical Chemistry and Laboratory Medicine
IFG, impaired fasting glucose
LDL, low density cholesterol
IMT, carotid intima-media thickness
MESA, Multi-Ethnic Study of Atherosclerosis
NGSP, National Glycohemoglobin Standardization Program
NHANES-III, Third National Health and Nutrition Examination Survey
NRI, Net Reclassification Improvement
PCI, percutaneous coronary intervention
RERI, relative excess risk due to interaction
ROC, receiver operating curve
SBP, systolic blood pressure
SCORE, Systemic Coronary Risk Evaluation
TWPAS, Typical Week Physical Activity Survey
ABSTRACT

In this thesis, we will seek to evaluate the role of a biomarker, hemoglobin A1c, as a predictor of incident cardiovascular disease and all-cause mortality in a multi-ethnic population free of cardiovascular disease and diabetes at the baseline. Atherosclerotic cardiovascular disease is a significant cause of morbidity and mortality and the prevalence continues to rise globally. The economic burden to care for cardiovascular disease is prohibitive. There has been growing interest to identify individuals who are at higher risk of developing cardiovascular disease so that targeted interventions can be administered. Existing models to predict cardiovascular disease are not very helpful in a large number of individuals and falsely categorize their cardiovascular risk profile. There is an opportunity to identify and use additional and novel biomarkers in order to improve our ability to predict cardiovascular disease. This knowledge may translate into targeted and efficient use of health care resources and implementation of preventive interventions in high risk individuals. The first chapter of our thesis will contain background, literature review and discussion of previous work done on this subject and will provide the foundation for our work. The second chapter will be the manuscript that will be submitted to a journal for publication. The last chapter will discuss additional analyses and further discussion of our findings.

Keywords: Hemoglobin A1c, cardiovascular disease, mortality, proportion, analysis of variance, relative risk, risk ratio, p-value, multiplicative interaction, additive interaction, survival analysis, Kaplan Meier analysis, Cox regression analysis, receiver operating curve analysis, net reclassification improvement.
CHAPTER – I: BACKGROUND

Epidemiology of Cardiovascular Disease

Cardiovascular disease is a major health problem in the United States and worldwide. It continues to be a leading cause of morbidity and mortality and a significant burden on healthcare resources. According to current estimates, cardiovascular disease affects almost 83.6 million US adults. Among this group, 77.9 million individuals have diagnosed hypertension and 15.4 million have coronary heart disease. Despite the overall decline in cardiovascular disease mortality, it remains the leading cause of death accounting for one of every three deaths in the United States. All of this translates into an estimated direct and indirect cost of 312.6 billion dollars or almost 17% of national health expenditure. The incidence and prevalence of cardiovascular disease and associated morbidity and mortality increases with age and clinical risk factors like high blood pressure, disorders of glucose homeostasis, dyslipidemia, smoking, physical inactivity, unhealthy dietary habits, and increasing body mass index. Recent prevalence of many of these risk factors means that the burden of cardiovascular is expected to rise over time.

Cardiovascular Risk Assessment

Cardiovascular risk is the chance that an individual with presence or absence of one or more characteristics will develop some type of cardiovascular disease in a pre-defined amount of time. The ability to better predict cardiovascular risk and to identify high risk individuals and populations is an important clinical goal. First, individuals empowered by
this information may become motivated to adopt healthy lifestyle to lower their risk. Second, healthcare providers may be able to use valuable healthcare resources more efficiently by targeted application of preventive interventions in high risk individuals. Traditional cardiovascular risk factors include genetic predisposition, smoking, diabetes and related disorders of glucose homeostasis, high blood pressure, abnormal lipids profile, and excess body weight. Risk prediction models utilize a combination of these traditional risk factors to identify individuals in different risk categories for the sake of implementing risk-appropriate interventions.

Limitations of Existing Prediction Models

A number of risk assessment models are currently in use and recommended by scientific guidelines for cardiovascular risk assessment. Among all, Framingham Risk Score (FRS) is most widely used in clinical practice. However, Framingham risk score was developed based upon the findings in a Caucasian cohort of a small geographic area and questions have been raised about its generalizability in some studies while others have reported that the Framingham Risk Score can be used in other ethnic groups after proper recalibration. Furthermore, Framingham risk score has not been able to accurately define risk profile of a large number of individuals, especially those who fall in the intermediate risk category. In addition, there is wide risk discrepancy among different prediction models when estimating the risk in a given population. A number of studies have been reported in the literature highlighting these limitations. For example, in a middle-aged Italian population followed for twenty five years, Menotti et al reported that the Framingham Risk function overestimated the coronary risk especially in countries
characterized by a lower incidence of coronary events.\textsuperscript{10} In another study including 608 patients in Spain between the ages of 40-65 years and after a 10 year of follow up, Barroso et al reported that although both Framingham Risk Score and European Systemic Coronary Risk Evaluation (SCORE) overestimated the coronary risk, Framingham function did worse by overestimating the cardiovascular risk by 64\% when compared to 40\% for SCORE function.\textsuperscript{4} Similar findings were reported in UK cohorts as well.\textsuperscript{11} Therefore, efforts are underway to assess the usefulness of new and novel biomarkers in addition to the existing risk prediction models in improving our ability to better predict cardiovascular risk. In this regard, it is recommended that the new biomarker should at least have an independent association with outcomes of interest after adjusting for other established, inexpensive and readily available risk factors.\textsuperscript{12}

A number of limitations of existing risk prediction models are now being recognized. One of them is the baseline assumption that the effect of risk factors remains constant across wide spectrum of age and level of other risk factors which are included in the model. For example, a young person may still be classified in the low risk category despite having one or more other cardiovascular risk factors.\textsuperscript{13} Furthermore, there is growing concern that the intermediate risk group may, in fact, contain certain high-risk individuals which may benefit from more aggressive medical therapy and some with low risk in whom lifestyle modification is sufficient. This has important implications in terms of management decisions since over-prediction of risk may result in inappropriate use of recommended therapies and health care resources. On the other hand, if the risk is underestimated, then certain high risk individuals may be unjustly denied much needed
preventive interventions. Therefore, additional risk factors are being studied to assess the improvement in our predictive abilities.\textsuperscript{14}

\textbf{Emerging Biomarkers and Prediction of Cardiovascular Disease Risk}

In the recent years, a number of new biomarkers have been reported to be associated with cardiovascular risk. However, their use in clinical practice remains less clear. High sensitivity C-reactive protein (hs-CRP), coronary artery calcium (CAC), carotid intima-media thickness (IMT), ankle-brachial index (ABI), and many other novel serum and imaging biomarkers have been reported in literature to be useful in predicting cardiovascular risk. Among these, CAC has shown a consistent association with incident cardiovascular disease. American College of Cardiology Foundation/American Heart Association has recommended that CAC be used for risk stratification of individuals who are at intermediate risk for CHD by Framingham Risk Score.\textsuperscript{15} In 2008, Folsom et al reported that in a multi-ethnic population free of CVD at baseline, CAC was a better predictor of incident CVD when compared to IMT.\textsuperscript{16} In addition, Yeboah et al recently compared ABI, brachial flow-mediated dilation (FMD), CAC, IMT, family history, and hs-CRP and found that all except IMT and FMD were independent predictors of CHD after adjusting for traditional risk factors. However, CAC had the strongest association. Similarly, addition of CAC to FRS resulted in significant improvement in area under the curve (AUC) as well net reclassification improvement.\textsuperscript{14} In a recent study, Cross et al developed a coronary heart disease risk assessment algorithm (CHDRA) by including clinical risk factors (age, gender, and family history of premature myocardial infarction) and seven biomarkers associated with atherosclerosis (CTACK, Eotaxin, Fas Ligand,
HGF, IL-16, MCP-3, and sFas) and validated in Multi-Ethnic Study of Atherosclerosis. They reported that CHDRA was an independent predictor of acute coronary events (hazard ratio = 2.17, p < 0.001) even after adjustment for Framingham risk factors.17

Abnormal Glucose Homeostasis and Risk of Cardiovascular Disease

Diabetes is a significant risk factor for cardiovascular disease and mortality.18-22 A number of cohort studies have demonstrated an association between presence of diabetes and incident cardiovascular disease and cardiovascular- and all-cause mortality. Traditionally, fasting blood glucose and 2-hour glucose tolerance tests have been used to diagnose diabetes. It is argued that the risk associated with abnormal glucose homeostasis may be continuous even at levels below the diagnostic cut offs for diabetes mellitus.23, 24 Therefore, opportunities exist to study the role of aggressive preventive interventions even in individuals who fall in the spectrum of abnormal glucose homeostasis without having the diagnosis of diabetes mellitus.

Hemoglobin A1c

Formation of Hemoglobin A1c: Non-enzymatic glycation is a process by which saccharide derivatives, like glucose and fructose, interact with macromolecules (proteins, carbohydrates, nucleic acids, and lipids). This results in the formation of early (EGP) and advanced glycation end products (AGE). HbA1c is an example of EGP and, like other EGP, is a precursor of an AGE, Hemoglobin-AGE (Hb-AGE).25 AGEs have been suggested to play a key role in the micro- and macrovascular diabetic complications as well as aging.26, 27
**Hemoglobin A1c Testing:** For many years, hemoglobin A1c has been used as a standard test to estimate level of glycemia over a prolonged period and to monitor effectiveness of diabetes treatment and control as reflected in the recommendations of American Diabetes Association. This practice is based upon the finding of the association of higher levels of hemoglobin A1c with microvascular complications, particularly retinopathy. Recently, hemoglobin A1c testing has been standardized and agreed upon by American Diabetes Association, European Association for the Study of Diabetes, International Diabetes Federation, and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in a consensus statement. The important recommendations made in that statement are: “(1) The new IFCC reference system for A1C represents the only valid anchor to implement standardization of the measurement. (2) A1C results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-National Glycohemoglobin Standardization Program (NGSP) master equation. (3) If the ongoing “average plasma glucose study” fulfills its a priori–specified criteria, an A1C-derived average glucose (ADAG) value calculated from the A1C result will also be reported as an interpretation of the A1C results. (4) Glycemic goals appearing in clinical guidelines should be expressed in IFCC units, derived NGSP units, and as ADAG.” The goal of these recommendations is to implement testing and reporting of hemoglobin A1c which is comparable worldwide and more useful to the healthcare providers.
Racial/Ethnic Differences in Hemoglobin A1c: It has been reported that the hemoglobin A1c levels in different racial and ethnic groups may not be similar despite same degree of glycemia. The exact mechanisms for these differences remain unclear. Ziemer et al analysed data of two population studies, Screening for Impaired Glucose Tolerance and Third National Health and Nutrition Examination Survey, and found that African-Americans had higher levels of hemoglobin A1c when compared to Caucasian-Americans after adjusting for plasma glucose. They also noted that these differences were amplified with worsening of glucose intolerance. However, it is not clear if these racial/ethnic differences in glycation are associated with varying degrees of cardiovascular risk.

Hemoglobin A1c and Diagnosis of Diabetes: In 2010, American Diabetes Association (ADA) has included HbA1c as one of the diagnostic criteria for classification of diabetes. The HbA1c cut points, like FBG, are based upon its association with microvascular complications, especially with retinopathy. Individuals with HbA1c values > 6.5% are now considered to have diabetes and those with HbA1c values 5.7% to 6.4% are considered be at high risk for diabetes; a level below 5.7% is considered normal. In a meta-analysis of over forty-four thousand participants, Colagiuri et al demonstrated that diabetes-specific retinopathy was significantly more prevalent for the participants with HbA1c above 6.4%. Similarly, in a European cohort study, Eskesen and colleagues found a significantly increased risk of incident diabetes associated with every single unit increase in HbA1c level (HR[95%CI]: 4.19[2.01-8.71]).
Hemoglobin A1c as a Predictive Marker of Cardiovascular Disease

Diabetes mellitus is a well-recognized risk factor for cardiovascular disease\textsuperscript{37-39} and is considered a coronary heart disease risk equivalent. In other words, the risk of major cardiovascular events in patients with type 2 diabetes may be similar to someone who does not have diabetes but has established coronary heart disease.\textsuperscript{40, 41} In fact, cardiovascular complications account for the majority of diabetes-related morbidity and mortality. The reason for increased cardiovascular disease with diabetes is only partially understood and is due to a combination of chronic hyperglycemia on cardiovascular system and co-existence of other risk factors. Furthermore, it also known that higher level of HbA1c in diabetics is associated with microvascular complications\textsuperscript{35} and incident cardiovascular disease.\textsuperscript{42-44} However, the association of HbA1c and cardiovascular disease events in persons without diabetes is not clear and we do not know if HbA1c has a role for screening people for cardiovascular disease risk prediction.

A number of studies assessing the utility of HbA1c as a risk marker in individuals without diabetes have produced mixed results. In one population study of community dwellers without diabetes, Matsushita found that increasing levels of hemoglobin A1c (5.5\% or above) was associated with new onset heart failure after adjusting for potential confounders.\textsuperscript{45} Then, in 2004, Khaw et al reported that the HbA1c significantly predicted all-cause mortality, cardiovascular disease, and coronary heart disease independent of traditional risk factors in a European cohort from Norfolk, England.\textsuperscript{46} Later, in 2008, from the same cohort and 8.5 years of follow up, Simmons et al showed that addition of HbA1c made a small but statistically significant improvement in cardiovascular risk
prediction in men only.\textsuperscript{47} Adams et al described in 2009 that elevated HbA1c is related to new onset cardiovascular over a relatively short follow-up period in both men and women without diabetes and who do not develop diabetes, after adjustment for other major risk factors. However, the study was limited by short follow-up of 3.5 years and mostly native population of Southern Australia. In another study, Chonchol et al concluded that HbA1c was not an independent predictor of all-cause and cardiovascular mortality in an elderly population of Cardiovascular Health Study (CHS).\textsuperscript{48} Recently, in a large biracial cohort (Atherosclerosis Risk in Communities [ARIC]) with more than ten thousand participants, around 22\% of whom were African-Americans, and a 14 years of follow up, Selvin et al found that HbA1c was significantly associated with increased risk of diabetes as well as cardiovascular events and deaths\textsuperscript{49} and that race did not modify this association. In 2011, Dheir reported increased risk of cardiovascular disease events in non-diabetic dialysis patients with high levels of HbA1c but the sample was very small and follow-up was only 5 years.\textsuperscript{50} In another study involving only American Indians, Wang et al concluded that there was no independent association of HbA1c and cardiovascular disease risk for HbA1c in the prediabetic range despite high prevalence of obesity and diabetes in this population.\textsuperscript{51} In a large Danish cohort (Copenhagen City Heart Study) involving more than five thousand participants, Eskesen et al reported that elevated levels of hemoglobin A1c was associated with incident cardiovascular disease events in participants without diabetes after adjusting for traditional risk factors (Hazard ratio[95\% Confidence Interval]: 1.31 [1.05-1.64]).
Most of the previous studies did not have racial/ethnic diversity and did not produce consistent results. To build on the existing data and to address some of the limitations of previous work, we are proposing to use Multi-Ethnic Study of Atherosclerosis (MESA) data to further explore the association of HbA1c and incident cardiovascular events and all-cause mortality in individuals without DM. Since HbA1c was measured during the second MESA exam, this will be used as baseline visit for our study. Multi-Ethnic Study of Atherosclerosis represents a racially diverse population and has seven years of adjudicated cardiovascular events for our analyses. Therefore, MESA is an excellent cohort to be used for this important study.

**Hemoglobin A1c and Subclinical Cardiovascular Disease**

Atherosclerotic cardiovascular disease generally has a long asymptomatic phase. Therefore, recognition of subclinical changes and aggressive control of risk factors in this stage can slow the progression, and perhaps, reverse some of the pathophysiological changes of cardiovascular system. Most of the previous studies were aimed at studying the association of hemoglobin A1c and clinical cardiovascular disease. However, with advancements in existing diagnostic tools and emergence of newer imaging modalities, like cardiac CT and MRI, the interest has grown to study the association of risk factors with subclinical CVD so that clinical events can be prevented by appropriate and evidence based interventions. In this context, McNeely et al reported that increasing levels of hemoglobin A1c were significantly associated with common and internal carotid intimal-medial wall thickness in a multi-ethnic cohort free of diabetes after adjusting for other cardiovascular risk factors.\(^{52}\) Van Der Meer et al has reported that type 2 diabetes was
associated with abnormal pulse wave velocity and distensibility independent of blood pressure.\textsuperscript{53} Somewhat similar results were reported in individuals without diabetes by Stokes et al. who concluded that elevated levels of glycosylated hemoglobin in the non-diabetic range were associated with aortic stiffening and increased left ventricular mass.\textsuperscript{54} In another study, Lukich et al found that worsening glucose homeostasis was associated with increased arterial stiffness.\textsuperscript{55}

**Role of Hemoglobin A1c in the Pathogenesis of Cardiovascular Disease**

The exact mechanisms by which hemoglobin A1c exerts its role in the causation of microvascular (retinopathy and nephropathy) and macrovascular (atherosclerotic CVD) complications are not completely understood. However, it is suggested that the formation of advanced glycation end products (AGE) is one of the important pathophysiological processes which is correlated with complications of abnormal glucose homeostasis. In a review article by Brownlee, it is proposed that the AGE precursors cause covalent modification of intracellular, matrix, as well as plasma proteins resulting in malfunction of cellular proteins, abnormal inter-cellular interactions, and altered gene expression with accelerated formation of chemokines known to play a central role in atherosclerosis (Figure-1).\textsuperscript{27} It is also suggested that HbA1c is an example of early glycation product and a precursor of Hemoglobin-AGE.\textsuperscript{25}
**Figure-1:** Advanced Glycation End-products (AGE) and pathogenesis of vascular complications. (Figure taken from "Biochemistry and molecular cell biology of diabetic complications." Brownlee, M. Nature 2001:414;6865; 813-820)
HYPOTHESIS AND SPECIFIC AIMS

Cardiovascular disease is a significant health problem and the associated morbidity and mortality and cost is overwhelming. Current prediction models, while useful in many patients, are inadequate in risk stratification in a large number of individuals. The overarching goal of our thesis project is to attempt to improve upon the existing Framingham Risk Score for prediction of cardiovascular disease in a multi-ethnic population free of diabetes and cardiovascular disease at baseline. More specifically, we sought to address the following questions:

1. Does hemoglobin A1c predict incident cardiovascular disease, coronary heart disease, and all-cause mortality above and beyond traditional risk factors in a multi-ethnic population free of diabetes and cardiovascular disease at the baseline?

2. Does the relationship between hemoglobin A1c and cardiovascular disease, coronary heart disease, and all-cause mortality vary by race and ethnicity?

To address these questions, we will perform secondary data analysis of an ongoing prospective cohort study, Multi-Ethnic Study of Atherosclerosis (MESA). We will use second MESA exam as our baseline since HbA1c was tested during this visit and adjust time to event accordingly. For the purpose of this study, incident cardiovascular disease is defined as the composite of coronary heart disease death, resuscitated cardiac arrest,
incident myocardial infarction, definite angina, probable angina (if followed by coronary artery bypass grafting and percutaneous coronary intervention), ischemic stroke, stroke death, other atherosclerotic death, or other cardiovascular disease death as defined by the MESA protocol. Incident coronary heart disease event is defined as a composite of coronary heart disease death, resuscitated cardiac arrest, incident MI, definite angina, or probable angina (if followed by coronary artery bypass grafting and percutaneous coronary intervention).

If successful, this project will provide valuable information regarding the use of an easily available, less expensive, and non-invasive biomarker, HbA1c, to predict incident cardiovascular disease and mortality in a multi-ethnic population. Additionally, it may improve the performance of the existing prediction model, Framingham Risk Score, thereby identifying individuals into accurate risk categories which may result in the appropriate use of preventive interventions.
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ASSOCIATION OF HEMOGLOBIN A1C WITH CARDIOVASCULAR DISEASE AND ALL-CAUSE MORTALITY IN INDIVIDUALS WITHOUT DIABETES – The Multi-Ethnic Study of Atherosclerosis

Amir Azeem\textsuperscript{a}, Joseph Yeboah\textsuperscript{a}, Alain G. Bertoni\textsuperscript{b}, Gregory L. Burke\textsuperscript{c}, Evrim B. Turkbey\textsuperscript{d}, Moyses Szklo\textsuperscript{e}, David M. Herrington\textsuperscript{a}

From the Departments of \textsuperscript{a}Internal Medicine / Cardiology, \textsuperscript{b}Epidemiology and Prevention, \textsuperscript{c}Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC, \textsuperscript{d}Radiology and Imaging Sciences, National Institutes of Health, Bethesda, MD, \textsuperscript{e}Epidemiology and Medicine, Johns Hopkins University, Baltimore, MD
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Address correspondence and reprints to Amir Azeem, MD, Department of Internal Medicine/Cardiology, Wake Forest School of Medicine, Medical Center Blvd., Winston-Salem, North Carolina 27157.
Phone: (336) 716-4357; Fax: (336) 716-8333; Email: aazeem@wakehealth.edu
Abstract:

Background: Currently, ACCF/AHA recommends HbA1c for cardiovascular risk assessment in adults without diabetes. However, the predictive ability of HbA1c, especially in different ethnic groups, remains unclear. We examined the hypothesis that HbA1c predicts cardiovascular disease (CVD), coronary heart disease (CHD), and all-cause mortality in a multi-ethnic population free of diabetes and CVD at baseline, and assessed whether this association varies by race/ethnicity, in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods: During a maximum of 7.1 years (median 5.9 years) of follow-up, 178 participants had an adjudicated coronary heart disease (CHD) event defined as MI, definite angina, probable angina if followed by PCI/CABG, resuscitated cardiac arrest and CHD death; 244 had an adjudicated cardiovascular (CVD) event defined as CHD, stroke, stroke death or CVD death; and 228 died. Cox regression was used to estimate the association of HbA1c with CVD/CHD/all-cause mortality after adjusting for age, gender, race/ethnicity, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, body mass index, urine albumin-creatinine ratio, education level, and cigarette smoking. Receiver Operating Curve analysis (ROC) was used to examine the predictive value of HbA1c for cardiovascular risk assessment. Similar models were used to evaluate these associations stratified by ethnicity.

Results: Among 5069 non-diabetic participants, 53% were women, 43% Caucasian, 25% African American and 20% Hispanic, and 11.6% Chinese. In the total cohort, HbA1c was
not significantly associated with incident CVD, CHD, or mortality (HR [95%CI]: 1.31 [0.90-1.89], 1.44 [0.93-2.21], and 1.16 [0.80-1.68] respectively). There was significant interaction between HbA1c and ethnicity with respect to incident CVD (p=0.02). In stratified analysis, HbA1c was independently and significantly associated with CVD only in Caucasians (2.21 [1.31-3.72]) but not in other ethnicities. Overall, addition of HbA1c to Framingham Risk Score did not improve area under the curve for incident CVD (0.7024 vs. 0.7028; p= 0.92). In Caucasians the addition of HbA1c to the Framingham Risk Score also did not improve area under the curve (0.7013 vs. 0.7163; p =0.35) for incident CVD. Similar results were obtained for the outcome of CHD.

**Conclusion:** Overall, HbA1c was not an independent predictor of incident CVD, CHD or all-cause mortality in adults without diabetes after a median follow up of 5.9 years. In subgroup analysis, HbA1c was significantly associated with incident CVD and CHD only in Caucasians. However, it failed to provide any significant incremental predictive information above and beyond traditional risk factors in the entire cohort as well as in Caucasians.

**Key Words:** Hemoglobin A1c, Cardiovascular Disease, Death, Population
BACKGROUND:

Hemoglobin A1c (HbA1c) has been used as a glycemic marker for monitoring of long-term glycemic control in patients with diabetes\(^1\). It represents average blood glucose over an extended period of time.\(^2\) Compared to fasting blood glucose (FBG), testing for HbA1c is more convenient as there is no need for overnight fast, HbA1c is more stable at room temperature, and between- and within-subject coefficients of variation are much lower.\(^3\) Furthermore, HbA1c test has been standardized and is now widely available.\(^4\) As a result, in 2010, American Diabetes Association (ADA) has included HbA1c as one of the diagnostic criteria for classification of diabetes and, like FBG, the cut points are based upon its association with microvascular complications.\(^5\) Individuals with HbA1c values $\geq 6.5\%$ are now considered to have diabetes and those with HbA1c values 5.7\% to 6.4\% are considered have prediabetes; below 5.7\%, it is categorized as normal.\(^5\)

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality.\(^6\) Current risk prediction models do not perform well for a large proportion of individuals at risk for CVD and death,\(^7\) in particular for ethnic minorities. Thus, there is a need to explore additional potentially modifiable biomarkers that can improve CVD prediction.

Diabetes mellitus is a strong risk factor for cardiovascular disease (CVD) and mortality\(^8\)\(^-\)\(^11\) and considered a coronary heart disease (CHD) risk equivalent\(^12\)\(^,\)\(^13\)\(^,\)\(^14\) \ Higher levels of HbA1c in patients with diabetes are associated with microvascular complications\(^15\) and CVD.\(^16\)\(^-\)\(^18\) Even in the non-diabetic range, HbA1c seems to be correlated with adverse cardiovascular outcomes.\(^19\)\(^,\)\(^20\) Currently, it is recommended that HbA1c may be used for
cardiovascular risk stratification in individuals without diabetes.\textsuperscript{21} However, the evidence justifying its use for risk stratification for cardiovascular events is much less abundant than the evidence supporting its use to predict development of diabetes – particularly when considering non-Caucasian populations.\textsuperscript{22}

Accordingly, we used Multi-Ethnic Study of Atherosclerosis (MESA) data to test the hypothesis that HbA1c is an independent predictor of CVD and CHD events and all-cause mortality in participants without diabetes above and beyond traditional CVD risk factors. While previous studies were done in populations representing one \textsuperscript{19, 23} or two \textsuperscript{20} ethnic groups, our analyses include diverse ethnic groups in a single cohort for the first time. The main secondary aim of this study was to assess whether the association between HbA1c and CVD and mortality varied by race and ethnicity.

METHODS:

Study Population:

The details of the objectives and design of the MESA study have been published previously.\textsuperscript{24} In brief, the MESA is a cohort study of community dwellers to investigate the prevalence, correlates, and progression of subclinical atherosclerotic disease. The MESA cohort at baseline consisted of 6,814 women and men, aged 45-84 years, who were recruited between July 2000 and August 2002 from six U.S. communities: Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; northern Manhattan, New York; and St. Paul, Minnesota. Cohort participants are 38% white, 28% African American, 22% Hispanic, and 12% Chinese.
Persons with a history of physician-diagnosed myocardial infarction, angina, heart failure, stroke, or transient ischemic attack, or who had undergone an invasive procedure for CVD (coronary artery bypass graft surgery, angioplasty, valve replacement, pacemaker placement, or other vascular surgeries) were excluded. All participants provided written informed consent and the study was approved by the institutional review boards of each study site.

For the present study, we used the second MESA examination (2002-2004) as baseline since HbA1c was measured during that visit. Out of 6233 participants who attended exam 2, we included 5069 participants who did not have known history of doctor-diagnosed diabetes, were not taking medications for diabetes, had FBG<126 mg/dl and HbA1c<6.5%, and who did not have missing HbA1c values and follow up information (Figure-1). The cohort was followed through 2011 for this report.

**Data Collection:**

Data points/variables obtained during second MESA exam were used for this analysis unless noted otherwise. MESA participants provided demographic information, lifestyle information, and medical history by self-report. For measure of baseline physical activity, Typical Week Physical Activity Survey (TWPAS) was used. Participants were considered to be active smokers if they smoked at least one cigarette in the last 30 days. Socioeconomic status was estimated by educational level and total household income of the family. We defined educational levels categorically attending up to 11th grade, graduating from high school or passing GED or attending some college without a degree,
having technical school certificate of an associate degree, and having a bachelor’s degree or attending graduate or a professional school. Medication bottles were reviewed to obtain information regarding use of antihypertensive, diabetes and other medications. Height, weight, and waist circumference were measured by trained personal. Body mass index (BMI) was calculated as weight (kg) divided by height (m$^2$). Three readings of systolic and diastolic blood pressure were measured at rest and the average of second and third values were recorded. Hypertension was defined as use of hypertensive medication(s), mean resting systolic blood pressure $\geq$140 mm Hg or diastolic blood pressure $\geq$90 mmHg. Total cholesterol (Chol) and high density lipoprotein (HDL) cholesterol were measured from blood samples obtained after a 12-h fast. Low-density lipoprotein (LDL) cholesterol was estimated by the Friedewald equation$^{26}$. Fasting blood glucose (FBG; serum) was measured by the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, New York). HbA1c was measured by high-performance liquid chromatography (HPLC), as used in the Diabetes Control and Complications Trial. Diabetes was defined as history of doctor-diagnosed diabetes, use of anti-hyperglycemic medications, FBG $\geq$126 mg/dl or HbA1c $\geq$6.5%. Prediabetes was defined as FBG 100 to 125 mg/dl or HbA1c 5.7% to 6.4%. Framingham risk scores were calculated based upon ATP-III guidelines (JAMA 2001).

**Ascertainment of Outcomes:**

All outcomes were adjudicated by a MESA study committee that included cardiologists, physician epidemiologists, and neurologists.$^{24}$ For the purpose of this study, we used CVD events, CHD events, and death due to any cause as outcomes. Cardiovascular
disease event was defined as composite of CHD death, resuscitated cardiac arrest, incident myocardial infarction (MI), definite angina, probable angina (if followed by coronary artery bypass grafting [CABG] or percutaneous coronary intervention [PCI]), ischemic stroke, stroke death, other atherosclerotic death, or other CVD death as defined by the MESA protocol. Coronary heart disease event was defined as a composite of CHD death, resuscitated cardiac arrest, incident MI, definite angina, or probable angina (if followed by CABG or PCI).

**Statistical Analysis:**
Baseline characteristics of the whole cohort and different ethnic groups are presented. Continuous variables are reported as means and standard deviations, and categorical variables as frequencies and percentages. Distribution of continuous variables was assessed by examining the histograms, box-whisker plots and normal curves. Analysis of variance (ANOVA) was used to compare unadjusted means across ethnic groups for continuous variables and Chi-Square test for categorical variables. Time to events was calculated from the second MESA exam for all participants until new event or end of the year 2011. For our main analyses, HbA1c was used as a continuous variable. Crude incidence rates for outcomes of interest were calculated during the cumulative time at risk and presented per thousand person-years. Cox regression was used to estimate the association of 1% increases in HbA1c (as continuous variable) with CVD and CHD events and all-cause mortality before and after adjustment for traditional risk factors. Covariates were selected based upon previous evidence of association with outcomes. The linearity assumption was assessed by inspecting the plot of the residuals against HbA1c. The
assumption of proportionality was tested by creating time dependent covariates (interaction of predictor with log [time]), including them in the full model to build an extended model, and then using a global proportionality test statement. Time dependent covariates were not included if the proportionality test was not significant. We tested for interaction (both multiplicative and additive) between HbA1c and ethnicity. For additive interaction, we used relative excess risk due to interaction (RERI); a value above zero suggests presence of interaction. We performed Receiver Operating Curve (ROC) analysis and calculated the area under the receiver operating characteristic curve (AUC) statistic to assess discrimination (i.e. the ability of the risk-prediction model to differentiate between patients who experienced a cardiovascular disease event during the study and those who did not) by traditional risk factors (Framingham Risk Score) with and without the addition of HbA1c in the models; a value of 1 represents perfect discrimination. We used 10-year Framingham Risk Score (FRS) cut-off of 10% and 20% to describe low, intermediate, and high risk categories. Significance level was set at alpha of 0.05 (two-sided). All analyses were conducted using SAS software, version 9.2 (SAS Institute, Cary, North Carolina).

RESULTS:
Among 5069 non-diabetic participants, 43% were Caucasians, 25% African American, 20% Hispanics, and 12% Chinese (Table-1). Fifty three percent were female, 41% hypertensive, and 41% former and 11% current smoker. The mean age, BMI, and HbA1c were 63 years, 28 kg/m², and 5.4% respectively. Caucasians had lowest mean HbA1c while Chinese had lowest BMI. Hypertension was more prevalent in African Americans.
There was modest but significant correlation between FBG and HbA1c (Pearson coefficient 0.40; p <0.0001). HbA1c identified more individuals as having prediabetes as compared to FBG (26% vs 18%). Participants with HbA1c levels in the prediabetes range were older, mostly non-Caucasians, had higher BMI, and had higher prevalence of other CV risk factors. HbA1c reclassified 831 (20%) individuals with normal FBG as having prediabetes and reclassified 434 (46.7%) participants with prediabetes based on FBG as normal. This reclassification was more pronounced in ethnic minorities. During a median follow up of 5.9 years (maximum: 7.1 years) there were 244 (4.8%) adjudicated CVD events, 178 (3.5%) incident CHD and 228 (4.5%) deaths. At baseline, 267 (5.37%) participants had a Framingham Risk Score (FRS) above 20% and, of these, only 34 (12.7%) developed CVD and 27 (10.1%) developed CHD events. The unadjusted incidence rates for CVD and CHD were higher in Caucasians while the all-cause mortality rate was highest in African Americans (Table-2).

**HbA1c and CVD, CHD, and All-Cause Mortality in the Total Cohort:**

For every 1-percent increase in HbA1c, CVD, CHD, and all-cause mortality risk was 1.59, 1.71 and 1.76 times higher in univariate analyses, respectively (Table-3). However, in multivariate analyses controlling for age, gender, race/ethnicity, education level, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, body mass index, urine albumin-creatinine ratio and cigarette smoking the hazard ratios for CVD, CHD, and all-cause mortality were attenuated and no longer statistically significant (HR [95%CI] for CVD, CHD, and all-cause mortality: 1.31 [0.90-1.89], 1.44 [0.93-2.21], and 1.16 [0.80-1.68]). In the full
model, we also observed that the age, male gender, current smoking, total cholesterol, systolic blood pressure, anti-hypertension medication use, and urine albumin-creatinine ratio were independently associated with incident CVD and CHD events while age, systolic blood pressure, hypertensive medication use, and urine albumin-creatinine ratio were independently predictive of incident stroke (not shown). The linearity assumption of the Cox regression model was not violated for the outcomes of CVD, CHD and all-cause mortality. The assumption of proportionality was also met for CVD (global proportionality test $p=0.23$), CHD ($p=0.24$), MI ($p=0.21$), angina ($p=0.75$), stroke ($p=0.78$) and all-cause mortality ($p=0.73$). In the ROC analyses, the addition of HbA1c to FRS did not improve the AUC for incident CVD ($0.7024$ vs. $0.7028$, $p=0.92$; Fig-2).

**HbA1c and CVD, CHD, and All-Cause Mortality Stratified by Ethnicity:**

There was evidence of effect modification by race/ethnicity; the multiplicative interaction between HbA1c and ethnicity in the full model was significant for CVD ($p=0.02$ for overall heterogeneity among ethnic groups and $p=0.009$ for Caucasian versus the other groups combined). For additive interaction we used Relative Excess Risk due to Interaction (RERI) in Caucasians (versus the other groups combined) and used the median HbA1c in the whole population as a cut-point to create a dichotomous variable. The RERI was found to be significant (RERI [95%CI]: $1.37 \ [0.74-\ 2.0]$, $p <0.001$) suggesting that the relative risk of having CVD in Caucasians was 1.37 times higher with each 1 percent increase in HbA1c due to HbA1c-ethnicity interaction than if there were no interaction between HbA1c and ethnicity.
For CHD, multiplicative interaction for overall heterogeneity was significant (p=0.02) and was also trending towards significance for Caucasians versus other groups (p=0.10). The additive interaction between HbA1c as a categorical variable and ethnicity (Caucasians versus other ethnic groups) was significant (RERI [95%CI]: 1.52 [0.71-2.32], p<0.001).

For all-cause mortality, multiplicative interaction for overall heterogeneity was significant (p=0.04) and was also trending towards significance for Caucasians versus other groups (p=0.09). For additive interaction between HbA1c as a categorical variable with median as a cut point and Caucasians (versus other groups combined), the RERI was significant (0.56 [0.05-1.07], p=0.03).

When Cox regression models were repeated stratified by ethnicity, the HbA1c was found to be an independent predictor of CVD only in Caucasians (Table-4); every 1-percent increase in HbA1c was associated with doubling the risk for CVD (HR=2.21; p=0.003). Similar results were obtained when CHD was used as the outcome (HR=2.06; p=0.01). However, despite a positive association of HbA1c with incident CVD events in fully adjusted Cox regression model, the addition of HbA1c to Framingham Risk Score failed to provide any significant incremental information to predict new CVD (AUC: 0.7013 vs. 0.7163, p =0.35).
DISCUSSION:

In this paper, we report that HbA1c is not an independent predictor of incident CVD, CHD, or all-cause mortality in a community-based, multi-ethnic cohort free of clinical CVD or diabetes at the baseline after a median follow up of almost 6 years. In subgroup analysis, we found a statistically significant association of HbA1c with CVD and CHD after adjusting for traditional risk factors only in Caucasians. However, the addition of HbA1c to FRS failed to significantly improve AUC in this ethnic group as well.

Our findings, while adding to the existing knowledge, are in contrast to some but not all studies done previously evaluating the role of HbA1c as a biomarker to predict adverse cardiovascular outcomes and mortality in non-diabetics. Kehl et al evaluated Third National Health and Nutrition Examination Survey (NHANES-III) data and reported that the higher levels of HbA1c in the non-diabetic range were associated with all-cause and CVD mortality in non-Hispanic whites but not in Mexican-Americans or African Americans. Similarly, Silbernagel et al reported that HbA1c was associated with cardiovascular and all-cause mortality in a German cohort of 2500 participants; however, compared to our study, there was no ethnic diversity, more than 50 % of participants had documented coronary artery disease (CAD) at baseline, and the association was significant for the HbA1c only in the diabetic range in multivariate analysis. In contrast, all participants in our study have HbA1c in the non-diabetic range. In 2004, Khaw et al reported that the HbA1c significantly predicted all-cause mortality, CVD, and CHD independent of traditional risk factors in a European cohort from Norfolk, England. Later, in 2008, based on the same cohort and 8.5 years of follow up,
Simmons et al showed that addition of HbA1c made a small but statistically significant improvement in CVD risk prediction only in men. In a biracial cohort (Atherosclerosis Risk in Communities [ARIC]) of more than ten thousand participants with 22% African-Americans and 14 years of follow up, Selvin et al found that higher levels of HbA1c were associated with increased risk of diabetes as well as CVD events, and that the ethnicity did not modify that association although the level of HbA1c was higher in African Americans. In another study involving only American Indians, Wang et al concluded that there was no independent association of HbA1c with CVD risk for HbA1c in the prediabetic range. Lastly, in a subanalysis from a clinical trial from Japan, Nishimura et al reported a linear relationship between HbA1c and CVD risk independent of use of statins, thus suggesting additional management for HbA1c on top of statin use. Currently, it is recommended that HbA1c may be used for assessment of cardiovascular risk in non-diabetics in 2010. However, our present study does not support this recommendation after a maximum follow up of 7 years.

While studies mentioned earlier examined the association of HbA1c with clinical outcomes, McNeely et al assessed the association between HbA1c and subclinical CVD. They reported that the highest quartile of HbA1c in non-diabetic MESA participants had a weak positive association with common and internal carotid intimal-medial wall thickness but no association with prevalent coronary artery calcium (CAC). This observation may be consistent with our findings, since CAC is highly correlated with CVD and CHD events.
The observation of different prevalence rates of prediabetes depending upon whether HbA1c or FBG criteria were used, particularly in minority groups, was similar to that of previous reports.\textsuperscript{36-38} These findings may have important clinical significance since individuals in the prediabetic group not only are at higher risk of developing diabetes\textsuperscript{39}, but they also have higher burden of other cardiovascular risk factors. Thus, intervention strategies targeting HbA1c to prevent diabetes may also have a favorable impact on the cardiovascular outcomes. However, this hypothesis needs confirmation. Lastly, we observed that the strong univariate correlation of HbA1c to CVD and CHD events and mortality was attenuated by addition of cardiovascular risk factors which may suggest that the increased risk associated with HbA1c may be due to its coexistence with known clinical risk factors. This observation further supports the need for rigorous control of modifiable risk factors to improve cardiovascular health.

**Strengths and Limitations:**

Our study had a number of limitations. Our median follow up time was only 5.9 years (maximum: 7.1 years) and the total number of events was low, thus, resulting in limited statistical power. Only a single measure of HbA1c was available at baseline. However, our results were consistent for the association of HbA1c with outcomes even after excluding participants who developed incident diabetes during the follow up period. We have adjusted for known clinical risk factors; however, residual confounding may still be present as ours was an observational study. In addition, participants were free of clinical CVD at the baseline which limits our study’s external validity. Strengths of our study
include ethnic diversity of the study cohort, standardized data collection and adjudicated event classification.

CONCLUSION:
In this multi-ethnic population free of diabetes or clinical CVD at baseline, HbA1c was not an independent predictor of incident CVD, CHD or all-cause mortality after a median follow up of 5.9 years. In subgroup analysis, HbA1c was significantly associated with incident CVD and CHD only in Caucasians; however, it failed to provide significant predictive information above and beyond traditional risk factors in this ethnic group. Our observations call into question the appropriateness of current American Heart Association recommendation to use HbA1c as a potential risk marker to predict incident cardiovascular disease since it remains unclear whether HbA1c can improve the predictive power of existing models to assess cardiovascular risk. Further work is needed to assess the utility of HbA1c to provide any incremental information in predicting cardiovascular risk in individuals without diabetes mellitus.
<table>
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<th>Characteristic</th>
<th>Overall</th>
<th>Caucasians</th>
<th>Afr. Ameri.</th>
<th>Hispanics</th>
<th>Chinese</th>
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<tr>
<td>Participants, n (%)</td>
<td>5069 (100)</td>
<td>2187 (43.1)</td>
<td>1269 (25.0)</td>
<td>1023 (20.2)</td>
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<td>Men, n (%)</td>
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<td>1033 (47.2)</td>
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<td>3738 (73.7)</td>
<td>1836 (83.9)</td>
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<td>1331 (26.3)</td>
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<td>91.7 (9.4)</td>
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<td>4131 (81.6)</td>
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<td>267 (5.3)</td>
<td>102 (4.7)</td>
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<td>2082 (41.4)</td>
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<td>Chol&lt;sub&gt;e&lt;/sub&gt;, mg/dl [mean (std)]</td>
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<td>54.9 (15.4)</td>
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<tr>
<td>Trig&lt;sub&gt;g&lt;/sub&gt;, mg/dl [mean (std)]</td>
<td>126.8 (74.5)</td>
<td>127.9 (74.5)</td>
<td>101.3 (54.4)</td>
<td>146.6 (80.3)</td>
<td>142.8 (85.3)</td>
<td>&lt;0.0001</td>
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<tr>
<td>LDL&lt;sub&gt;h&lt;/sub&gt;, mg/dl [mean (std)]</td>
<td>115.4 (31.8)</td>
<td>114.7 (31.5)</td>
<td>115.7 (33.9)</td>
<td>117.7 (31.6)</td>
<td>113.0 (28.3)</td>
<td>0.02</td>
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<tr>
<td>Lipid lowering drugs [n (%)]</td>
<td>942 (19.4)</td>
<td>497 (24.1)</td>
<td>206 (16.7)</td>
<td>151 (15.4)</td>
<td>88 (15.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urine Alb/Cr ratio, mg/g [mean (std)]</td>
<td>15.5 (74.7)</td>
<td>13.2 (59.1)</td>
<td>15.7 (71.7)</td>
<td>15.4 (94.2)</td>
<td>23.9 (130.4)</td>
<td>0.02</td>
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<td>Smoking Current [n (%)]</td>
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<tr>
<td>Never</td>
<td>2360 (46.9)</td>
<td>896 (41.3)</td>
<td>528 (42)</td>
<td>504 (49.6)</td>
<td>432 (73.5)</td>
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<td>Former</td>
<td>2100 (41.7)</td>
<td>1047 (48.2)</td>
<td>522 (41.5)</td>
<td>400 (39.4)</td>
<td>131 (22.3)</td>
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<td>Current</td>
<td>572 (11.4)</td>
<td>228 (10.5)</td>
<td>207 (16.5)</td>
<td>112 (11.0)</td>
<td>25 (4.3)</td>
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<tr>
<td>Education [n (%)]</td>
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<td>&lt;0.0001</td>
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<td>Level 1</td>
<td>750 (14.8)</td>
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<td>124 (9.8)</td>
<td>415 (40.6)</td>
<td>117 (19.8)</td>
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<td>Level 2</td>
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<td>493 (39.1)</td>
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<td>153 (25.9)</td>
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<td>Level 3</td>
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<td>182 (14.4)</td>
<td>126 (12.3)</td>
<td>71 (12.1)</td>
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<td>1963 (38.8)</td>
<td>1134 (51.9)</td>
<td>462 (36.6)</td>
<td>119 (11.6)</td>
<td>248 (42.1)</td>
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Table-1 Baseline Characteristics of participants in entire cohort and in different ethnic groups – Multi-Ethnic Study of Atherosclerosis
*Unadjusted comparison across ethnic groups

\( ^{a} \)Fasting blood glucose, \( ^{b} \)systolic blood pressure, \( ^{c} \)diastolic blood pressure, \( ^{d} \)hypertension,

\( ^{e} \)total cholesterol, \( ^{f} \)high density cholesterol, \( ^{g} \)triglyceride, \( ^{h} \)low density cholesterol;

Level 1: Attending up to 11th grade education;

Level 2: High school graduate or attending some college without college degree;

Level 3: Technical school certificate or an associate degree;

Level 4: Bachelor’s degree or attended graduate/professional school
### Table-2: Unadjusted Incidence Rates – Multi-Ethnic Study of Atherosclerosis

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<th>Sample Size (N)</th>
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<th>Person-Years (PYs)</th>
<th>Incidence Rate (per 1000 PYs)</th>
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<tr>
<td>Entire Cohort</td>
<td>5069</td>
<td>244 (4.8)</td>
<td>36715.8</td>
<td>6.64</td>
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<tr>
<td>Caucasian</td>
<td>2187</td>
<td>136 (3.2)</td>
<td>15911.4</td>
<td>8.55</td>
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<tr>
<td>AA</td>
<td>1269</td>
<td>53 (4.2)</td>
<td>9084.5</td>
<td>5.83</td>
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<td>7368.2</td>
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<tr>
<td>Chinese</td>
<td>590</td>
<td>10 (1.7)</td>
<td>4351.7</td>
<td>2.29</td>
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<td><strong>CHD</strong></td>
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<td>37 (3.0)</td>
<td>9116.4</td>
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<td>7398.8</td>
<td>4.19</td>
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<td>Chinese</td>
<td>590</td>
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<td>4355.3</td>
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<td>83 (1.6)</td>
<td>37076.6</td>
<td>2.24</td>
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<td>16115.6</td>
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<td><strong>All-Cause Mortality</strong></td>
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<td></td>
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<td>37574.5</td>
<td>6.07</td>
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<td>9294.7</td>
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Table-3: Risk of CVD, CHD, and All-Cause Mortality with every 1% increase in HbA1c in a multi-ethnic population free of CVD and diabetes – Multi-Ethnic Study of Atherosclerosis

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<th>HR*</th>
<th>95% CI**</th>
<th>P value</th>
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<td>244</td>
<td>1.59</td>
<td>1.15-2.22</td>
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<tr>
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<td>83</td>
<td>1.27</td>
<td>0.72-2.22</td>
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<td>105</td>
<td>1.90</td>
<td>1.15-3.13</td>
<td>0.012</td>
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<td>66</td>
<td>1.35</td>
<td>0.72-2.54</td>
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<td>228</td>
<td>1.76</td>
<td>1.25-2.47</td>
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<td>244</td>
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<td>1.06-2.12</td>
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<td>83</td>
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<td>0.66-2.18</td>
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<td>105</td>
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<td>CVD</td>
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<td>0.90-1.89</td>
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<td>100</td>
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*HR, Hazard Ratio; **CI, Confidence interval

CVD: composite of myocardial infarction (MI), resuscitated cardiac arrest, coronary heart disease (CHD) death, angina or probable angina, stroke, stroke death, other CVD death, or other atherosclerotic death

CHD: composite of myocardial infarction (MI), resuscitated cardiac arrest, CHD death, angina, or probable angina

Model-1: Unadjusted

Model-2: Adjusted for age, gender, and race/ethnicity
Model-3: Additionally adjusted for body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level
Table-4: Risk of CVD, CHD, and All-Cause Mortality with every 1% increase in HbA1c stratified by race/ethnicity in a multi-ethnic population free of CVD and diabetes – Multi-Ethnic Study of Atherosclerosis

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<th>Hispanics</th>
<th>Chinese</th>
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<td>N (E)</td>
<td>N (E)</td>
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<td>1023 (45)</td>
<td>590 (10)</td>
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<tr>
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<td>1.53 (0.69-3.36), 0.29</td>
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<td>1269 (53)</td>
<td>1023 (45)</td>
<td>590 (10)</td>
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<td>578 (10)</td>
</tr>
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<td>1023 (31)</td>
<td>590 (7)</td>
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<td>590 (7)</td>
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<td>All-Cause Mortality</td>
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<td>1023 (31)</td>
<td>590 (20)</td>
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<td>578 (20)</td>
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<td>3.36 (0.67-16.83), 0.25</td>
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*Sample size; b Number of events;

CVD: composite of myocardial infarction (MI), resuscitated cardiac arrest, coronary heart disease (CHD) death, angina or probable angina, stroke, stroke death, other CVD death, or other atherosclerotic death

CHD: composite of myocardial infarction (MI), resuscitated cardiac arrest, CHD death, angina, or probable angina

Model-1: Unadjusted

Model-2: Adjusted for age and gender
Model-3: Additionally adjusted for body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level
Figure 1: Study Flow Diagram – Multi-Ethnic Study of Atherosclerosis (MESA)

MESA Exam 2
n=6233

- Missing HbA1c a = 91
- Missing follow up = 5

n=6137

- HbA1c ≥ 6.5% = 698

n=5439

- History of diabetes
- Diabetes medications
- FBG b ≥ 126 mg/dl

N=5069

a Hemoglobin A1c

b Fasting blood glucose
Figure-2: Receiver-Operating Curve analysis including all participants to assess the discrimination ability of HbA1c when added to Framingham Risk Score (FRS) - Multi-Ethnic Study of Atherosclerosis
**Figure-3:** Receiver-Operating Curve analysis in Caucasians to assess the discrimination ability of HbA1c when added to Framingham Risk Score (FRS) - Multi-Ethnic Study of Atherosclerosis.
Appendix Table- : Differential classification of Normal and Prediabetes status by fasting blood glucose (FBG) and Hemoglobin A1c (HbA1c) – Multi-Ethnic Study of Atherosclerosis

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<th>HbA1c</th>
<th></th>
<th></th>
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<td>Prediabetes</td>
<td>Total</td>
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</tr>
<tr>
<td>Prediabetes</td>
<td>434</td>
<td>500</td>
<td>934 (18.4%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3734</td>
<td>1331 (26.3%)</td>
<td>5065</td>
<td></td>
</tr>
</tbody>
</table>

Number of missing FBG values: 4
Normal: FBG <100 mg/dl or HbA1c <5.7%
Prediabetes: FBG 100 to 125 mg/dl or HbA1c 5.7 to 6.4%
REFERENCES:


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CHAPTER-III: ANCILLARY ANALYSES

In a multi-ethnic adult population free of cardiovascular disease (CVD) and diabetes at the baseline exam in the Multi-ethnic Study of Atherosclerosis (MESA), HbA1c (as a continuous variable) was not an independent predictor of CVD, coronary heart disease (CHD), and all-cause mortality after adjusting for potential confounders. Our findings are in contrast to some,\textsuperscript{1, 2} but not all,\textsuperscript{3} of the previous reports. Furthermore, we did find an independent and significant association of HbA1c with cardiovascular events only in Caucasians but not in other ethnic groups. The positive association of HbA1c with incident cardiovascular disease and all-cause mortality reported in two previous large cohort studies, Atherosclerosis Risk in Communities (ARIC) and European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk), may very well be due to the fact that almost 80\% of the participants in the former and 100\% in the later were Caucasians in contrast to our study which had 43\% Caucasian Americans. Furthermore, we did assess the predictive ability of HbA1c when added to Framingham Risk Score by performing Receiver Operating Curve (ROC) analysis and observed that HbA1c did not improve area under the curve (AUC) in the total cohort (0.7024 versus 0.7028; \(p = 0.92\)). In Caucasians, there was slight improvement in AUC which was statistically nonsignificant (0.7013 versus 0.7163; \(p = 0.35\)). We performed additional analyses to assess the distribution of HbA1c in different ethnic groups and gender in addition to further clarify the association of HbA1c with outcomes of interest as demonstrated in the next few tables and figures. These include the new and emerging net reclassification improvement (NRI) analysis to assess whether our biomarker being assessed for
cardiovascular risk prediction, HbA1c, improves the performance of existing prediction model based upon Framingham study.
Figure-1: Racial/ethnic differences in the mean HbA1c after adjusting for age, gender, BMI, and fasting blood glucose - Multi-Ethnic Study of Atherosclerosis

Trend p value <0.0001 across age categories; CA: Caucasian Americans; AA: African Americans

We performed generalized linear model (least square methods) to calculate the mean HbA1c in different age categories, stratified by race/ethnicity, after adjusting for covariates. There seems to be a relation between increasing age and HbA1c levels. Furthermore, non-Caucasians, especially, African Americans, were found to have higher levels of HbA1c compared to Caucasians even after adjusting for age, gender, body mass index, and fastig blood glucose. Although the exact mechanisms for these ethnic differences are not clear, genetic factors affecting the rate of non-enzymatic glycation and higher degree of dysglycemia may explain some of this variation.
Figure-2: Gender differences in mean HbA1c after adjusting for age, race/ethnicity, BMI, and fasting blood glucose - Multi-Ethnic Study of Atherosclerosis (MESA)

Trend p value <0.001 across age categories

We performed generalized linear model (least square methods) to calculate the mean HbA1c in different age categories, stratified by gender, after adjusting for covariates. We observed that women had higher levels of HbA1c until the age 75. The exact mechanisms governing these gender differences are not entirely clear and it is not clear whether exposure to different sex hormones in earlier age categories have any effect on these variations.
Table 1: Risk of CVD, CHD, and mortality with every 1% increase in HbA1c within the categories of HbA1c in a multi-ethnic population free of CVD and diabetes – Multi-Ethnic Study of Atherosclerosis

<table>
<thead>
<tr>
<th>HbA1c Category</th>
<th>N</th>
<th>HbA1c, % Mean (SD)</th>
<th>#Events</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CVD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>542</td>
<td>4.73 (0.2)</td>
<td>19</td>
<td>10.58</td>
<td>0.25- 453</td>
<td>0.22</td>
</tr>
<tr>
<td>5.0 - &lt;5.5</td>
<td>2171</td>
<td>5.23 (0.13)</td>
<td>91</td>
<td>0.72</td>
<td>0.14-3.82</td>
<td>0.69</td>
</tr>
<tr>
<td>5.5 - &lt;6.0</td>
<td>1910</td>
<td>5.66 (0.13)</td>
<td>107</td>
<td>1.88</td>
<td>0.39-8.88</td>
<td>0.43</td>
</tr>
<tr>
<td>6.0 - &lt;6.5</td>
<td>446</td>
<td>6.12 (0.13)</td>
<td>27</td>
<td>0.67</td>
<td>0.03-15.89</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>CHD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>542</td>
<td>4.73 (0.2)</td>
<td>12</td>
<td>14.72</td>
<td>0.11- 2059</td>
<td>0.29</td>
</tr>
<tr>
<td>5.0 - &lt;5.5</td>
<td>2171</td>
<td>5.23 (0.13)</td>
<td>66</td>
<td>0.55</td>
<td>0.08-3.88</td>
<td>0.55</td>
</tr>
<tr>
<td>5.5 - &lt;6.0</td>
<td>1910</td>
<td>5.66 (0.13)</td>
<td>81</td>
<td>2.75</td>
<td>0.47-16.07</td>
<td>0.26</td>
</tr>
<tr>
<td>6.0 - &lt;6.5</td>
<td>446</td>
<td>6.12 (0.13)</td>
<td>19</td>
<td>0.13</td>
<td>0.002- 9.06</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>All-Cause Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>542</td>
<td>4.73 (0.2)</td>
<td>21</td>
<td>5.1</td>
<td>0.38-82.58</td>
<td>0.21</td>
</tr>
<tr>
<td>5.0 - &lt;5.5</td>
<td>2171</td>
<td>5.23 (0.13)</td>
<td>73</td>
<td>0.3</td>
<td>0.04-1.94</td>
<td>0.20</td>
</tr>
<tr>
<td>5.5 - &lt;6.0</td>
<td>1910</td>
<td>5.66 (0.13)</td>
<td>109</td>
<td>4.38</td>
<td>1.02-18.87</td>
<td>0.04</td>
</tr>
<tr>
<td>6.0 - &lt;6.5</td>
<td>446</td>
<td>6.12 (0.13)</td>
<td>25</td>
<td>3.41</td>
<td>0.13-87.61</td>
<td>0.46</td>
</tr>
</tbody>
</table>

All models adjusted for age, gender, and race/ethnicity, body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level.

There was some suggestion of an association in the HbA1c category of 5.5 to <6.0% although statistical significance could only be achieved for the outcome of all-cause mortality. These results should be interpreted with caution because of low power.
**Table-2:** Risk of CVD, CHD, and mortality across categories of HbA1c when compared to a reference category (HbA1c 5.0 to ≤5.5) in a multi-ethnic population free of CVD and diabetes – Multi-Ethnic Study of Atherosclerosis

<table>
<thead>
<tr>
<th>HbA1c Category</th>
<th>N</th>
<th>HbA1c, % Mean (SD)</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CVD (244)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>542</td>
<td>4.73 (0.2)</td>
<td>1.003</td>
<td>0.60-1.67</td>
<td>0.99</td>
</tr>
<tr>
<td>5.0 - &lt;5.5 (Ref)</td>
<td>2171</td>
<td>5.23 (0.13)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.5 - &lt;6.0</td>
<td>1910</td>
<td>5.66 (0.13)</td>
<td>1.19</td>
<td>0.88-1.61</td>
<td>0.25</td>
</tr>
<tr>
<td>6.0 - &lt;6.5</td>
<td>446</td>
<td>6.12 (0.13)</td>
<td>1.29</td>
<td>0.81-2.04</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td></td>
<td></td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CHD (178)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>542</td>
<td>4.73 (0.2)</td>
<td>0.91</td>
<td>0.49-1.69</td>
<td>0.76</td>
</tr>
<tr>
<td>5.0 - &lt;5.5 (Ref)</td>
<td>2171</td>
<td>5.23 (0.13)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.5 - &lt;6.0</td>
<td>1910</td>
<td>5.66 (0.13)</td>
<td>1.33</td>
<td>0.93-1.88</td>
<td>0.11</td>
</tr>
<tr>
<td>6.0 - &lt;6.5</td>
<td>446</td>
<td>6.12 (0.13)</td>
<td>1.30</td>
<td>0.75-2.24</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All-Cause Mortality (228)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5.0</td>
<td>542</td>
<td>4.73 (0.2)</td>
<td>1.34</td>
<td>0.82-2.19</td>
<td>0.24</td>
</tr>
<tr>
<td>5.0 - &lt;5.5 (Ref)</td>
<td>2171</td>
<td>5.23 (0.13)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.5 - &lt;6.0</td>
<td>1910</td>
<td>5.66 (0.13)</td>
<td>1.34</td>
<td>0.98-1.84</td>
<td>0.07</td>
</tr>
<tr>
<td>6.0 - &lt;6.5</td>
<td>446</td>
<td>6.12 (0.13)</td>
<td>1.13</td>
<td>0.69-1.86</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td></td>
<td></td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*DF=3

All models adjusted for age, gender, and race/ethnicity, body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level

We did not observe any significant association between any category of HbA1c (compared to reference category) and incident outcomes. These observations are in...
contrast to some previously reported.\textsuperscript{1,2} Interestingly, we also noted a J-shaped trend for
the association between HbA1c and CVD and all-cause mortality although the CI did not
reach statistical significance. Similar patterns were reported in the some previous
studies.\textsuperscript{4}
Table-3: Risk of CVD, CHD, and mortality with every 1% increase in HbA1c within categories of Normal and Prediabetes HbA1c in a multi-ethnic population free of CVD and diabetes – Multi-Ethnic Study of Atherosclerosis

<table>
<thead>
<tr>
<th>HbA1c Category</th>
<th>N</th>
<th>HbA1c, % Mean (SD)</th>
<th>#Events</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CVD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3738</td>
<td>5.25 (0.28)</td>
<td>165</td>
<td>1.23</td>
<td>0.69-2.39</td>
<td>0.42</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>1331</td>
<td>5.89 (0.19)</td>
<td>79</td>
<td>1.49</td>
<td>0.45-4.94</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>CHD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3738</td>
<td>5.25 (0.28)</td>
<td>119</td>
<td>1.51</td>
<td>0.72-3.16</td>
<td>0.28</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>1331</td>
<td>5.89 (0.19)</td>
<td>59</td>
<td>0.92</td>
<td>0.22-3.86</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>All-Cause Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>3738</td>
<td>5.25 (0.28)</td>
<td>142</td>
<td>0.85</td>
<td>0.47-1.53</td>
<td>0.58</td>
</tr>
<tr>
<td>Prediabetes</td>
<td>1331</td>
<td>5.89 (0.19)</td>
<td>86</td>
<td>0.79</td>
<td>0.24-2.64</td>
<td>0.71</td>
</tr>
</tbody>
</table>

All models adjusted for age, gender, and race/ethnicity, body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level.

The point estimates were suggestive of a positive association between HbA1c and incident CVD in individuals with prediabetic HbA1c although the results did not reach statistical significance.
Table-4: Risk of CVD, CHD, and mortality for Prediabetic HbA1c versus Normal HbA1c in a multi-ethnic population free of CVD and diabetes – Multi-Ethnic Study of Atherosclerosis

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>N</th>
<th>#Events</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CVD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prediabetes vs. Normal</td>
<td>5069</td>
<td>224</td>
<td>1.20</td>
<td>0.89-1.61</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prediabetes vs. Normal</td>
<td>5069</td>
<td>178</td>
<td>1.32</td>
<td>0.94-1.85</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>All-Cause Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prediabetes vs. Normal</td>
<td>5069</td>
<td>228</td>
<td>1.35</td>
<td>1.01-1.80</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

All models adjusted for age, gender, and race/ethnicity, body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level.

We defined prediabetes as HbA1c 5.7% to <6.5% and compared the risk associated with prediabetes (versus normal). We noted a significant association between prediabetes and all-cause mortality in fully adjusted models and the point estimates suggested a possible association between prediabetes status based on HbA1c and CVD and CHD although the CI did not reach statistical significance. many, but not all, previous studies have suggested prediabetes to be a risk factor for incident CVD and mortality and advocated aggressive risk factor control in this group.
For additional analyses, we examined the risk of incident events based upon baseline diabetes status (diabetes versus no diabetes). After excluding missing values of HbA1c and follow up time, there were 969 individuals who met the definition diabetes mellitus (FBG ≥126 mg/dl, HbA1c ≥6.5%, history of diabetes mellitus, use of diabetic medications) and of those 84 developed incident CVD and 63 had incident CHD. The number of participants in the nondiabetic group was 5069 and of those 244 developed new CVD events. We observed a significant association between diabetes (versus no diabetes) and risk of incident CVD in unadjusted (1.88 [1.47-2.41]) as well as fully adjusted models (1.35 [1.02-1.77]). Similar results were obtained for the outcome of CHD but not with all-cause mortality. This may suggest that the association of HbA1c with CVD may be linked to the diabetes status and similar patterns were seen by Yeboah et al for the association of impaired fasting glucose and incident CVD.5
Table-5: Risk of CVD, CHD, and mortality for every 1% increase in HbA1c after excluding participants who developed new diabetes (n=123) during the follow-up period – Multi-Ethnic Study of Atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th># Events</th>
<th>HR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CVD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>4946</td>
<td>212</td>
<td>0.97</td>
<td>0.65-1.44</td>
<td>0.87</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2142</td>
<td>122</td>
<td>1.82</td>
<td>1.03-3.20</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>African Americans</td>
<td>1224</td>
<td>48</td>
<td>0.49</td>
<td>0.25-0.98</td>
<td>0.04</td>
</tr>
<tr>
<td>Hispanics</td>
<td>995</td>
<td>32</td>
<td>0.51</td>
<td>0.18-1.49</td>
<td>0.22</td>
</tr>
<tr>
<td>Chinese</td>
<td>585</td>
<td>10</td>
<td>1.47</td>
<td>0.17-12.35</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>CHD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>4946</td>
<td>156</td>
<td>1.16</td>
<td>0.73-1.85</td>
<td>0.53</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2142</td>
<td>93</td>
<td>2.04</td>
<td>1.07-3.90</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>African Americans</td>
<td>1224</td>
<td>34</td>
<td>0.49</td>
<td>0.22-1.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Hispanics</td>
<td>995</td>
<td>22</td>
<td>0.74</td>
<td>0.20-2.72</td>
<td>0.65</td>
</tr>
<tr>
<td>Chinese</td>
<td>585</td>
<td>7</td>
<td>15.37</td>
<td>0.81-292</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>All-Cause Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>4946</td>
<td>218</td>
<td>1.16</td>
<td>0.79-1.69</td>
<td>0.44</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2142</td>
<td>96</td>
<td>1.72</td>
<td>0.91-3.27</td>
<td>0.09</td>
</tr>
<tr>
<td>African Americans</td>
<td>1224</td>
<td>76</td>
<td>0.69</td>
<td>0.39-1.18</td>
<td>0.17</td>
</tr>
<tr>
<td>Hispanics</td>
<td>995</td>
<td>26</td>
<td>1.91</td>
<td>0.56-6.54</td>
<td>0.30</td>
</tr>
<tr>
<td>Chinese</td>
<td>585</td>
<td>20</td>
<td>3.55</td>
<td>0.69-18.10</td>
<td>0.13</td>
</tr>
</tbody>
</table>

All models adjusted for age, gender, and race/ethnicity, body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level.

In order to eliminate the possibility of confounding effect of new onset of diabetes on the association of HbA1c with outcomes, we excluded all participants who developed incident diabetes during the follow up period. However, our results remain similar;
HbA1c was independently associated with new CVD and CHD events only in Caucasians and not in the entire cohort or other ethnic groups after adjusting for covariates in the fully adjusted model. In addition, there was some suggestion of an association of HbA1c with all-cause mortality in Caucasians; however the estimate did not achieve statistical significance.
Table-6: Risk of CVD, CHD, and All-Cause Mortality with every 1% increase in HbA1c in participants with HbA1c <5.7%, FBG <100, and no history of diabetes or use of diabetic medications – Multi-Ethnic Study of Atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th># Events</th>
<th>HR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CVD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>3304</td>
<td>145</td>
<td>1.08</td>
<td>0.57-2.03</td>
<td>0.81</td>
</tr>
<tr>
<td>Caucasians</td>
<td>1620</td>
<td>80</td>
<td>2.14</td>
<td>0.79-5.73</td>
<td>0.13</td>
</tr>
<tr>
<td>African Americans</td>
<td>679</td>
<td>35</td>
<td>0.88</td>
<td>0.29-2.63</td>
<td>0.83</td>
</tr>
<tr>
<td>Hispanics</td>
<td>631</td>
<td>26</td>
<td>0.53</td>
<td>0.12-2.28</td>
<td>0.39</td>
</tr>
<tr>
<td>Chinese*</td>
<td>374</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>CHD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>3304</td>
<td>103</td>
<td>1.26</td>
<td>0.59-2.69</td>
<td>0.54</td>
</tr>
<tr>
<td>Caucasians</td>
<td>1620</td>
<td>58</td>
<td>2.25</td>
<td>0.71-7.14</td>
<td>0.17</td>
</tr>
<tr>
<td>African Americans</td>
<td>679</td>
<td>25</td>
<td>0.89</td>
<td>0.24-3.23</td>
<td>0.86</td>
</tr>
<tr>
<td>Hispanics</td>
<td>631</td>
<td>17</td>
<td>0.81</td>
<td>0.12-5.44</td>
<td>0.83</td>
</tr>
<tr>
<td>Chinese</td>
<td>374</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>All-Cause Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>3304</td>
<td>123</td>
<td>0.74</td>
<td>0.39-1.38</td>
<td>0.34</td>
</tr>
<tr>
<td>Caucasians</td>
<td>1620</td>
<td>50</td>
<td>0.36</td>
<td>0.13-1.02</td>
<td>0.05</td>
</tr>
<tr>
<td>African Americans</td>
<td>679</td>
<td>45</td>
<td>0.73</td>
<td>0.28-1.86</td>
<td>0.50</td>
</tr>
<tr>
<td>Hispanics</td>
<td>631</td>
<td>16</td>
<td>1.42</td>
<td>0.16-12.93</td>
<td>0.75</td>
</tr>
<tr>
<td>Chinese</td>
<td>374</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Data did not fit the model for this group

All models adjusted for age, gender, and race/ethnicity, body mass index, systolic blood pressure, anti-hypertensive medication use, total and HDL cholesterol, use of lipid lowering medications, urine albumin/creatinine ratio, smoking status, and educational level

We repeated our analyses after excluding all participants who had history of diabetes, who were taking diabetes medications, and who had FBG or HbA1c in the prediabetes
range. The HbA1c did not show any significant association with any of the outcomes of interest in the fully adjusted models. There was some suggestion of an increased risk of CVD and CHD only in Caucasians but the estimates failed to reach statistical significance. We do acknowledge the possibility of loss of power after excluding a large number of participants for these analyses to come to any meaningful conclusion.
The next section contains figures and tables demonstrating the Net Reclassification Analyses of our data. We used 10% and 20% cut off of Framingham Risk Score to define risk categories (ATP-III, JAMA 2001). In the entire cohort, addition of HbA1c did not improve net reclassification index for incident CVD (NRI= -0.020; p=0.21) or CHD (NRI= -0.01; p= -0.66) and actually seems to have made it worse (minus sign). In Caucasians (since associations were only significant in Caucasian in subgroup analysis, we only performed these additional analysis in Caucasians), there was 9% net reclassification improvement when HbA1c was added to the Framingham Risk Score for incident CVD although the results overlap the null slightly (NRI= 0.09; p=0.06). Similar results were obtained in for CHD outcome (NRI=0.08, p=0.10). As noted in Chapter-II, we found comparable results with receiver operating curve analyses for incremental predictive information obtained by adding HbA1c to Framingham Risk Score. These findings may suggest utility of HbA1c as an additional predictive marker in Caucasians, although more research is needed to support this hypothesis.
**Figure-3:** Risk stratification ability of the model with and without HbA1c for incident CVD in the entire cohort (N=5069) with low\(^{a}\), intermediate\(^{b}\), and high\(^{c}\) Framingham Risk Score at baseline after a maximum follow up of 7 years - Multi-Ethnic Study of Atherosclerosis (MESA)

\(^{a}\)<10\%, \(^{b}\)10\% to <20\%, \(^{c}\)≥20\%
Table-7: Reclassification Table for the outcome of CVD in the entire cohort - Multi-Ethnic Study of Atherosclerosis (MESA)

Entire cohort (N=5069)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>3791</td>
<td>105</td>
</tr>
<tr>
<td>Intermediate</td>
<td>71</td>
<td>823</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>3862</td>
<td>941</td>
</tr>
</tbody>
</table>

Entire cohort with CVD events (n=244)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>122</td>
<td>3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7</td>
<td>75</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>80</td>
</tr>
</tbody>
</table>

Entire cohort with no CVD events (n=4825)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>3669</td>
<td>102</td>
</tr>
<tr>
<td>Intermediate</td>
<td>64</td>
<td>748</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>3733</td>
<td>861</td>
</tr>
</tbody>
</table>

Categories of Framingham Risk Score (FRS) defined as per ATP-III (JAMA 2001)

Low: <10% 10-year risk

Intermediate: 10% to <20% 10-year risk

High: ≥20% 10-year risk
Reclassification in the entire cohort with Events:

= (#events moving up - #events moving down) / #events

= (6 -9) / 244 = - 0.01229 or -1.23%

Events z statistic = -0.7746; p value = 0.44

Reclassification in the entire cohort with No events:

= (#nonevents moving down - #nonevents moving up) / #nonevents

= (75 -114) / 4825 = - 0.00829 or -0.83%

No events z statistic = -2.8368; p value = 0.004

NRI = (-0.01229) + (-0.00829) = - 0.02058 or approximately – 2.1%

NRI 95% CI = - 0.052 to 0.0112 or approximately -5.2% to1.1%

NRI z statistic = - 1.2636

NRI p value = 0.21
Figure-4: Risk stratification ability of the model with and without HbA1c for incident CVD in Caucasians with low\textsuperscript{a}, intermediate\textsuperscript{b}, and high\textsuperscript{c} Framingham Risk Score at baseline after a maximum follow up of 7 years - Multi-Ethnic Study of Atherosclerosis (MESA)

\textsuperscript{a}<10\%, \textsuperscript{b}10\% to <20\%, \textsuperscript{c}\geq 20\%
Table-8: Reclassification Table for the outcome of CVD in Caucasians - Multi-Ethnic Study of Atherosclerosis (MESA)

All Caucasians (N=2187)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>1537</td>
<td>189</td>
</tr>
<tr>
<td>Intermediate</td>
<td>87</td>
<td>226</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>1624</td>
<td>436</td>
</tr>
</tbody>
</table>

Caucasians with CVD events (n=136)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>57</td>
<td>18</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>45</td>
</tr>
</tbody>
</table>

Caucasians with no CVD events (n=2051)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>1480</td>
<td>171</td>
</tr>
<tr>
<td>Intermediate</td>
<td>80</td>
<td>202</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>1560</td>
<td>391</td>
</tr>
</tbody>
</table>

FRS: Framingham Risk Score

Low: <10% 10-year risk

Intermediate: 10% to <20% 10-year risk

High: ≥20% 10-year risk
Reclassification in Caucasians with events:

\[
= \frac{(#\text{events moving up} - #\text{events moving down})}{#\text{events}}
\]

\[
= \frac{29 - 10}{136} = 0.13971 \text{ or } 14\%
\]

Events z statistic = 3.0424; p value = \textbf{0.002}

Reclassification in Caucasians with no events:

\[
= \frac{(#\text{nonevents moving down} - #\text{nonevents moving up})}{#\text{nonevents}}
\]

\[
= \frac{98 - 206}{2051} = -0.05265 \text{ or } -5.26\%
\]

No events z statistic = -6.1942; p value = \textless \textbf{0.0001}

\[
\text{NRI} = (0.13971) + (-0.05265) = 0.08706 \text{ or approximately } 9\%
\]

\[
\text{NRI 95\% CI} = -0.0045 \text{ to } 0.1786 \text{ or approximately } -0.4\% \text{ to } 17.8\%
\]

\[
\text{NRI z statistic} = 1.864
\]

\[
\text{NRI p value} = 0.06
\]
Figure-5: Risk stratification ability of the model with and without HbA1c for incident CHD in the entire cohort with low\textsuperscript{a}, intermediate\textsuperscript{b}, and high\textsuperscript{c} Framingham Risk Score at baseline after a maximum follow up of 7 years - Multi-Ethnic Study of Atherosclerosis (MESA)

\begin{itemize}
\item \textbf{Entire Cohort (N=5069)}
\item \textbf{CHD Events (N=178)}
\item \textbf{No CHD Events (N=4891)}
\end{itemize}

\textsuperscript{a}<10\%, \textsuperscript{b}10\% to <20\%, \textsuperscript{c}\geq20\%
Table-9: Reclassification Table for the outcome of CHD in the entire cohort - Multi-Ethnic Study of Atherosclerosis (MESA)

**Entire cohort (N=5069)**

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>3752</td>
<td>144</td>
</tr>
<tr>
<td>Intermediate</td>
<td>96</td>
<td>790</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>3848</td>
<td>957</td>
</tr>
</tbody>
</table>

**Entire cohort with CHD events (n=178)**

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>85</td>
<td>5</td>
</tr>
<tr>
<td>Intermediate</td>
<td>6</td>
<td>52</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>59</td>
</tr>
</tbody>
</table>

**Entire cohort with no CHD events (n=4891)**

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>3667</td>
<td>139</td>
</tr>
<tr>
<td>Intermediate</td>
<td>90</td>
<td>738</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>3757</td>
<td>898</td>
</tr>
</tbody>
</table>

FRS: Framingham Risk Score

Low: <10% 10-year risk

Intermediate: 10% to <20% 10-year risk

High: ≥20% 10-year risk
Reclassification in entire cohort with events:

\[
= \frac{\text{(#events moving up} - \text{#events moving down})}{\text{#events}}
\]

\[
= \frac{(8 - 8)}{178} = 0.0 \text{ or } 0%
\]

Events z statistic = 0; p = 1

Reclassification in entire cohort with no events:

\[
= \frac{\text{(#nonevents moving down} - \text{#nonevents moving up})}{\text{#nonevents}}
\]

\[
= \frac{(111 - 159)}{4825} = -0.00981 \text{ or } 1%
\]

No events z statistic = -2.9212; p value = **0.003**

\[
\text{NRI} = (0.0) + (-0.00981) = -0.00981 \text{ or approximately } -1%
\]

\[
\text{NRI 95% CI} = -0.0543 \text{ to } 0.0347 \text{ or approximately } -5.4\% \text{ to } 3.5\%
\]

\[
\text{NRI z statistic} = -0.4319
\]

\[
\text{NRI p value} = 0.66
\]
Figure-6: Risk stratification ability of the model with and without HbA1c for incident CHD in Caucasians with low\(^a\), intermediate\(^b\), and high\(^c\) Framingham Risk Score at baseline after a maximum follow up of 7 years - Multi-Ethnic Study of Atherosclerosis (MESA)

\(^a<10\%\), \(^b10\%\) to \(<20\%\), \(^c\geq20\%\)
**Table-10**: Reclassification Table for the outcome of CHD in Caucasians - Multi-Ethnic Study of Atherosclerosis (MESA)

All Caucasians (n=2187)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>1558</td>
<td>164</td>
</tr>
<tr>
<td>Intermediate</td>
<td>82</td>
<td>238</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>1640</td>
<td>423</td>
</tr>
</tbody>
</table>

Caucasians with CHD events (n=103)

<table>
<thead>
<tr>
<th>FRS</th>
<th>FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>45</td>
<td>11</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>31</td>
</tr>
</tbody>
</table>

Caucasians with no CHD events (n=2084)

<table>
<thead>
<tr>
<th>FRS</th>
<th>0FRS + HbA1c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Low</td>
<td>1513</td>
<td>153</td>
</tr>
<tr>
<td>Intermediate</td>
<td>77</td>
<td>220</td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>1590</td>
<td>392</td>
</tr>
</tbody>
</table>

FRS: Framingham Risk Score

Low: <10% 10-year risk

Intermediate: 10% to <20% 10-year risk

High: ≥20% 10-year risk
Reclassification in Caucasians with Events:

= (#events moving up - #events moving down) / #events

= (20 – 7) / 103 = 0.12621 or 12.6%

Events z statistic = 2.5018; p value = 0.01

Reclassification in Caucasians with No events:

= (#nonevents moving down - #nonevents moving up) / #nonevents

= (96 – 188) / 2084 = -0.04414 or -4.4%

No events z statistic = -5.3218; p = <0.0001

NRI = (0.12621) + (-0.04414) = 0.08270 or approximately 8%

NRI 95% CI = -0.0171 to 0.1832 or approximately 1.7% to 18.3%

NRI z statistic = 1.625

NRI p value = 0.10
NET RECLASSIFICATION IMPROVEMENT

Computation of Cardiovascular Risk

Individuals with established cardiovascular disease or certain medical conditions, like diabetes, are considered to be at higher cardiovascular risk and warrant aggressive primary and secondary preventive interventions. For all other apparently asymptomatic individuals, predictions models are recommended to be used to determine their approximate total cardiovascular risk. Traditional cardiovascular risk factors include age, gender, smoking status, systolic blood pressure, total cholesterol, and HDL cholesterol. These risk factors have been used to develop risk prediction models to estimate the risk of cardiovascular disease. In the US, Framingham Risk Score (FRS) has been widely used while Systemic Coronary Risk Evaluation (SCORE) has been developed using multiple European cohorts and is recommended by European guidelines. The main objective of these risk prediction models is to classify individuals in different cardiovascular risk categories so that preventive interventions can be much more aggressively implemented in individuals who are at a higher risk of developing cardiovascular disease.

Despite a widespread use of existing prediction models, it has been observed that a large number of individuals get classified into inappropriate risk categories. This is particularly concerning for individuals who are considered to have intermediate risk of cardiovascular disease. There is growing evidence that many individuals in the intermediate risk category may, in fact, have a higher risk, thus in need of preventive interventions, or may
belong to a lower risk category in which case lifestyle modifications alone are sufficient.\textsuperscript{13}

In order to improve the predictive ability of an existing prediction model, new and novel biomarkers are being identified to be used as predictors of cardiovascular risk. In this context, the challenge is to determine how to incorporate and interpret the information provided by adding a new biomarker into existing risk stratification models.\textsuperscript{14, 15} In an excellent review article, Lloyd-Jones and colleagues discussed the basic concepts and fundamental principles related to the assessment of new biomarkers when added to existing prediction models like Framingham Risk Score. They recommend that at the very minimum a significant statistical association is desirable between the new biomarker and outcomes of interest, although it is by no means enough to suggest that there is also a clinically significant association since large samples may produce significant p values even in the absence of a true association.\textsuperscript{13, 14} Furthermore, the observation of an independent and significant statistical association does not verify that the new marker also adds any incremental value to the existing models in the prediction of cardiovascular risk.\textsuperscript{12, 13} Receiver operating curve (ROC) analysis has been well known and widely used in cardiovascular epidemiology.\textsuperscript{16} It measures the area under the curve (AUC) or C statistics as an estimate of the ability of a prediction model to discriminate cases from non-cases based upon observed event rates in prospective studies.\textsuperscript{16} The receiver operating curve is constructed by plotting sensitivity against 1-specificity (false positive rates) and the output includes area under the curve (AUC) and corresponding C statistic.\textsuperscript{14} An AUC of 0.5 means no discrimination and 1.0 reflects a perfect discrimination. To
assess the added predictive information that can be obtained by a novel risk factor, we compare the models with and without the new risk marker and test for the difference between the two AUC. However, ROC suffers from certain limitations.\textsuperscript{17, 18} Notably, an AUC merely ranks the risks among cases and noncases and does not provide information regarding differences in risks between them.\textsuperscript{16} In addition, it is very difficult for a new marker to increase AUC since large values of odds ratios are usually required to bring about this change.\textsuperscript{17, 18} Lastly, it does not assess the calibration and accuracy of the prediction model.\textsuperscript{15}

**Net Reclassification Improvement**

To address some limitations inherent to AUC, new statistical methods are being proposed. One way is to examine the global reclassification of individuals in different risk categories in the entire cohort after addition of a novel biomarker.\textsuperscript{16} However, this simple approach fails to provide any meaningful information regarding improvement in model performance since some individuals (both cases and non-cases) may still be reclassified in inaccurate risk categories.\textsuperscript{14} Net Reclassification Improvement (NRI), has been described by Pencina et al to better evaluate the predictive ability of a new marker above and beyond traditional risk markers.\textsuperscript{14} The NRI represents the ability of a new marker to reclassify individuals into more accurate risk categories when added to existing prediction model thereby improving the model performance.\textsuperscript{14, 19}

The NRI can be calculated by the following formula:
In other words, NRI is based upon the upward and downward movement of predicted risk across risk categories when a new risk marker is added to the model. Net Reclassification Improvement can also be stated as:

\[
NRI = 
\left[ \frac{\text{Prob}(\text{being correctly reclassified to a higher risk category})}{\text{events}} \right] - \left[ \frac{\text{Prob}(\text{being incorrectly reclassified to a lower risk category})}{\text{events}} \right] + 
\left[ \frac{\text{Prob}(\text{being correctly reclassified to a lower risk category})}{\text{nonevent}} \right] - 
\left[ \frac{\text{Prob}(\text{being incorrectly reclassified to a higher risk category})}{\text{nonevent}} \right]
\]

That is to say the NRI is the “sum of differences in proportions of individuals moving up minus the proportion moving down for people who develop events, and the proportion of individuals moving down minus the proportion moving up for people who do not develop events”. A statistically significant NRI would mean that appropriate reclassification occurs more than the inappropriate reclassification by the addition of the new marker in the model. In their paper, Pencina and colleagues demonstrated that addition of a new risk marker to a prediction model may produce significant NRI despite a trivial and
nonsignificant change in AUC.\textsuperscript{14} Certin limitations have been acknowledged regarding use of NRI in assessment of risk. First, NRI depends on the event status and therefore the information regarding calibration of the estimated risk cannot be obtained from this measure.\textsuperscript{20} Second, use of NRI in survival data is also a challenge and not very clear. Third, NRI depends on the number of categories and how we define those using arbitrary or clinically relevant cut-points.\textsuperscript{14, 20, 21} And lastly, it has been suggested that the NRI is inherently biased towards a false positive result and, therefore, produces exceedingly optimistic results.\textsuperscript{22}

** Modifications of Net Reclassification Improvement**

To address the limitations of NRI, to enhance its methodology, and to increase its utility, several modifications have been proposed in the past few years.\textsuperscript{21-23} These include the applications of NRI to survival and case-control data and use of category-free NRI the details of which are beyond the scope of this thesis. The interested reader may refer to the references cited for more in-depth understanding of these extensions of NRI. However, in the next section we will briefly discuss a recently proposed method to calculate the bias corrected-clinical NRI (intermediate risk category only).\textsuperscript{22}

**Bias-Corrected-Net Reclassification Improvement in Intermediate Risk Group**

Existing prediction models divide the individuals into low, intermediate, and high risk categories.\textsuperscript{7} There is growing interest in assessing the role of novel risk factors in
reclassification of individuals who fall into the intermediate risk category by the base model. It is argued that the treatment guidelines are much clearer for individuals at low or high risk but not so much for persons in the intermediate risk category. In addition, many individuals in the intermediate risk category in fact belong to either low or high risk hence may qualify for more different and more specific interventions. All of this may also have implications related to cost and clinical utility.

In a recent paper, Paynter and Cook have proposed methods to estimate the bias-corrected clinical NRI (cNRI). The NRI by previously proposed method is first calculated for the intermediate risk category and is named naïve cNRI. Then, expected reclassification table is constructed as described in their paper with the assumption that the expected risk will be evenly distributed around diagonal when null hypothesis is valid. The expected NRI is then estimated using this expected cell frequency table. And finally, the unbiased cNRI is calculated by using the following formula:

\[ cNRI = \text{naïve cNRI} - \text{expected cNRI} \]

As an example, Paynter and Cook demonstrated that the addition of HDL cholesterol to the baseline prediction model using Women’s Health Study data resulted in an overly optimistic naïve cNRI of 17% (p = 0.001). However, bias-corrected cNRI was more realistic, 4% (p = 0.24), and was similar to the overall NRI of the entire cohort (all risk categories combined). This unbiased cNRI, may prove to be a valuable tool to assess the reclassification improvement of individuals in the intermediate risk category by a novel risk marker thereby allowing appropriate use of indicated preventive interventions.
Calculation of Unbiased cNRI in Our Data:

We used the Multi-Ethnic Study of Atherosclerosis (MESA) data of adults without diabetes and who were free of CVD at the baseline to calculate the unbiased cNRI when HbA1c is added to the Framingham Risk Score for the composite outcome of CVD separately in the entire cohort (N=5069) and then only in Caucasians (N=2187). We divided our study cohort into three risk categories based upon the Framingham risk score using same cut-offs as mentioned earlier in this chapter for traditional NRI: Low (<10%), Intermediate (10% to <20%), and High (≥20%). The reclassification tables and related calculations of naïve cNRI, expected cNRI, and bias-corrected cNRI are demonstrated in the next few tables. As shown, the naïve cNRI for incident CVD was statistically significant in Caucasians but not the unbiased cNRI.
Table-11: Calculation of unbiased cNRI - Risk stratification ability of the model with and without HbA1c for incident CVD in the entire cohort (N=5069) with low (<10%), intermediate (10% to <20%), and high (>20%) Framingham Risk Score (FRS) at baseline after a maximum follow up of 7 years - Multi-Ethnic Study of Atherosclerosis (MESA)

**Observed Reclassification: Events (n=244) / No events (n=4825)**

<table>
<thead>
<tr>
<th>FRS</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FRS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>122/3669</td>
<td>3/102</td>
<td>0/0</td>
<td>125/3771</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7/64</td>
<td>75/748</td>
<td>3/12</td>
<td>85/824</td>
</tr>
<tr>
<td>High</td>
<td>0/0</td>
<td>2/11</td>
<td>32/219</td>
<td>34/230</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>129/3733</td>
<td>80/861</td>
<td>35/231</td>
<td>244/4825</td>
</tr>
</tbody>
</table>

**Expected Reclassification: Events (n=244) / No events (n=4825)**

<table>
<thead>
<tr>
<th>FRS</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FRS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>122/3669</td>
<td>5/83</td>
<td>0/0</td>
<td>127/3752</td>
</tr>
<tr>
<td>Intermediate</td>
<td>5/83</td>
<td>75/748</td>
<td>2.5/11.5</td>
<td>82.5/842.5</td>
</tr>
<tr>
<td>High</td>
<td>0/0</td>
<td>2.5/11.5</td>
<td>32/219</td>
<td>34.5/230.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>127/3752</td>
<td>82.5/842.5</td>
<td>34.5/230.5</td>
<td>244/4825</td>
</tr>
</tbody>
</table>

Reclassification in the entire cohort with Events:

= (#events moving up - #events moving down) / #events

= (6 –9) / 244 = - 0.01229 or -1.23%

Events z statistic = -0.7746; p value = 0.44

Reclassification in the entire cohort with No events:

= (#nonevents moving down - #nonevents moving up) / #nonevents

= (75 –114) / 4825 = - 0.00829 or -0.83%
No events $z$ statistic = -2.8368; $p$ value = 0.004

Overall NRI = (-0.01229) + (-0.00829) = - 0.02058 or approximately – 2.1%
Overall NRI 95% CI = - 0.052 to 0.0112 or approximately -5.2% to 1.1%
Overall NRI $z$ statistic = - 1.2636; Overall NRI $p$ value = 0.21

Naïve cNRI (using only intermediate risk category at baseline):

Naïve cNRI = \[
\frac{(#\text{events moving up} - #\text{events moving down})}{#\text{events}} + \frac{(#\text{nonevents moving down} - #\text{nonevents moving up})}{#\text{nonevents}}
\]

\[
= \frac{(3-7)}{85} + \frac{(64-12)}{824}
\]

\[
= [(-0.047)] + [0.063] = 0.016 \text{ or } 1.6%
\]

Naïve cNRI $z$ statistic = 0.4149; naïve cNRI $p$ value = 0.68

Expected cNRI (using only intermediate risk category at baseline):

Expected cNRI = \[
\frac{(#\text{events moving up} - #\text{events moving down})}{#\text{events}} + \frac{(#\text{nonevents moving down} - #\text{nonevents moving up})}{#\text{nonevents}}
\]

\[
= \frac{(2.5-5)}{82.5} + \frac{(83-11.5)}{842.5}
\]

\[
= [(-0.0303)] + [0.0848] = 0.0545 \text{ or } 5.4%
\]

Expected cNRI $z$ statistic = 1.5525; expected cNRI $p$ value = 0.12

cNRI = naïve cNRI – expected cNRI = 0.016 – 0.054 = -0.038 or -3.8%
cNRI $z$ statistic = -0.9824; cNRI $p$ value = 0.32
Table-12: Calculation of unbiased cNRI - Risk stratification ability of the model with and without HbA1c for incident CVD in Caucasians (n=2187) with low (<10%), intermediate (10% to <20%), and high (≥20%) Framingham Risk Score (FRS) at baseline after a maximum follow up of 7 years - Multi-Ethnic Study of Atherosclerosis (MESA)

Observed Reclassification in Caucasians: events (136) / no events (2051)

<table>
<thead>
<tr>
<th>FRS</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>57/1480</td>
<td>18/171</td>
<td>0/2</td>
<td>75/1653</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7/80</td>
<td>24/202</td>
<td>11/33</td>
<td>42/315</td>
</tr>
<tr>
<td>High</td>
<td>0/0</td>
<td>3/18</td>
<td>16/65</td>
<td>19/83</td>
</tr>
<tr>
<td>Total</td>
<td>64/1560</td>
<td>45/391</td>
<td>27/100</td>
<td>136/2051</td>
</tr>
</tbody>
</table>

Expected Reclassification in Caucasians: events (136) / no events (2051)

<table>
<thead>
<tr>
<th>FRS</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>57/1480</td>
<td>12.5/125.5</td>
<td>0/1</td>
<td>69.5/1606.5</td>
</tr>
<tr>
<td>Intermediate</td>
<td>12.5/125.5</td>
<td>24/202</td>
<td>7/25.5</td>
<td>43.5/353</td>
</tr>
<tr>
<td>High</td>
<td>0/1</td>
<td>7/25.5</td>
<td>16/65</td>
<td>23/91.5</td>
</tr>
<tr>
<td>Total</td>
<td>69.5/1606.5</td>
<td>43.5/353</td>
<td>23/91.5</td>
<td>136/2051</td>
</tr>
</tbody>
</table>

Reclassification in Caucasians with events:

= (#events moving up - #events moving down) / #events

= (29 – 10) / 136 = 0.13971 or 14%

Events z statistic = 3.0424; p value = 0.002

Reclassification in Caucasians with no events:

= (#nonevents moving down - #nonevents moving up) / #nonevents

= (98 – 206) / 2051 = - 0.05265 or -5.26%
No events $z$ statistic = -6.1942; $p$ value = <0.0001

Overall NRI = $(0.13971) + (-0.05265) = 0.08706$ or approximately 9%

Overall NRI 95% CI = - 0.0045 to 0.1786 or approximately -0.4% to 17.8%

Overall NRI $z$ statistic = 1.864; Overall NRI $p$ value = 0.06

Naïve cNRI (using only intermediate risk category at baseline):

Naïve cNRI = $\left[ \frac{(#\text{events moving up} - #\text{events moving down})}{#\text{events}} \right] + \left[ \frac{(#\text{nonevents moving down} - #\text{nonevents moving up})}{#\text{nonevents}} \right]$

\[ = \left[ \frac{11-7}{42} \right] + \left[ \frac{80-33}{315} \right] \]

\[ = [0.0952] + [0.1492] = 0.2444 \text{ or } 24.44\% \]

Naïve cNRI $z$ statistic = 2.2952; naïve cNRI $p$ value = 0.02

Expected cNRI (using only intermediate risk category at baseline):

Expected cNRI = $\left[ \frac{(#\text{events moving up} - #\text{events moving down})}{#\text{events}} \right] + \left[ \frac{(#\text{nonevents moving down} - #\text{nonevents moving up})}{#\text{nonevents}} \right]$

\[ = \left[ \frac{7-12.5}{43.5} \right] + \left[ \frac{125.5-25.5}{353} \right] \]

\[ = [(-0.1264)] + [0.2832] = 0.1568 \text{ or } 15.7\% \]

Expected cNRI $z$ statistic = 1.4615; expected cNRI $p$ value = 0.14

$cNRI = \text{ naïve cNRI} - \text{ expected cNRI} = 0.2444 - 0.1568 = 0.0876 \text{ or } 8.76\%$

$cNRI$ $z$ statistic = 0.8225; $cNRI$ $p$ value = 0.41
The methods to assess the performance of prediction models, when a new risk marker is added, continue to evolve and merits and disadvantages of various strategies are highly discussed in the statistical and medical literature.\textsuperscript{25, 26} A positive expected outcome of this ongoing debate is the emergence of refined techniques to examine the predictive ability and clinical utility of novel risk markers.
CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, cardiovascular disease is a major cause of morbidity, mortality, and a significant burden on the healthcare resources in the United States and around the world. With increasing prevalence of cardiovascular risk factors, this problem is expected to get worse over time. Therefore, it is prudent that we enhance our capacity to accurately identify individuals harboring a higher level of cardiovascular risk so that valuable resources can be utilized in a more efficient manner to decrease this risk. In our study of adult participants free of diabetes or cardiovascular disease at the baseline, HbA1c was not significantly associated with cardiovascular disease, coronary heart disease, and all-cause mortality in contrast to some previous reports. In subgroup analyses, the HbA1c was independently associated with increased risk of cardiovascular disease and coronary heart disease events only in Caucasians but not in any other ethnic groups in line with some of the previous studies. However, addition of HbA1c failed to improve the predictive ability of existing model – Framingham Risk Score – in the entire cohort or in Caucasians. Our findings seriously call into question the validity of current American Heart Association recommendation regarding use of HbA1c as a potential biomarker for cardiovascular risk stratification in individuals without diabetes as it may have implications related to resource utilization and risks associated with further testing and treatment. We did, however, reconfirm that the baseline diabetes status is a significant risk factor for cardiovascular disease and coronary heart disease. We have also shown that the baseline prediabetes status based upon HbA1c increases the mortality risk. The role of HbA1c in screening individuals for cardiovascular risk free of diabetes remains less clear due to conflicting evidence. Further research is warranted to assess the utility of
HbA1c in cardiovascular risk prediction in individuals free of diabetes and cardiovascular disease at the baseline, especially in ethnic minorities. Futures analyses in Multi-Ethnic Study of Atherosclerosis and other cohort studies may provide more information in clarify this. In addition, work should be continued to explore new, easily available and less expensive biomarkers to enhance our ability to accurately identify high risk individuals who may get benefit from primary and secondary prevention.
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Amir Azeem, MD, FACP
6031 Claudias Ln, Apt 102, Winston-Salem, NC 27103
Mobile Ph.: (910) 922-4689 / Pager: (336) 806-9492
aazeem@wakehealth.edu / aazeem@umc.edu

CURRENT POSITIONS & ENROLLMENTS

Fellow - Cardiology
University of Mississippi Medical Center
Program Director: Thomas N. Skelton, MD
July 01, 2013 -

Graduate Student
Master of Science (MS) in Clinical and Population Translational Science (CPTS)
Graduate School of Arts and Sciences
Wake Forest University, Winston-Salem, NC
Program Directors: Robert P. Byington, MPH, PhD and Ann M. Geiger, MPH, PhD
Aug, 2011 –

PROFESSIONAL ADDRESS

Division of Cardiovascular Diseases
University of Mississippi Medical Center
2500 N. State Street, Jackson, MS 39216
Program Director: Thomas N. Skelton, MD

PREVIOUS EDUCATION

Bachelor of Medicine and Surgery (MBBS) – MD equivalent
Dow Medical College, University of Karachi
Baba-E-Urdu Road, Karachi 74200 Pakistan
Honors: Biochemistry and Ophthalmology
May 1994-Sep 1999

Higher Secondary Certificate (HSC) – Pre-medical
Adamjee Government Science College, Board of Intermediate Education Karachi
Business Recorder Road, Karachi, Pakistan
Distinctions: Chemistry, Physics, Biology
April 1991-May 1993
PREVIOUS POST-DOCTORAL TRAINING

Post-Doc Fellow
Clinical Cardiovascular Research Program
Focus on cardiovascular disease epidemiology
Wake Forest School of Medicine, Winston-Salem, NC
Program Director: David M. Herrington, MD, MHS
Jul, 2011 – Jun 2013

Residency – Internal Medicine
Michigan State University Internal Medicine Residency Program
788 Service Road, Room B-301, East Lansing, MI 48824 USA
Program Director: Heather Laird-Fick, MD, MPH (previously Davoren Chick, MD)

Internship – Internal Medicine
Michigan State University Internal Medicine Residency Program
788 Service Road, Room B-301, East Lansing, MI 48824 USA
Program Director: Heather Laird-Fick, MD, MPH (previously Davoren Chick, MD)
Jul 2003 – Jun 2004

Externship
Department Of Rheumatology (Supervisor: Raza Jaffry, MD)
Liaquat National Post-Graduate Medical Center
Stadium Road, Karachi, Pakistan
Dec 2000-Feb 2001

HONORS & AWARDS

Fellow of American College of Physicians (FACP)
Sep 2012 -

Outstanding Senior Resident Award
Jun 2006
By the Michigan State University Internal Medicine Residency Program
East Lansing, MI, for the residency year 2005-2006

Outstanding Internal Medicine Senior Resident Award
Jun 2006
By the teaching faculty and staff of Sparrow Hospital System, Lansing, MI,
for the residency year 2005-2006

Outstanding Teaching Resident Award
Mar 2006
By the graduating Class of 2006, College of Human Medicine,
Michigan State University, East Lansing, MI

“Lifetime membership”
1999
Patients Welfare Association (PWA),
Dow Medical College & Civil Hospital, Karachi, Pakistan

Honors in Biochemistry, and Ophthalmology
1994-1999
Dow Medical College, University of Karachi, Pakistan
Distinctions in Chemistry, Physics, Biology  1991-1993
Adamjee Government Science College, Karachi, Pakistan

PROFESSIONAL LICENSURE

- Mississippi State Board of Medical Licensure (Physician’s License)  Jun 2013-
- North Carolina Medical Board (Physician’s License)  2006-
- Drug Enforcement Agency (DEA) Registration  2006-
- Pakistan Medical & Dental Council (PMDC), Pakistan – Inactive  1999-2003

SPECIALTY CERTIFICATION

- Diplomate American Board of Internal Medicine (ABIM)  Nov 2006-

OTHER CERTIFICATIONS

- NIH online course on the Protection of Human Research participants  Jul 2011
- Medical Education Research Certification (MERC) - by AAMC  Jan 3-5, 2011
- Basic Life Support (BLS)  Jun 2013-
- Advance Cardiac Life Support (ACLS)  Oct 2011-
- Educational Commission of Foreign Medical Graduates certification  Oct 2002-

PREVIOUS ACADEMIC APPOINTMENTS

Assistant Professor – Part time  Sep, 2011 – Jun 2013
Department of Internal Medicine, Section on Hospital Medicine
Wake Forest School of Medicine, Winston-Salem, NC

Assistant Professor (Full time)  Aug 01, 2010 – Jun 30, 2011
Department of Internal Medicine
Section on General Internal Medicine (Hospital Medicine)
Wake Forest School of Medicine, Winston-Salem, NC

Clinical Instructor (Internal Medicine Resident)  2003- 2006
Department of Medicine; College of Human Medicine
Michigan State University, East Lansing, MI
EMPLOYMENT

**General Internist/Hospitalist**  
Scotland Medical Center, PA  
102 Pine Street, Maxton, NC 28364 USA  
Direct patient care at Scotland Memorial Hospital, including ICU, Office, Nursing Home (Edwin Morgan Center) and in the Homes of some home-bound patients

**Medical Officer – General Internist**  
Liaquat Ali Khan College of Dentistry and Darul-Sehat Hospital,  
Karachi, Pakistan

**Medical Officer – General Internist**  
Taher Medical Center  
419-A, Amir Khusro Road, C.P. Berar Society, Karachi, Pakistan

**Medical Officer – General Internist**  
North Karachi Hospital (Pvt.) Ltd.  
122-C, Sector 11-B, North Karachi Township, Karachi Pakistan

PROFESSIONAL APPOINTMENTS & ACTIVITIES

**Staff Physician – Internal Medicine (Locum Hospitalist)**  
Morehead Memorial Hospital  
Eden, NC  
Dec 2012-

**Staff Physician – Internal Medicine (Locum Hospitalist)**  
First Health Moore Regional Hospital  
Pinehurst, NC  
Feb 2013-

**Staff Physician – Internal Medicine (Hospital Medicine)**  
Wake Forest Baptist Health – Lexington Medical Center  
Lexington, NC 27292  
Sep 2012-

**Staff Physician – Internal Medicine (Hospital Medicine)**  
Wake Forest Baptist Medical Center  
Medical Center Boulevard, Winston Salem, NC 27157  
Aug 2010-

**Staff Physician – Internal Medicine**  
Scotland Memorial Hospital, Laurinburg, NC 28352 USA  
Acute inpatient care, including ICU  
Aug 2006-Jul 2010

**Staff Physician**  
Edwin Morgan Center - Skilled Nursing Facility  
Scotland Health Care System, 517 Peden Street, Laurinburg, NC  
Aug 2006-Jul 2010
INSTITUTIONAL SERVICE

Interviewer for Internal Medicine Residency Candidates  
Wake Forest School of Medicine, Winston Salem, NC  
Nov 16, 2012

Interviewer for Internal Medicine Residency Candidates  
Wake Forest School of Medicine, Winston Salem, NC  
Dec, 2011

Interviewer for Internal Medicine Residency Candidates  
Wake Forest School of Medicine, Winston Salem, NC  
Nov 16, 2011

Member-Infection Control committee  
Wake Forest Baptist Medical Center, Winston Salem, NC  
Jan 2011-

Member-Internal Medicine Quality Improvement Plan  
Acute MI Readmission Team  
Wake Forest Baptist Medical Center, Winston Salem, NC  
Sep 2010-Mar 2011

Member Medical Care Committee  
Scotland Memorial Hospital, Laurinburg, NC  
Sep 2006-Jul 2010

Member Critical Care Committee  
Scotland Memorial Hospital, Laurinburg, NC  
Jul 2007-Jul 2010

ACLS Instructor  
Scotland Memorial Hospital, Laurinburg, NC  
Jun 2009-Jul 2010

Heart Failure Program Management Group  
Quality improvement project. Sparrow Hospital, Lansing, MI  
2004-2006

Quality Improvement Project at a Nursing Home  
Dec 2004

Policy and Procedure concerning the use of COX-2 Inhibitors or an NSAID in Long-Term Care Facilities. Mentor: Larry Lawhorne, MD, Geriatric Education Center of Michigan, Michigan State University, East Lansing, MI. Completed literature review and created a handout for the residents of Masonic Pathways (Nursing Home), Alma, MI

Curriculum Development: Electrophysiology elective for senior medical residents, a collaborative effort with Roshan B. Patel, MD, Kristofer Dosh, MD, and Dr. Ranjun Thakur, MD. MSU Internal Medicine Residency, East Lansing, MI

Adult Code Blue Committee  
Sparrow Hospital, Lansing, MI  

Recruitment Committee  
Michigan State University Internal Medicine Residency Program,  
2003 – 2006
PROFESSIONAL MEMBERSHIPS

American Heart Association (AHA)
American College of Cardiology (ACC)
American College of Physicians (ACP)
American Medical Association (AMA)
Society of Hospital Medicine (SHM) - Inactive
Association of Physicians of Pakistani Descent of North America (APPNA)
North Carolina Medical Society (NCMS) - Inactive
Michigan State Medical Society (MSMS) - Inactive
Pakistan Medical Association (PMA) – inactive

PUBLICATIONS


NATIONAL MEETING PRESENTATIONS

- Primary investigator/lead author/data analyst
- Poster presented at the American Heart Association Scientific Sessions 2013/Cardiovascular Disease Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism (AHA/Epi-NPAM) at New Orleans Marriott, New Orleans, LA held from Mar 19-22, 2013
- Oral presentation at National Heart Lung Blood Institute (NHLBI) Cardiovascular,
Premature Ventricular Complexes Predict Mortality in a Population with Type 2 Diabetes – The Diabetes Heart Study. Sebastiano Virgadamo, Amir Azeem, Joseph Yeboah, Carlos J. Rodriguez, Amanda J. Cox, Donald W. Bowden, David M. Herrington. -Second author/data analyst
-Poster presented at Seventh Annual Residents and Fellow Research Day, Department of Internal Medicine, Wake Forest School of Medicine on May 7, 2013; first prize for Resident Clinical Research category
-Poster presented at AHA/Epi-NPAM Sessions in New Orleans, LA on Mar 2013
-Poster presented at ACP North Carolina Chapter Sessions at Durham, NC on Feb 2013

Heart Rate Corrected QTc Interval is an Independent Predictor of Mortality in a Diabetic Population – The Diabetes Heart Study. Amir Azeem, Joseph Yeboah, Elsayed Z. Soliman, Amanda Cox, Alain Bertoni, David Herrington, Donald Bowden.
-Primary investigator/lead author/data analyst
-Poster presented at the American Heart Association Scientific Session 2012 at Los Angeles Convention Center, Los Angeles, CA held in Nov 2012
-Manuscript has been submitted to Diabetes Care in Jun 2013 for peer review.


Cat-scratch disease presenting as mono-ocular blindness. Raza S, Pervaiz M, Azeem A, Mohmand A, Armstrong J. Abstract accepted for poster presentation at ACP Michigan Chapter Scientific Meeting, Acme, MI. September 2005

An Interesting Case of Pneumomediastinum and Subcutaneous Emphysema after severe Retching. Roshan B. Patel, MD, Dwarakraj Soundarraj, MD, Kristofer M. Dosh, MD, Amir Azeem, MD, Gary Ferenchick, MD. Michigan State University, Internal Medicine Residency Program, East Lansing, MI. Poster presentation at ACP Michigan Chapter Scientific Meeting, Acme MI Sep 2004

A case report of HTLV-I associated myelopathy / tropical spastic paraparesis (HAM/TSP) in Michigan. Amir Azeem, MD, Kristofer Dosh, MD, Roshan Patel, MD, Vivek Kak, MD. Michigan State University, Internal Medicine Residency Program, East Lansing, MI. Poster presentation at ACP Michigan Chapter Scientific Meeting Sep 2004

An unusual case of TTP-HUS resulting from low-estrogen containing Oral contraceptive pills. Sundeep Sohal, MD, Amir Azeem, MD, Kristofer Dosh, MD, Vivek Kak, MD. Department of Medicine, Michigan State University, East Lansing, Michigan; Poster presentation at ACP Michigan Chapter Scientific Meeting, Sep 30-Oct 02, 2004

OTHER RESEARCH EXPERIENCE

Evaluation and Management of Hypertension in Pregnancy. Ralph Watson, MD, FACP, Amir Azeem, MD, Harold J. Sauer, MD, Donald J. DiPette, MD, Stephanie W. Watts, MD, Alex Chen, MD, PhD, Gregory D. Fink, PhD. I did literature search and actively participated in writing of the manuscript. Mentor: Ralph Watson, MD, Michigan State University, East Lansing, MI. Unpublished. 2006

Management of High Blood Pressure in Acute Stroke. Ralph Watson, MD, FACP, Amir Azeem, MD, Arshad Majid, MD, Donald J. DiPette, MD, Stephanie W. Watts, MD, Alex Chen, MD, PhD, Gregory D. Fink, PhD. I did literature search and actively participated in the manuscript for this review article. Mentor: Ralph Watson, MD, Michigan State University, East Lansing, MI. Unpublished. 2006

Rate of Use of Secondary Preventive Agents in Patients with Ischemic Heart Disease and Diabetes Mellitus. Ingham County Research Group Department of Medicine, Michigan State University, East Lansing, MI. 2004-2006 Mentor: Adesuwa Olomu, MD. Actively participated in project initiation, created Chart Abstraction Manual and Database Form. The purpose of the study was to determine the rate of use and factors affecting the use of recommended medications in low socioeconomic and minority populations of Ingham County, MI

Analysis of Limited Resuscitations in Patients Suffering In-Hospital Cardiac Arrest. Actively participated in data abstraction from charts. “Acknowledgments We are very thankful for the help provided by Julie Bey, Suzanne Leialoha, and Stacy Near as well as Arman Raza, MD, Amir Azeem, MD, Kwsai Al-Rahhal, MD, and Dwarakraj Soundarraj, MD who assisted by collecting the data for this study.” Resuscitation. 2009 Sep;80(9):985-9. Epub 2009 Jul 5

CONTINUED MEDICAL EDUCATION PRESENTATIONS

Cardiology Research Conference Jan 4, 2012
Department of Internal Medicine, Section on Cardiology.
Wake Forest School of Medicine, Winston Salem, NC
“HbA1c and Cardiovascular Disease Risk.”

Hospital Medicine Noon Conference Jun 16, 2011
Department of Internal Medicine, Section on Hospital Medicine,
Wake Forest Baptist Medical Center, Winston Salem, NC
“Perioperative Medical Evaluation-Cardiovascular Risk Stratification and Prevention of Complications.”

PGY-3 Medicine Grand Round
Sparrow Health System (St. Lawrence Campus), Lansing, MI
Michigan State University, Internal Medicine Residency
“Diabetes Mellitus - Diagnostic and Management Considerations in General Population and in Psychiatric Patients.”

TEACHING ACTIVITIES

Preceptor for Clinical Experience (Adult Inpatient: General Internal Medicine)
For MD/PA students and Internal Medicine residents of Wake Forest School of Medicine at Wake Forest Baptist Medical Center, Winston Salem, NC - Aug 2010- Jun 2013

Preceptor for Clinical Experience (Adult Outpatient: General Internal Medicine)
For MD/PA Students of Wake Forest School of Medicine at Delivering Equal Access to Care (DEAC; a free clinic) in Winston Salem, NC - Aug 2011-Jun 2013

Evaluator for Procedures Objective Structured Clinical Exam (OSCE)
For medical students (class of 2013) of Wake Forest School of Medicine, Winston Salem, NC on May 21, 2012 (2 hours)

Evaluator for Standardized Patient Assessment (SPA)-II
For medical students (class of 2015), Wake Forest School of Medicine, Winston Salem, NC - May 4, 2012 (2 hours)

Preceptor for Bedside Teaching of 2nd year MD students (Group B-13) of Wake Forest School of Medicine at Wake Forest Baptist Medical Center. Sep 2010 through Dec 2010 (Total five sessions of 3 hours each)

Preceptor for Clinical Experience (Adult Inpatient: General Internal Medicine)
For 3rd and 4th year medical students of College of Human Medicine, Michigan State University, East Lansing, MI during various General Medicine and Medical/Cardiac Critical Care rotations. Jul 2003 to Jun 2006

Preceptor for Clinical Experience (Adult Inpatient: General Internal Medicine)
For 2nd Year medical students of College of Human Medicine, Michigan State University (fall semester) at Sparrow Hospital, Lansing, MI. Five students per day for two days. Sep 13, 2005 & Sep 20, 2005.

Preceptor for Clinical Experience (Adult Outpatient: Pulmonary Examination)
For 2nd Year medical students of College of Human Medicine, Michigan State University (fall semester) at Clinical Center, East Lansing, MI. Total of 3 hours (4 groups of 4 students each) on Nov 29, 2005; 3 hours (4 groups of 3 students each) on Nov 30, 2005

Preceptor for Clinical Experience (Adult Inpatient: General Internal Medicine)
For 2nd Year medical students of College of Human Medicine, Michigan State University (fall semester) at Ingham Regional Medical Center and Sparrow Hospital, Lansing, MI. Total 3 hours. Sep 14-16, 2004.

Preceptor for Clinical Experience (Adult Outpatient: Pulmonary Examination)
For 2nd Year medical students of College of Human Medicine, Michigan State University (fall semester) at Clinical Center, East Lansing, MI. Nov 30, 2004.

CONFERENCES & WORKSHOP ATTENDED

Cardiovascular Disease Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism (NPAM) at New Orleans Marriott, New Orleans, LA

- ACP North Carolina Chapter Scientific Sessions, Durham, NC Feb 2013

- Faculty Development Workshop Nov 28, 2012
Understanding Medical Education; Moderators: David Manthey, MD and Christopher Godshall, MD; Wake Forest School of Medicine, Winston Salem, NC

- AHA Scientific Sessions, Los Angeles, CA Nov 4-7, 2012


- AHA Scientific Sessions, Orlando, FL Nov 12-16, 2011

- Multi-ethnic Study of Atherosclerosis (MESA) Annual Meeting Oct 2011
2 days - Washington DC

- AHA 37th Ten-Day Seminar on Jul 24-Aug 5, 2011
Epidemiology and Prevention of Cardiovascular Disease. Lake Tahoe, CA.

- 11th Annual Southern Hospital Medicine Conference, Atlanta, GA. Oct 20-23, 2010
Emory University School of Medicine.

- ACP Michigan Chapter Meeting, at Acme, Michigan Sep 22-Sep 25 2005

- ACP Michigan Chapter Meeting, at Acme, Michigan  
  Sep 30-Oct 01 2004

- Faculty Development Workshop  
  Organized by Department of Medicine, College of Human Medicine, Michigan State University, at Henry Center for Executive Development, Lansing, MI.  
  April 20, 2005

COMMUNITY SERVICE

**Delivering Equal Access to Care (DEAC) – Free clinic**  
Volunteer physician and preceptor for medical students of Wake Forest School of Medicine at this students-led free clinic in Winston Salem, NC  
Aug 2011-

**Scotland County Health Department - Free Clinic**  
Volunteer physician in Laurinburg, NC  
July 2010

**Pre-participation sports physical**  
6th to 8th grade students Sycamore Lane School, Laurinburg, NC  
May 1, 2009

**Patients Welfare Association (PWA)**  
Run by students of Dow Medical College and Civil Hospital, Karachi, Pakistan. Positions held: Director Drug Bank (1st and 2nd year), Vice President Publications (3rd year), Treasurer (4th year), Senior Advisor (5th year), and now a “Lifetime Member.”  
May 1994-

PERSONAL INTERESTS

Squash, tennis, running, reading and traveling  
Languages other than English: Urdu/Hindi/Punjabi

REFERENCES

Upon request.