MODIFICATION OF THE EFFECT OF GLYCEMIC STATUS
ON AORTIC DISTENSIBILITY BY AGE IN THE
MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

BY

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ABSTRACT

Richard Brandon Stacey

MODIFICATION OF THE EFFECT OF GLYCEMIC STATUS ON AORTIC DISTENSIBILITY BY AGE IN THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

Thesis under the direction of David M. Herrington, M.D., M.H.S., Professor of Medicine

Background: Elevated serum glucose from diabetes mellitus (DM) or impaired fasting glucose (IFG) shares many mechanisms with aging that decrease aortic distensibility (AD), such as glycation of the extra-cellular matrix. However, little data compares the simultaneous effects of elevated serum glucose and aging on AD. To study this, we examined the relationship between fasting glucose status, age, and AD in the Multi-Ethnic Study of Atherosclerosis (MESA): a multi-ethnic cohort of individuals aged 45-84 years without clinical cardiovascular disease.

Methods: In MESA, participants with normal fasting glucose (NFG; n = 2270), IFG (n = 870), and DM (n = 412) underwent MRI assessment of proximal thoracic aortic distensibility. This sample was 46% male, 42% white, 30% AA, 11% Asian, and 17% Hispanic. The relationship between glucose status, age, and AD was analyzed with general linear models by adjusting for factors influential on AD. An interaction term was used to determine if age modified the effect of glucose status on AD.

Results: AD was lowest among those with DM. The interaction term was significant
(p = 0.024). Comparing participants less than 65 years of age, AD was different between NFG and DM (p < 0.01), and between NFG and IFG (p = 0.02). In those older than 65, fasting glucose group was no longer a significant predictor of AD.

**Conclusions:** Our data indicate that there are overall differences in AD between DM, IFG, and NFG. However, age modified the effect of glucose status such that differences between the groups diminished with advancing age.
Chapter I

Introduction

Diabetes mellitus is one of the strongest risk factors for cardiovascular disease. Prior studies demonstrate that having diabetes mellitus carries the same risk as having had a previous myocardial infarction.\(^1\) While diabetes mellitus has well established relationships with myocardial infarctions, heart failure,\(^2\) and aortic stiffness,\(^3\) the role of impaired fasting glucose is still being determined. The prevalence of both diabetes mellitus and impaired fasting glucose continue to rise.\(^4\)

To compound the growing problem with the rise of diabetes and impaired fasting glucose, the population continues to age. By 2040 in the United States, individuals 65 years old or over will double.\(^5\) This group accounts for over 80% of deaths related to coronary artery disease, as well as 75% of the total population diagnosed with heart failure.\(^6\) One well-described cardiovascular change that occurs with aging is increased aortic stiffness. Aortic stiffness is a strong, independent predictor of cardiovascular mortality in elderly patients.\(^7\) Both diabetes and aging cause glycation of several components in the blood vessel wall.\(^8, 9, 10, 11\) Prior researchers have described that increased age decreased the difference in pulse wave velocities between diabetics and non-diabetics.\(^12\) However, no major studies have confirmed this, nor have any studies addressed whether pre-diabetes (impaired fasting glucose) carries a similar interaction with aging.

To determine the effect of a possible interaction between fasting glucose status and aging on aortic stiffness, we turned to the Multi-Ethnic Study of Atherosclerosis...
(MESA). With over 3,500 MRI scans measuring aortic distensibility, MESA provides an opportunity to describe these relationships with more precise imaging techniques. MESA recruited individuals from different ethnic backgrounds aged 45-84 without prior cardiovascular disease, and of these participants, approximately 25% have impaired fasting glucose.

Aging Population

One of the most remarkable developments in recent history is the expansion of an older population. For the first time in known history, individuals older than 65 years of age outnumber children under the age of 5. In the United States alone by 2040, the population older than 65 years of age is expected to double from 40 million to 80 million. This growth is also occurring in other developed and less developed regions of the world. The increase in longevity has enabled this to happen. In east Asia, the life expectancy went from 45 in 1950 to 72 today. Much of this advance is due to better treatment of infectious diseases with improved medical therapy and sanitation. Likewise, this advance has been enabled by better medical treatment of more chronic diseases, such as hypertension, diabetes mellitus, and coronary artery disease.

Increase in Diabetes Mellitus and Impaired Fasting Glucose

Parallel to the expansion of the older demographic, the prevalence type 2 diabetes mellitus has also greatly expanded. In France, the prevalence of diabetes mellitus increased from 2.78% in 1997 to 3.5% in 2005. In the United States in 2005, the prevalence of diabetes mellitus was 7%. This increased from 4.9% in 1990. However, these trends are not isolated to developed nations. In 2005, the prevalence of pharmacologically treated diabetes equaled 10.1% in Guadeloupe, 7.9% in Martinique
3.7. and 7.4% in La Reunion Island. Expressed in number of patients, the total number of adult diabetic patients in the world will increase from 135 to 300 million between 1995 and 2030, mainly due to a tremendous increase of 171% in developing countries, from 84 to 228 million, while it will increase only 41% in developed countries, from 51 to 72 million. This is concerning due to the increased burden of cardiac disease that will be precipitated by the growth of diabetes.

In the late 1980’s and 1990’s, the metabolic syndrome became the focus of a significant amount of research. Several different professional societies have developed definitions. Most base their criteria on fasting glucose levels, waist-hip ratio, body mass index, blood pressure, triglycerides, and HDL levels. Citing the importance of abnormal glucose metabolism, the World Health Organization defines the metabolic syndrome as having impaired fasting glucose with at least 2 additional abnormal criteria from above. Using the ATP-III definition of the metabolic syndrome, investigators using NHANES (1988-1994) demonstrated that the overall prevalence of the metabolic syndrome was approximately 23%. This study also demonstrated that the prevalence among those aged 20-29 years was approximately 7%, while the prevalence was over 40% in people aged 60-69 years. The metabolic syndrome defined by multiple definitions predicts cardiovascular disease mortality in elderly patients in Finland. Likewise, coronary heart disease, cardiovascular disease, and total mortality are significantly higher in US adults with metabolic syndrome than in those without it.

Impaired fasting glucose is regarded as one of the chief components of the metabolic syndrome. Impaired fasting glucose is defined as a fasting glucose level from 100 mg/dl to 125 mg/dl. Whereas the prevalence of diabetes in the U.S. is
approximately 7%, the prevalence of impaired fasting glucose is 26% in a cross-section sample of U.S. adults aged 20 to 74 years (tested from 1988 to 1994). In individuals aged 20-39, the prevalence of impaired fasting glucose was 16%, but in individuals aged 65 years or more, the prevalence of impaired fasting glucose was 39.1%. In elderly individuals, impaired fasting glucose increased the risk of myocardial infarction, stroke, or coronary death by 66% compared to elderly individuals with normal fasting glucose.

Diabetes mellitus has long been recognized as a significant risk factor for cardiovascular disease, and as such, evidence-based guidelines assist clinicians in reducing their cardiac disease risk. A growing body of literature suggests that having impaired fasting glucose also increases the risk of developing cardiovascular disease. However, few evidence-based guidelines are available to help clinicians to address the increased risk associated with impaired fasting glucose.

**Aortic stiffness**

Aortic stiffness provides us with additional data to consider when evaluating vascular fitness and function. It has been shown to be an independent predictor of cardiovascular mortality in different subgroups including hypertensive, elderly, and chronic renal patients. Aortic stiffness also indicates an increased risk of fatal and non-fatal cardiovascular disease in patients with impaired fasting glucose and diabetes mellitus.

Aortic stiffness is an important component in the pathogenesis of heart failure. In stable, compensated heart failure, left ventricular afterload is elevated by increased wave reflection from the periphery. In acute, decompensated heart failure, pulse wave velocity is increased, and with treatment, particularly diuresis, the pulse wave velocity
decreases to levels seen in those with stable, compensated heart failure. One possible mechanism for these observations is activation of the renin-angiotensin-aldosterone system. Activation of this system results in a measured decrease in arterial compliance. The presence of aortic stiffness on MRI stress testing correlates to decreased exercise tolerance and poor functional capacity in patients with systolic heart failure. In patients with diastolic heart failure, increased aortic stiffness also corresponds to severe exercise intolerance.

One relationship being studied is the relationship between ascending aortic stiffness and coronary flow reserve. Coronary flow reserve represents the coronary circulation’s ability to increase blood flow to meet increased metabolic needs. It is measured during transesophageal echocardiographic examination by using pulse wave Doppler to assess blood flow velocity in the left anterior descending coronary artery. To measure hyper-metabolic coronary flow, the coronary flow is measured during administration of adenosine or dipyridamole. Several studies have demonstrated a consistent correlation between increased aortic stiffness and reduced coronary flow reserve in patients with hypertension, diabetes mellitus, hypercholesterolemia, coronary artery disease, and aortic valvular stenosis. A cardiac catheterization study reported that aortic stiffness was associated with reduced coronary blood flow, a lower hyperemic coronary blood flow, and reduced the improvement in coronary blood flow after successful percutaneous coronary intervention. Likewise, older individuals who presented with chest pain who had a normal coronary angiogram had reduced coronary flow reserve and increased aortic stiffness when compared to younger individuals with normal coronary angiograms. Since coronary blood flow occurs during diastole, this
has caused some to speculate that aortic stiffness may actually impair coronary filling due to reduced aortic elastic recoil. However, there is no temporal data yet to confirm this.

**Aging and Aortic Stiffness**

It is well-established that aging increases aortic stiffness. Early studies focused on autopsy comparisons between the young and elderly. In 1937, one such study used pressure-volume experiments from cadaver aortas to demonstrate that with aging, the aorta becomes less compliant. More recently with MRI, researchers have demonstrated that compliance in the aorta falls with age, and its decrease is most obvious in the ascending aorta.\(^{48}\) Comparing patients less than 55 years of age to individuals older than 55, it has been demonstrated that the aorta stiffens, and this is most evident in the proximal aorta.\(^{49}\) Histologically with aging, the aorta has a decrease in elastin, an increase in collagen content, and an increase in mucopolysaccharides.\(^{50}\)

**Diabetes and Aortic Stiffness**

Diabetes mellitus is also a major catalyst for increasing aortic stiffness. Diabetic patients develop aortic stiffness early in their disease course.\(^{51}\) Compared to their normal glycemic counterparts, children with type I diabetes mellitus already demonstrate some degree of aortic stiffness.\(^{52}\) In patients with type 2 diabetes mellitus, aortic stiffness increases independent from blood pressure and correlates with diastolic left ventricular function.\(^{53}\) In the Hoorn study, both impaired fasting glucose and type II diabetes mellitus were associated with aortic stiffness.\(^{54}\) Diabetes increases aortic stiffness in a manner similar to aging.\(^{12}\)
Pathways from Aging and Diabetes to Aortic Stiffness

Understanding the extra-cellular matrix is important to understanding the function of the vascular system. Elastin is most concentrated and organized in the proximal ascending aorta, but as one travels distally, elastin content and organization deteriorate.\textsuperscript{55} Conversely, vascular smooth muscle is present in only a small amount in the ascending aorta, but it greatly increases in content as one goes to the peripheral arteries. However, collagen is very organized and ubiquitous throughout the vascular tree.

The effect of aging and diabetes on elastin and collagen present in large arteries may help explain their effect on aortic stiffness. Elastin is cross-linked with collagen via the amino acid aldosine. The aldosine content increases in the aorta until mammals reach maturity, and at that point, it decreases with aging as elastin is replaced with collagen.\textsuperscript{56} Mammalian aortas have less aldosine in diabetic specimens, which suggests that there is less elastin in the aortas of those with diabetes. Likewise, desmosine and isodesmosine are specific amino acids found only in elastin.\textsuperscript{57} In autopsy studies, diabetic aortas had a lower desmosine to hydroxyproline ratio than non-diabetic aortas, which represents less elastin in relation to collagen. Not only does aging and diabetes decrease the amount of elastin, but collagen cross-linking with glucose, also called glycation, is increased. This is known to occur in both diabetic patients and through the process of aging. Collagen cross-linking with glucose increases the tensile strength of collagen.\textsuperscript{58}

In addition to glycation, there are other mechanisms through which aging and diabetes affect the vasculature. First, both aging and diabetes increase atherosclerosis. Atherosclerosis is almost exclusively a condition that manifests itself later in life. Likewise, diabetics often encounter more clinical disease from atherosclerotic burden.
Second, general inflammation increases as individuals age.\textsuperscript{59} Likewise, those individuals with diabetes mellitus have increased systemic inflammation.\textsuperscript{60} Finally, endothelial function is affected both by aging and diabetes.\textsuperscript{61,62}

**Measures of Aortic Stiffness**

There are several measures used to describe aortic stiffness. One widely-used measure is arterial tonometry due to its reliability and cost-effectiveness.\textsuperscript{63} Several indices have been proposed, but 2 measures are highly reproducible and have been widely studied: (1) Pulse wave velocity, and (2) Augmentation index.\textsuperscript{64} Dr. Bramwell was one of the first researchers to describe pulse wave velocity, and in particular, the relationship between aging and increased pulse wave velocity, which is due to aortic stiffness.\textsuperscript{65} The augmentation index is a measure of how much the reflected wave augments central pressure. It helps to describe the overall interaction between the arterial tree and the left ventricle.\textsuperscript{66} Arterial tonometry provides an estimate of central pressure, which may be more relevant to evaluating left ventricular afterload since it is the central pressure to which the left ventricle is exposed. At present, arterial tonometry measurement of pulse wave velocity is regarded by some as the standard when evaluating aortic stiffness.\textsuperscript{67}

Other measures are also used by investigators to describe vascular stiffness, such as distensibility of a particular artery. Aortic distensibility was first derived from experiments conducted during open-heart surgery in the early 1960’s.\textsuperscript{68} It is calculated by using the following validated formula.\textsuperscript{69,70,38}
Aortic Distensibility = \( \frac{\text{max. aortic area} - \text{min. aortic area}}{\text{minimum aortic area}} \)

Pulse Pressure

Many studies have investigated the relationship between aortic distensibility and different cardiovascular risk factors, including smoking\(^71\), hypertension\(^70\), and hyperlipidemia\(^72\).

The primary reservation against non-invasive measures of aortic stiffness is use of the brachial or radial pulse pressure.\(^73\) However, cardiac catheterization provides these measures with the added benefit that central aortic pulse pressure is directly measured rather than inferred. Using echocardiography, investigators demonstrated a high correlation (\(r=0.94; p <0.001\)) between echocardiographic determination of aortic distensibility calculated with brachial pulse pressure and invasive measurement with cardiac catheterization. Hence, non-invasive measures of aortic distensibility are suitable for interpretation and analysis.

MRI assessment of aortic distensibility and vascular function is highly correlated with ultrasound and echocardiographic measurement, and it provides the benefit of acquiring multiple measures at the same time.\(^74\) In addition, cardiac MRI is a highly reproducible measure with lower interobserver and intra-observer variability when compared with echocardiography, a widely-used imaging modality.\(^75\)

**Multi-Ethnic Study of Atherosclerosis**

MESA is a longitudinal epidemiological study of 6,814 men and women. The MESA recruitment protocol has been previously published.\(^76\) In summary, MESA recruited individuals aged 45-84 years who were free of clinical cardiovascular disease. It contains significant representation from 4 major ethnic groups: Caucasian, African-
American, Hispanic, and Chinese. The patients were recruited from 6 clinical sites that included: Forsyth County, NC; New York, NY; Baltimore, MD; Chicago, IL; Minneapolis, MN; Los Angeles, CA. Participation in MESA included 4 exam visits, which were scheduled over a 3-5 year period. Exam 1 occurred from July 2000 to August 2002, and it contained multiple questionnaires, history and physical measurements, lab analysis, a CT scan, and a cardiac MRI scan. Of the individuals enrolled, approximately 3,500 underwent MRI scans from which aortic distensibility was measured.

With a broad age range and diverse representation, MESA presents an opportunity to evaluate aortic distensibility prior to the manifestation of cardiac disease. As such, these results will be generalizable to a middle-aged and elderly population without cardiac disease.

As previously stated, an older population will present new challenges for health care. In addition, changes in lifestyle have precipitated an unprecedented rise in diabetes mellitus, as well as impaired fasting glucose, which represents a pre-diabetic state. The process of aging and diabetes affect the vasculature through shared mechanisms, such as glycation of the vessel wall. However, little data describes the interaction between aging and diabetes on cardiovascular disease. No major studies describe the possibility of an interaction between age and impaired fasting glucose on cardiovascular disease. The purpose of our study is to fill these gaps and to contribute knowledge on how impaired fasting glucose acts as a risk factor for cardiovascular disease. To accomplish these goals, we will turn to the Multi-Ethnic Study of Atherosclerosis to determine if an interaction between age and glycemia status affects aortic distensibility.
**Hypothesis:** Age modifies the effect of fasting glucose status on aortic distensibility.

**Specific Aims:** This study will perform the following specific aims through a secondary analysis of MESA:

I. **Primary Specific Aim:** To determine if age modifies the effect of diabetes mellitus on aortic distensibility.

II. **Secondary Specific Aims:**

   i. To determine if age modifies the effect of impaired fasting glucose on aortic distensibility.

   ii. To determine if there are differences between men and women in how age modifies the effect of fasting glucose status on aortic distensibility.

   iii. To determine if there are differences between ethnicities in how age modifies the effect of fasting glucose status on aortic distensibility.

**Significance:** In light of the aging U.S. population, this study seeks to add to our knowledge of the relationship between fasting glucose level and aortic distensibility, particularly as this relationship may be modified by age. With 25% of the U.S. adult population having impaired fasting glucose, even a small increase in cardiovascular risk can have significant public health ramifications.
Reference List


(30) van d, V, de WO, Gillebert TC, de SJ. Diabetes and impaired fasting glucose as predictors of morbidity and mortality in male coronary artery disease patients with reduced left ventricular function. *Acta Cardiol* 2006 April;61(2):137-43.


(61) Lauer T, Heiss C, Balzer J, Kehmeier E, Mangold S, Leyendecker T, Rottler J, Meyer C, Merx MW, Kelm M, Rassaf T. Age-dependent endothelial dysfunction is associated with


(65) Bramwell J, Hill A, McSwinney B. The velocity of the pulse wave in man in relation to age as measured by the hot wire sphygmograph. Heart 10, 233-256. 1923. Ref Type: Generic


Chapter II

Modification of the Effect of Glycemic Status on Aortic Distensibility by Age in the Multi-Ethnic Study of Atherosclerosis

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Introduction

The aging population will present difficult challenges for health care providers in the coming decades. By 2040 in the United States, individuals who are 65 years old or over will double from 40 million to over 80 million people.\(^1\) This age group accounts for over 80\% of deaths related to coronary artery disease in the United States, as well as 75\% of the total population diagnosed with heart failure.\(^2\)

One well-described change that occurs with aging is aortic stiffness. Aortic stiffness is a strong, independent predictor of cardiovascular mortality in elderly patients.\(^3\) Aging and diabetes mellitus affect the vasculature through glycation of the vascular wall.\(^4,5,6,7\) Prior researchers have described an interaction between age and diabetes in which increasing age decreased the difference in pulse wave velocity between diabetics and non-diabetics.\(^8\) However, this has not been replicated. In addition, no major studies have addressed whether impaired fasting glucose also modifies this relationship.

With almost 3,500 MRI scans measuring aortic distensibility, the Multi-Ethnic Study of Atherosclerosis (MESA) provides an opportunity to revisit these relationships with more precise imaging techniques and a larger study population. The purpose of this study is to determine if age modifies the effect of diabetes mellitus on aortic distensibility. In addition, we also seek to determine if age modifies the effect of impaired fasting glucose on aortic distensibility.
Methods

Study Participants

The recruitment criteria of participants in MESA has been previously published.9 The MESA study is a population-based cohort of 6,814 men and women aged 45-84 from four ethnic groups (Caucasian, African-American, Hispanic, and Chinese) who were free of clinical cardiovascular disease when recruited from 2000-2002. Of these, we excluded 1,810 who did not have cardiac MRI. We further excluded 1,443 participants without aortic distensibility measures, and 9 participants with missing diabetes status.

As part of the baseline exam, participants submitted fasting blood samples. Samples were collected at each clinical site and sent to the central laboratory for analysis. Glucose was measured in serum at the University of Minnesota Central Laboratory using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650). Total cholesterol and HDL-C were measured in EDTA plasma on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation, Indianapolis, IN) using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation). Before measurement of HDL-C, the non-HDL-C fractions were precipitated with magnetic 50,000 MW dextran sulfate and magnesium chloride. Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation). LDL-C was calculated in specimens having a triglyceride value <400 mg/dL using the Friedewald equation.

Individuals in this study were classified into 1 of 3 groups using criteria based on fasting glucose level established by the American Diabetes Association.10 These groups
included normal fasting glucose (NFG: fasting glucose level < 100 mg/dl), impaired fasting glucose (IFG: fasting glucose level 100-125 mg/dl), and diabetes mellitus (DM: fasting glucose level >126 mg/dl). Individuals with a history of diabetes were classified into the DM group without regard to their fasting glucose level.

Resting, seated systolic and diastolic blood pressure was measured 3 times using a Dinamap automated oscillometric sphygmomanometer (Model pro100l Critkon, Tampa Fl); the last 2 measures were averaged for analyses. Hypertension was defined on the basis of use of an antihypertensive medications or BP\(\geq\) 140/90. Use of lipid-lowering medication was used as an indicator of being diagnosed with high cholesterol. Cigarette use was divided into 3 groups: never, former, and current, which was defined as having smoked within the past 30 days.

**Magnetic Resonance Imaging Technique**

MRI studies were acquired at 6 participating sites using 1.5 Tesla magnets (3 were Siemens Medical Solutions [Erlangen, Germany] Symphony or Sonata platforms, and 3 were General Electric Medical Systems [Waukesha, WI] CV/I or LX platforms). Participants were scanned in a supine position using a torso phased array coil placed anteriorly and posteriorly.

Images of the proximal thoracic aorta were acquired axially at the level of the main pulmonary artery identified on a sagittal scout image. Imaging parameters included a phase-contrast gradient-echo sequence. Imaging parameters were as follows: repetition time = 10 msec; echo time = 1.9 msec; field of view = 34 cm; slice thickness = 8 mm; matrix size = 256 \times 224; 2 signal averages; temporal resolution = 20 ms; velocity encoding gradient = 150 cm/s in the superior to inferior direction; and receiver bandwidth \(\pm\) 32 kHz. Blood pressure
was measured in the supine position at the beginning and end of the 45 minute MRI session; the two results were averaged for the final blood pressure measurement.

**Ascending Aortic Stiffness**

To determine ascending thoracic aortic stiffness, aortic distensibility was calculated by using the following validated formula:

\[
\text{Aortic Distensibility} = \frac{(\text{max. aortic area} - \text{min. aortic area})}{\text{minimum aortic area}}
\]

\[
\text{Pulse Pressure} = \text{Systolic Blood Pressure} - \text{Diastolic Blood Pressure}
\]

**Statistical Analyses**

Baseline characteristics were described for each fasting glucose group (NFG, IFG, and DM). T-tests and chi-square tests were performed to identify statistical differences in baseline characteristics between the groups with normal fasting glucose serving as our reference. Next, we compared aortic distensibility between the fasting glucose groups through ANCOVA. From this point, multiple linear regression lines of aortic distensibility for each fasting glucose group on age were generated by PROC GLM in SAS Enterprise Guide 4.1 (Copyright by SAS Institute, Inc.). We first included fasting glucose group and age as well as an interaction term between the two in the model to predict aortic distensibility (Model 1--see below). Next, additional covariates were adjusted for their influence on the relationship between fasting glucose level and age.
Graphs depicting these relationships were generated with GraphPad Prism version 4.00 for Windows (GraphPad Software: San Diego California USA; www.graphpad.com).

There were 2 models fit to describe aortic distensibility. These models included:

Model 1: age, fasting glucose group, age*fasting glucose group (interaction term)

Model 2: Model 1 + adjustment for gender, race, body mass index, mean arterial pressure, use of anti-hypertensive medication, LDL, HDL, Triglycerides, use of lipid-lowering medications, smoking history, pack years, creatinine and C-reactive protein levels (CRP).

For these models, the interaction term was examined. If significant at level of 0.05, specific pairwise comparisons of the slopes of the regression lines on age were performed.

From this point, age was divided into 2 groups (45-64 years vs. 65-84 years) as a categorical variable to use for both a main effect as well as a part of an interaction term with fasting glucose group to interpret the interaction. First, the adjusted means of aortic distensibility were compared using model 1. Next, ANCOVA analyses were performed using model 2.

Results

After exclusions, our study population consisted of 3,552 participants. Their characteristics by glycemic status are shown in Table 1. Both the IFG and DM groups
had higher BMI, higher systolic blood pressure, and higher C-reactive protein than the NFG group. In addition, both IFG and DM groups had lower HDL cholesterol than the NFG group. In comparing demographic features, the NFG group was younger and composed of more women and white participants. A box plot of aortic distensibility by 5-year age groups is presented in Figure 1.
<table>
<thead>
<tr>
<th></th>
<th>Normal (n=2270)</th>
<th>IFG (n=870)</th>
<th>DM (n=412)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>59.4 ± 10.0</td>
<td>62.2 ± 9.9</td>
<td>63.5 ± 9.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 ± 4.7</td>
<td>28.8 ± 5.1</td>
<td>29.8 ± 5.3</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.0 ± 0.8</td>
<td>3.1 ± 0.8</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Trig (mmol/L)</td>
<td>1.3 ± 0.9</td>
<td>1.5 ± 0.9</td>
<td>1.7 ± 1.4</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.0 ± 0.3</td>
<td>5.9 ± 0.3</td>
<td>8.4 ± 2.8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.1 ± 20.3</td>
<td>128.2 ± 21.1</td>
<td>132.7 ± 22</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>71.3 ± 10.2</td>
<td>73.4 ± 10.4</td>
<td>72.8 ± 10.4</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>88.2 ± 12.3</td>
<td>91.7 ± 12.6</td>
<td>92.8 ± 12.6</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>80.8 ± 15.6</td>
<td>81.4 ± 16.7</td>
<td>87.4 ± 23.7</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>83.1 ± 17.7</td>
<td>85.7 ± 17.7</td>
<td>85.7 ± 51.3</td>
</tr>
<tr>
<td>Smoking pack years</td>
<td>10.3 ± 22.8</td>
<td>12.1 ± 22.4</td>
<td>12.1 ± 22.4</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/L)</td>
<td>3.3 ± 5.4</td>
<td>4.0 ± 6.4</td>
<td>4.5 ± 6.6</td>
</tr>
<tr>
<td>Aortic Distensibility (10^3 * mmHg^-1)</td>
<td>2.1 ± 1.5</td>
<td>1.9 ± 1.3</td>
<td>1.6 ± 1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number (%)</th>
<th>Number (%)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid medication use</td>
<td>278 (12%)</td>
<td>155 (18%)</td>
<td>116 (28%)</td>
</tr>
<tr>
<td>HTN medication use</td>
<td>623 (27%)</td>
<td>377 (43%)</td>
<td>258 (63%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Caucasian</td>
<td>1061 (47%)</td>
<td>337 (39%)</td>
<td>98 (24%)</td>
</tr>
<tr>
<td>-Hispanic</td>
<td>233 (10%)</td>
<td>111 (13%)</td>
<td>49 (12%)</td>
</tr>
<tr>
<td>-Afro-American</td>
<td>605 (27%)</td>
<td>267 (31%)</td>
<td>189 (46%)</td>
</tr>
<tr>
<td>-Chinese</td>
<td>372 (16%)</td>
<td>156 (18%)</td>
<td>77 (19%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Male</td>
<td>939 (41%)</td>
<td>475 (55%)</td>
<td>209 (51%)</td>
</tr>
<tr>
<td>-Female</td>
<td>1332 (59%)</td>
<td>396 (45%)</td>
<td>204 (49%)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Never</td>
<td>1188 (52%)</td>
<td>429 (49%)</td>
<td>202 (49%)</td>
</tr>
<tr>
<td>-Former</td>
<td>767 (34%)</td>
<td>326 (37%)</td>
<td>167 (40%)</td>
</tr>
<tr>
<td>-Current</td>
<td>308 (14%)</td>
<td>111 (13%)</td>
<td>43 (10%)</td>
</tr>
</tbody>
</table>

**Table 1.** Baseline characteristics; asterisk by either IFG or DM variable indicates difference with NFG with a p < 0.05.
In analyzing Model 1, the interaction term was statistically significant (p=0.04). Individually assessing the glucose status comparisons, the slope of age was different between the DM group and the NFG group (p= 0.031), but there was no difference in the slopes of age between the DM group and the IFG group (p = 0.39). There was a trend towards a difference in the slope of age between the IFG group and the NFG group (p = 0.088). These comparisons are shown in Table 2, and are presented graphically in Figure 2.
### Table 2

<table>
<thead>
<tr>
<th>Fasting glucose group</th>
<th>Model 1 Standardized Regression β Coefficient for Age</th>
<th>Model 2 Standardized Regression β Coefficient for Age</th>
<th>Aortic Distensibility Comparisons</th>
<th>Model 1 p-value</th>
<th>Model 2 p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFG</td>
<td>-0.43</td>
<td>-0.38</td>
<td>Age</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IFG</td>
<td>-0.36</td>
<td>-0.30</td>
<td>Overall interaction term</td>
<td>0.0412</td>
<td>0.024</td>
</tr>
<tr>
<td>DM</td>
<td>-0.32</td>
<td>-0.26</td>
<td>-NFG Age β vs. IFG Age β</td>
<td>0.088</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-IFG Age β vs. DM Age β</td>
<td>0.39</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-NFG Age β vs. DM Age β</td>
<td>0.031</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Table 2. Comparison of interaction standardized regression coefficients and corresponding p-values. NFG is reference group, except for IFG vs. DM, in which IFG serves as the reference group.

**Figure 2.** Model 1: Unadjusted linear regression lines of AD on age stratified by fasting glucose group.

Adjusting for additional covariates in model 2, the overall interaction term continued to be statistically significant (p = 0.024). There continued to be a difference in the slope of age between the NFG group and the DM group (p = 0.022). In addition, the trend towards a difference between slope of age in the IFG group and slope of age in the
NFG group was strengthened (p = 0.057). There was no difference between slopes of age in the IFG group and the DM group (p = 0.38). These comparisons are shown in Table 2, and they are represented graphically in Figure 3.

**Figure 3.** Model 2: Linear regression lines of AD on age stratified by fasting glucose group. Adjusted for race, gender, body mass index, mean arterial pressure, use of anti-hypertensive medication, LDL, HDL, Triglycerides, use of lipid-lowering medications, smoking history, pack years, and C-reactive protein levels (CRP).

To further interpret this interaction, age was divided into 2 groups (45-64 years vs. 65-84 years) to use as a categorical variable in an interaction term with fasting glucose group through two-way ANCOVA. The overall interaction term was significant (p = 0.04). In comparing aortic distensibility between the fasting glucose groups for the 2 age groups, it reflects the results obtained from our overall regression models (see table 3). In the younger group, NFG is different from IFG (Model 2 p-value: 0.024) and DM (Model 2 p-value: <0.0001). Analyzing the older group through model 2, NFG was no longer statistically different from IFG (Model 2 p-value: 0.5), nor was it significantly different from DM (Model 2 p-value: 0.2). For stratified comparisons, please refer to
Table 3 and Figure 4.

<table>
<thead>
<tr>
<th></th>
<th>NFG AD $(10^3 \text{mmHg}^{-1})$</th>
<th>IFG AD $(10^3 \text{mmHg}^{-1})$</th>
<th>DM AD $(10^3 \text{mmHg}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age &lt; 65 years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>2.42 (2.35, 2.48)</td>
<td>2.22 (2.1, 2.33) *</td>
<td>1.91 (1.74, 2.08) *</td>
</tr>
<tr>
<td>Model 2</td>
<td>2.31 (2.22, 2.39)</td>
<td>2.16 (2.03, 2.28) *</td>
<td>1.88 (1.71, 2.07) *</td>
</tr>
<tr>
<td><strong>Age ≥65 years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.47 (1.38, 1.57)</td>
<td>1.47 (1.35, 1.6)</td>
<td>1.24 (1.05, 1.41) *</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.52 (1.41, 1.63)</td>
<td>1.57 (1.43, 1.7)</td>
<td>1.38 (1.03, 1.40)</td>
</tr>
</tbody>
</table>

**Table 3.** Comparisons of the model 1 and model 2 AD means and 95% confidence intervals between fasting glucose group stratified by age 65. For comparisons, NFG serves as the reference. An asterisk (*) means statistically different at p-value < 0.05.

**Figure 4.** Model 2 stratified by age. Adjusted for race, gender, body mass index, mean arterial pressure, use of anti-hypertensive medication, LDL, HDL, Triglycerides, use of lipid-lowering medications, smoking history, pack years, and C-reactive protein levels (CRP). Black bars represent age < 65; Grey bars represent age > 65. Error bars represent 95% Confidence Interval.
To determine if these results differed by race or gender, three-way interaction terms were used. Using model 2, age, fasting glucose group, and race were used as the interaction term, but failed to reach statistical significance (p-value: 0.15). In a separate analysis using model 2, age, fasting glucose group, and gender were used as the interaction term, but likewise, the term failed to reach statistical significance (p-value: 0.2). Next, we stratified the cohort according to race and gender, separately. The absolute values in the differences between the fasting glucose groups in the 2 age categories for each ethnic group and gender paralleled those of the entire cohort.

To determine if the variables in the numerator (percent change in aortic area for proximal thoracic aorta) or the denominator (pulse pressure) of aortic distensibility were more influential in accounting for the results, additional analyses using Model 2 were performed. Overall, the interaction term continued to be significant (p = 0.002). After adjusting for the cardiac cycle dependent percent change in aortic area, aortic distensibility before age 65 was $2.18 \times 10^{-3} \text{ mmHg}^{-1}$ for NFG, $2.11 \times 10^{-3} \text{ mmHg}^{-1}$ for IFG (p=0.01 from NFG), and $2.00 \times 10^{-3} \text{ mmHg}^{-1}$ for DM (p < 0.001 from NFG), and after age 65, aortic distensibility was $1.75 \times 10^{-3} \text{ mmHg}^{-1}$ for NFG, $1.78 \times 10^{-3} \text{ mmHg}^{-1}$ for IFG (p=0.32 from NFG), and $1.75 \times 10^{-3} \text{ mmHg}^{-1}$ for DM (p > 0.4 from NFG). In separate analyses adjusting for pulse pressure, the interaction term was less significant (p = 0.054). After adjustment for pulse pressure, aortic distensibility before age 65 was $2.18 \times 10^{-3} \text{ mmHg}^{-1}$ for NFG, $2.06 \times 10^{-3} \text{ mmHg}^{-1}$ for IFG (p=0.06 from NFG), and $1.86 \times 10^{-3} \text{ mmHg}^{-1}$ for DM (p < 0.001 from NFG), and after age 65, aortic distensibility was $1.71 \times 10^{-3} \text{ mmHg}^{-1}$ for NFG, $1.76 \times 10^{-3} \text{ mmHg}^{-1}$ for IFG (p = 0.5 from NFG), and $1.67 \times 10^{-3} \text{ mmHg}^{-1}$ for DM (p=0.6 from NFG). Since many of the patterns noted between the groups in Model 2
remain, the results of these adjustments suggest that both values in the numerators and denominators are important for influencing the differences or similarities in aortic distensibility noted between the groups assessed in this study.

Finally, to determine the influence of aortic size on aortic distensibility, additional analyses using Model 2 adjusted for minimum aortic area were performed. The interaction term was no longer significant (p = 0.18). After adjusting for the minimum aortic area, aortic distensibility before age 65 was $2.27 \times 10^{-3} \text{ mmHg}^{-1}$ for NFG, $2.14 \times 10^{-3} \text{ mmHg}^{-1}$ for IFG (p=0.039 from NFG), and $1.85 \times 10^{-3} \text{ mmHg}^{-1}$ for DM (p < 0.001 from NFG), and after age 65, aortic distensibility was $1.67 \times 10^{-3} \text{ mmHg}^{-1}$ for NFG, $1.68 \times 10^{-3} \text{ mmHg}^{-1}$ for IFG (p=0.8 from NFG), and $1.44 \times 10^{-3} \text{ mmHg}^{-1}$ for DM (p=0.02 from NFG).

While the pattern persists, after age 65 NFG and DM become statistically different. This suggests that the lack of difference seen previously may be related to aortic size.

**Discussion**

The relationship between aging and aortic stiffness has been described for many years.\textsuperscript{14,15} In this study, we sought to determine if increasing age modified the effect of DM on aortic distensibility, and to determine if the effect of IFG on aortic distensibility would also be modified by increasing age. From this study, several relationships can be described. First, increasing age decreases the differences in aortic distensibility between fasting glucose groups. Second, at younger ages, IFG decreased aortic distensibility when compared to NFG, and as expected, it behaves as an intermediate between DM and NFG.

Multiple mechanisms describe how aging affects aortic distensibility. Glycation of the extra-cellular matrix, including both elastin and collagen, occurs even at normal
levels of glucose as a consequence of aging.\textsuperscript{16,17,18,19} This develops through a Maillard-browning-type reaction.\textsuperscript{20,21} Glycation of collagen results in less flexibility, greater strength\textsuperscript{22}, and an increased resistance to proteolysis.\textsuperscript{23} Though not as well studied as collagen, glycation of elastin results in fragmentation\textsuperscript{24} and loss of elasticity.\textsuperscript{25,26,27} These same non-enzymatic glycation processes occur with increased frequency to both collagen and elastin in hyperglycemic states.\textsuperscript{28,29}

In addition to glycation, there are other mechanisms through which aging and diabetes affect vasculature. First, atherosclerosis almost exclusively manifests itself later in life. Likewise, diabetics encounter more clinical disease from atherosclerotic burden, even at younger ages. Second, general inflammation increases as individuals age.\textsuperscript{30} Those with diabetes mellitus also have increased systemic inflammation.\textsuperscript{31} Finally, endothelial function is affected both by aging and diabetes.\textsuperscript{32,33}

Increased ascending aortic distensibility greatly reduces the energy cost of cardiac work.\textsuperscript{34} As such, therapeutic options to improve aortic distensibility may be an attractive option, particularly in heart failure. One such option, collagen cross-link breakers, failed to improve aortic distensibility.\textsuperscript{35} In the ascending aorta, elastin is at its highest concentration and organization, whereas collagen is uniformly-distributed throughout the vascular tree.\textsuperscript{36} Hence, in describing aortic distensibility from MRI measurements of the ascending aorta, the effects of age and diabetes on elastin may account for the behaviors seen in our study, and as such, may serve as a target of future therapy.

The interaction seen in this study is due to a greater association between fasting glucose group and aortic distensibility at younger ages than at older ages. Aortic distensibility started at a lower level in the DM and IFG groups than the normal group.
As such, both DM and IFG had lower slopes than the normal group with the differences between the 3 groups decreasing with increasing age. If this study were performed prospectively, one would expect a steep decline in aortic distensibility during the time of impaired fasting glucose and early diabetes that would be followed by the lower slopes observed in our analysis. In addition, studies in younger populations are needed to further describe the relationship between different measures of vascular stiffness and fasting glucose level. One issue that may have influenced our analysis is that many of our participants already had stiff vessels as a result of aging alone.

The shared mechanisms of action between diabetes and aging enable us to consider a previously published concept. Diabetes may act by increasing the physiologic age of the cardiovascular system. From our study, this influence can be described quantitatively from the regression models (see table 4). This provides us with an additional perspective to consider how diabetes mellitus often leads to conditions at younger ages, such as diastolic dysfunction, that otherwise are not encountered until later in life. In addition, it provides us with a vehicle to explain to patients how diabetes affects their vasculature.
Table 4. Based on the adjusted regression equations, for the predicted AD in a diabetic at a given age, the equivalent age for that predicted AD is given for the NFG and IFG groups.

<table>
<thead>
<tr>
<th>DM age (yrs)</th>
<th>Equivalent IFG Age (yrs)</th>
<th>Equivalent NFG Age (yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>50.2</td>
<td>53.6</td>
</tr>
<tr>
<td>50</td>
<td>54.4</td>
<td>57.2</td>
</tr>
<tr>
<td>55</td>
<td>58.6</td>
<td>60.8</td>
</tr>
<tr>
<td>60</td>
<td>62.8</td>
<td>64.4</td>
</tr>
<tr>
<td>65</td>
<td>66.9</td>
<td>67.9</td>
</tr>
<tr>
<td>70</td>
<td>71.1</td>
<td>71.5</td>
</tr>
<tr>
<td>75</td>
<td>75.3</td>
<td>75.1</td>
</tr>
</tbody>
</table>

The MESA study provided us with the unique opportunity to pursue these questions. Due to a balanced recruiting of individuals from major ethnic groups, our results are more generalizable to diverse populations. In addition, the MESA study afforded us the opportunity to investigate aortic stiffness using area measurements derived from MRI scans, a technique validated by prior research studies. Likewise, with 3,500 MRI scans describing aortic distensibility, the MESA study provided us with enough statistical power to develop and test more directed hypotheses.

There are several implications of our study. First, increasing age is a powerful and often under-appreciated risk factor in the development of cardiovascular disease. To demonstrate its significance, increasing age reduced the difference between normal fasting glucose and diabetes mellitus, a powerful risk factor for morbidity and mortality from cardiac pathology.
Second, a growing body of literature supports the place of aortic stiffness in the pathway between traditional cardiac risk factors and heart failure. A stiffened aorta is associated with diastolic dysfunction, which is one of the main features of diabetic cardiomyopathy. These changes in diastolic function start to occur in individuals with impaired fasting glucose. As age increases, the incidence ratios of congestive heart failure between diabetics and non-diabetics decrease from 11 before age 45 to 1.2 in the ninth decade of life. This parallels the relationship between age and fasting glucose status on aortic distensibility seen in our study.

Third, with 25% of the adult U.S. population having impaired fasting glucose, even a minor increase in risk can translate into a significant public health problem. Hence, the decrease in aortic distensibility at younger ages by even mild hyperglycemia may represent a major health care risk. Further studies are needed to determine if more aggressive lifestyle and/or pharmacological intervention in younger patients with IFG or DM prevents the premature morbidity and mortality that may result from aortic stiffness.

There are several limitations of this study. First, non-invasive blood pressure measurements were used to calculate aortic distensibility. While not ideal, previous studies have indicated that noninvasive measures are adequate approximations and that they do predict cardiac mortality. In addition, our results are consistent with prior invasive studies that demonstrated that central pulse pressure is different between those with normal fasting glucose and those with impaired fasting glucose. Second, a selection bias may be present in our study. The MESA study specifically recruited individuals without cardiovascular disease, and as such, our participants may have more
resistance or fewer risk factors than the general population to cardiovascular disease. Likewise, this limits the generalizability of this study to individuals without cardiac disease. Third, our analysis is limited by the cross-sectional nature of our data. As such, temporality cannot be assessed. Another weakness of cross-sectional data is trying to determine if covariates are confounders or mediators. Fourth, one measurement of fasting glucose was used to identify participants’ glycemic status, which may have resulted in misclassification.

**Conclusion**

There are differences in aortic distensibility between fasting glucose groups. However, increasing age decreases the differences in aortic distensibility between the fasting glucose groups. With 25% of the adult population having impaired fasting glucose, further studies are needed to see if correction of mild hyperglycemia at younger ages can prevent premature morbidity and mortality from aortic stiffness.
References


(13) Hundley WG, Kitzman DW, Morgan TM, Hamilton CA, Darty SN, Stewart KP, Herrington DM, Link KM, Little WC. Cardiac cycle-dependent changes in aortic area
and distensibility are reduced in older patients with isolated diastolic heart failure and correlate with exercise intolerance. *J Am Coll Cardiol* 2001 September;38(3):796-802.


Ref Type: Generic

Ref Type: Generic


Ref Type: Generic


(33) Mahmud FH, Earing MG, Lee RA, Lteif AN, Driscoll DJ, Lerman A. Altered endothelial function in asymptomatic male adolescents with type 1 diabetes. *Congenit Heart Dis* 2006 May;1(3):98-103.


Chapter III

Interaction Effects in Linear Regression Models

Introduction

The relationship described in the previous chapter is that of an interaction. Many epidemiological studies have used interactions to describe complex relationships. They often provide information that can highlight important differences. However, by not accounting for them, they can obscure important information.

Definition

Many individuals have confusion about what an interaction is, and often, the phenomenon of confounding is confused with an interaction. An interaction is when the relationship of interest is different at varying levels of the predictor variables.\(^1\) An interaction can be tested statistically to determine its significance. On the other hand, confounding exists when the relationship of interests varies when a certain independent variable is included or excluded.\(^1\) Confounding cannot be tested statistically.

Linear Regression Analysis

Linear regression is one of the most used statistical techniques to describe relationships between dependent and independent variables. Regression analysis seeks to find the linear estimate that produces the smallest sum of squares.\(^1\) Sum of squares helps to reflect the difference between the observed and predicted values. By squaring this difference, sum of squares finds the best prediction to find the least squares estimate. Linear regression uses the following formula to describe relationships:

\[
Y = \beta_0 + \beta_1 X + \beta_2 Z + \varepsilon
\]
As stated previously, interactions can be represented statistically. Interactions are inserted into the linear regression analysis by using the cross-product of 2 or more independent variables which have already been included in the equation:

\[ Y = \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 XZ + \varepsilon \]

The coefficient for the interaction term represents the difference in slopes between regression lines of its components. This combination results in 3 possibilities: 2 continuous variables as a cross-product, 2 categorical variables as a cross-product, or a hybrid categorical and continuous variable as a cross-product. Because of the difficulty in grasping some interactions, it is best to represent it graphical to facilitate understanding of the relationship. The statistical significance can be determined with F tests.

**Figure 5.** Example of an interaction: Gender modified the effect of the medication on Systolic blood pressure.
Classification of Interaction

There are several ways to classify interactions. In linear regression, interactions can be classified as ordinal (noncrossover) or disordinal (crossover). An ordinal interaction is one in which the regression lines do not cross within the possible range of values. A disordinal interaction occurs when the regression lines cross within the possible range of values. As such, the interaction between age and fasting glucose status on aortic distensibility represents a disordinal (crossover) interaction since the different regression lines cross at age 75 which is within the possible range from 45-84 years of age.

When analyzing interactions through table format, one can ascertain whether a multiplicative or additive interaction is present. A sub-additive or sub-multiplicative interaction is when the difference between columns for each row is different from the other row (see Table 1 below). In table format, if an interaction is not present, one would expect there to be an additive or multiplicative factor that would be similar in the difference between columns for each row. Additive interactions are typically approached with linear regression, and multiplicative interactions can best be described by logistic regression.

<table>
<thead>
<tr>
<th></th>
<th>Initial (mmHg)</th>
<th>Follow-up (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>136</td>
<td>137</td>
</tr>
<tr>
<td>Women</td>
<td>137</td>
<td>115</td>
</tr>
</tbody>
</table>

Table 5. Modification of the Effect Of Medication on SBP by Gender.

Centering of Variables

One of the difficulties in interpreting regression analyses with interaction terms is interpretation of the individual regression coefficients. To foster understanding, centering of variables can be used. To center a variable, one needs to subtract the mean value from the variable in order that the mean value occurs at 0. Centering of variables does not affect analyses.
related to slopes between different lines. However, it does alter analyses based on the intercept. In an un-centered regression equation, the regression coefficient $B_1$ continues to mean the relationship of $Y$ on $X$ at $Z=0$. However, this may not have meaning if $Z=0$ is not included in the range of values for $Z$. By centering the variables, the intercept becomes meaningful since $B_1$ represents the relationship of $Y$ on $X$ at $Z = \text{Mean}$. In addition, one can center variables at a particular value to determine the difference between lines at that specified value.

The $B_1$ and $B_2$, first order terms, are often referred to as “main effects.” In stating this, it is implied that there is a constant effect, which is not the case when a meaningful interaction is present.

**Interaction of 2 Continuous Variables**

One form of a 2 variable interaction is between 2 continuous variables, also known as a bilinear interaction. Based on the MESA cohort, a sample is constructed to search for an interaction between age and fasting glucose level on aortic distensibility. The regression equation is as follows:

\[ \text{Aortic Distensibility} = \beta_0 + \beta_1 \cdot \text{Age} + \beta_2 \cdot \text{Fasting glucose level} + \beta_3 \cdot \text{Product Term} + \varepsilon \]

In this example, the interaction term is not significant ($p = 0.3$). The most likely explanation is that those with diabetes mellitus have decent control of their blood sugar, which means fasting glucose level would not reflect previous damage to the aorta due to elevated blood sugar. With the cross-product term in the un-centered equation, fasting glucose level is not statistically significant ($p = 0.1$). However, by centering age and fasting glucose level, fasting glucose level is statistically significant ($p < 0.01$).
Interaction of 1 Categorical Variable with 1 Continuous Variable

A second form of a 2 variable interaction is between 1 categorical variable and 1 continuous variable. This type of interaction was analyzed in the previous chapter. The regression coefficients of the categorical variable are usually defined using dummy variables. The regression equation is:

\[ Y = \beta_0 + \beta_1 \text{Age} + \beta_2 \text{Fasting Glucose Group} + \beta_3 \text{Product term} + \varepsilon \]

By centering the variable of age within our available range, one can compare the fasting glucose groups at specific points. The following table is a comparison between values obtained for certain ages with linear regression formula on the left and intercept centered analysis at specified ages on the right:

<table>
<thead>
<tr>
<th>Age</th>
<th>NFG</th>
<th>IFG</th>
<th>DM</th>
<th>NFG</th>
<th>IFG</th>
<th>DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>2.95</td>
<td>2.73</td>
<td>2.38</td>
<td>2.95</td>
<td>2.73</td>
<td>2.38</td>
</tr>
<tr>
<td>50</td>
<td>2.66</td>
<td>2.49</td>
<td>2.16</td>
<td>2.66</td>
<td>2.49</td>
<td>2.16</td>
</tr>
<tr>
<td>55</td>
<td>2.37</td>
<td>2.24</td>
<td>1.95</td>
<td>2.37</td>
<td>2.25</td>
<td>1.95</td>
</tr>
<tr>
<td>60</td>
<td>2.08</td>
<td>1.99</td>
<td>1.73</td>
<td>2.08</td>
<td>2</td>
<td>1.73</td>
</tr>
<tr>
<td>65</td>
<td>1.79</td>
<td>1.75</td>
<td>1.52</td>
<td>1.79</td>
<td>1.76</td>
<td>1.52</td>
</tr>
<tr>
<td>70</td>
<td>1.5</td>
<td>1.5</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>75</td>
<td>1.21</td>
<td>1.26</td>
<td>1.09</td>
<td>1.21</td>
<td>1.27</td>
<td>1.09</td>
</tr>
<tr>
<td>80</td>
<td>0.92</td>
<td>1.01</td>
<td>0.87</td>
<td>0.92</td>
<td>1.02</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Table 6. Comparison of AD values obtained from regression formula on left vs. AD values obtained from Age-centered analysis on the right.

Trace differences due to rounding. In the next table, the p-values from the intercept-centered model are presented for NFG vs. IFG and IFG vs. DM:
### Table 7. p-values of comparisons between FGG at certain ages as obtained from intercept-centered analysis.

If statistical significance is taken to be a p-value <0.05, the results in the above table correspond with the 95% confidence intervals obtained from linear regression:

<table>
<thead>
<tr>
<th>Age</th>
<th>IFG vs NFG</th>
<th>IFG vs DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>0.027</td>
<td>0.027</td>
</tr>
<tr>
<td>50</td>
<td>0.026</td>
<td>0.01</td>
</tr>
<tr>
<td>55</td>
<td>0.035</td>
<td>0.003</td>
</tr>
<tr>
<td>60</td>
<td>0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>65</td>
<td>0.5</td>
<td>0.002</td>
</tr>
<tr>
<td>70</td>
<td>0.9</td>
<td>0.028</td>
</tr>
<tr>
<td>75</td>
<td>0.5</td>
<td>0.15</td>
</tr>
<tr>
<td>80</td>
<td>0.4</td>
<td>0.35</td>
</tr>
</tbody>
</table>
2 Categorical Variables with Analysis of Covariance (ANCOVA)

One method to evaluate an interaction between 2 categorical variables is with analysis of covariance. As with the categorical/continuous interaction previously mentioned, dummy variables are also usually employed to generate regression coefficients. For this example, age will be stratified into 2 groups: individuals < 65 vs. individuals > 65. The regression formula for this interaction is the following:
\[ Y = \beta_0 + \beta_1 \text{Age Group} + \beta_2 \text{Fasting Glucose Group} + \beta_3 \text{Product term} + \varepsilon \]

Using ANCOVA, the overall interaction term is significant (\( p = 0.048 \)). A table with the p-values adjusted for multiple comparisons using Tukey (\( p = 0.05 \) is significant) follows:

<table>
<thead>
<tr>
<th>Tukey-adjusted p-values</th>
<th>&lt;65</th>
<th>&gt;65</th>
<th>Aortic Distensibility (mmHg(^{-1}))</th>
<th>&lt;65 yrs</th>
<th>&gt;65 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFG vs IFG</td>
<td>0.035</td>
<td>1</td>
<td>NFG</td>
<td>2.42</td>
<td>1.47</td>
</tr>
<tr>
<td>IFG vs DM</td>
<td>0.041</td>
<td>0.27</td>
<td>IFG</td>
<td>2.22</td>
<td>1.48</td>
</tr>
<tr>
<td>NFG vs DM</td>
<td>&lt;0.001</td>
<td>0.21</td>
<td>DM</td>
<td>1.91</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Table 8. ANCOVA Analysis: p-values for comparison between FGG divided by age are presented on left. Adjusted means for aortic distensibility for each FGG divided by age are presented on right.

These results were presented in chapter 2.

Summary

There are multiple ways to explore and define statistical interactions. While it is possible to describe interactions with continuous variables, it may not always be feasible. There are methods to compare lines at different points, but it is often difficult to interpret the results. As such, these methods, though available, are often not used. Most studies which have analyzed interactions use a stratified approach or ANCOVA.
Reference List


**Curriculum Vitae**

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“Modification of the Effect of Glycemic Status on Aortic Distensibility by Age in the Multi-Ethnic Study of Atherosclerosis”
Cardiology Research Conference
June 2009

“Aortic Stiffness, Aging, and Diabetes”
Cardiology Research Conference
January 2008

“Cardiovascular Disease and Women”
Cardiovascular pathophysiology lecture for 2
\textsuperscript{nd} year medical students
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