Fibroblast Growth Factor-23 and Cardiac Magnetic Resonance Indices of Myocardial Fibrosis in the Multi-Ethnic Study of Atherosclerosis

By

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# TABLE OF CONTENTS

LIST OF ILLUSTRATIONS AND TABLES.....................................................V

LIST OF ABBREVIATIONS..............................................................................Vii

ABSTRACT........................................................................................................Viii

CHAPTER ONE: BACKGROUND.................................................................1

A. Introduction..............................................................................................1
B. Definition of CKD......................................................................................2
C. Cardiovascular disease in CKD population..............................................3
D. The pathophysiology of CVD in CKD....................................................4
E. Myocardial fibrosis in CKD.................................................................5
F. Fibroblast growth factor-23 in individuals with CKD............................9
G. The relationship between FGF-23 and all-cause and cardiovascular mortality….10
H. The role of FGF-23 in the pathogenesis of CVD in individuals with CKD……10
I. Cardiac Magnetic Resonance indices of myocardial fibrosis...............10
J. FGF-23 and CMR....................................................................................15
K. Conceptual Model...................................................................................15
L. Knowledge gap......................................................................................17
M. Aims and Hypotheses...........................................................................18
N. Methods.................................................................................................19
O. References.............................................................................................20
CHAPTER TWO: MANUSCRIPT

A. KEYWORDS ........................................................................................................25

B. ABSTRACT ........................................................................................................26

   Background........................................................................................................26

   Methods...........................................................................................................26

   Results.............................................................................................................26

   Conclusion.......................................................................................................27

C. INTRODUCTION ..............................................................................................28

D. METHODS .......................................................................................................29

   Study population............................................................................................29

   Study procedure: CMR imaging ......................................................................30

   Measurement of serum FGF-23 concentrations .............................................32

   Statistical Analysis..........................................................................................32

E. RESULTS ..........................................................................................................34

F. DISCUSSION ....................................................................................................35

G. LIMITATIONS ..................................................................................................38

H. CONCLUSION ...................................................................................................39

I. TABLES AND FIGURES ....................................................................................40

J. SUPPLEMENTAL MATERIAL ...........................................................................44

K. REFERENCES ....................................................................................................45
CAPTER 3: ANCILLARY ANALYSES

A. ABSTRACT
B. INTRODUCTION
C. METHODS
   Study population
   Study procedures: CMR imaging
   Measurement of serum FGF-23 concentrations
   Ascertainment of heart failure events
   Statistical Analysis
D. RESULTS
E. DISCUSSION
F. LIMITATIONS
G. CONCLUSION
H. TABLES AND FIGURES
I. SUPPLEMENTAL MATERIAL
J. REFERENCES
CURRICULUM VITAE
LIST OF ILLUSTRATIONS AND TABLES

CHAPTER ONE

Figure 1. Histological changes of the myocardial cells in CKD

Figure 2. Diffuse interstitial myocardial fibrosis in CKD (histologic evidence)

Figure 3. Patterns of myocardial fibrosis in CKD on cardiac magnetic resonance imaging

Figure 4. Regulation of FGF-23

Figure 5. Expected concentration range of FGF-23 as a function of eGFR

Figure 6. Conceptual model of the role of FGF-23 in the development of heart failure

CHAPTER TWO

Table I. Baseline characteristics by Fibroblast Growth Factor-23 quartiles

Table II. CMR indices of myocardial fibrosis by gender

Table III. Associations of FGF-23 with native myocardial T1 mapping, 12-min post-contrast T1 mapping, and extracellular volume

Figure 1. Participant enrolment in the current study

Figure 2. Associations between serum FGF-23 quartiles and native myocardial T1 mapping, 12-min post-contrast myocardial T1 mapping and ECV
SUPPLEMENTAL MATERIAL

Supplemental Table 1: Baseline participant’s characteristics by Fibroblast Growth Factor-23 Quartile for the participants with measured ECV

CHAPTER THREE

Table I. Baseline characteristics by Fibroblast Growth Factor-23 quartiles

Table IIA. Association of FGF-23 and incident heart failure, HFrEF and HFpEF.

Table IIB. Association of FGF-23 and incident heart failure, HFrEF and HFpEF (Hazard ratios per FGF-23 quartiles)

Figure 1. Hazards ratios of the associations of FGF-23 quartiles with incident heart failure, HFrEF and HFpEF.

Figure 2. Higher FGF-23 levels are associated with increased risk of incident heart failure. Participants were stratified into quartiles of FGF-23. (Kaplan-Meier curve with 95% Hall-Wellner Bands and log-rank test). Notice the Y axis start from 90% survival, X axis is the time to heart failure event (days).

Figure 3A. Higher FGF-23 levels are associated with increased risk of incident HFrEF. Participants were stratified into quartiles of FGF-23. (Kaplan-Meier curve with 95% Hall-Wellner Bands and log-rank test).

Figure 3B. Higher FGF-23 levels are associated with increased risk of incident HFpEF. Participants were stratified into quartiles of FGF-23. (Kaplan-Meier curve with 95% Hall-Wellner Bands and log-rank test).

Supplemental Table 1. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF stratified by gender (Hazard ratios per doubling of FGF-23).

Supplemental Table 2A. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF overall and stratified by gender without adjustment for LV mass.

Supplemental Table 2B. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF overall and stratified by gender with adjustment for LVH-ECG.
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
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<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
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<tr>
<td>CKD-MBD</td>
<td>Chronic kidney disease-Metabolic Bone disease</td>
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<tr>
<td>CMR</td>
<td>Cardiovascular magnetic resonance</td>
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<td>CV</td>
<td>Cardiovascular</td>
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<tr>
<td>DE</td>
<td>Delayed enhancement</td>
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<tr>
<td>ECV</td>
<td>Extracellular volume</td>
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<tr>
<td>eGFR</td>
<td>estimated Glomerular filtration rate</td>
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<tr>
<td>FGF-23</td>
<td>Fibroblast Growth Factor-23</td>
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<td>HFpEF</td>
<td>Heart failure with preserved ejection fraction</td>
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<td>HFrEF</td>
<td>Heart failure with reduced ejection fraction</td>
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<tr>
<td>LGE</td>
<td>Late gadolinium enhancement</td>
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<td>LV</td>
<td>left ventricle</td>
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<tr>
<td>MESA</td>
<td>Multi-Ethnic Study of Atherosclerosis</td>
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<tr>
<td>MOLLI</td>
<td>Modified Look-Locker inversion recovery</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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ABSTRACT

In this thesis, we will evaluate the association of Fibroblast Growth Factor-23 (FGF-23) with myocardial fibrosis using cardiovascular magnetic resonance (CMR) imaging techniques. Higher levels of Fibroblast Growth Factor-23 (FGF-23) have been linked to increased risk of all-cause mortality, cardiovascular mortality, left ventricular hypertrophy and heart failure. Histologic studies on rats suggest an association between higher levels of FGF-23 with myocardial fibrosis. However, it is unknown whether such relationship exists in humans.

We hypothesized that higher levels of FGF-23 would be associated with CMR indices of myocardial fibrosis. We performed analyses of the association of FGF-23 with pre-and post-contrast T1 mapping and extracellular volume (ECV) in participants from the Multi-Ethnic Study of Atherosclerosis (MESA). The first chapter of this thesis will be a literature review and will discuss the pathogenesis of myocardial fibrosis in individuals with high levels of FGF-23 such as patients with chronic kidney disease (CKD). The second chapter will be a manuscript of the associations of FGF-23 with CMR indices of myocardial fibrosis. The third chapter is comprised of ancillary studies and discussion of the association of serum FGF23 with incident heart failure with preserved ejection fraction (HFpEF) and with reduced ejection fraction (HFrEF).
CHAPTER ONE: BACKGROUND

Introduction

Heart failure is common among patients with chronic kidney disease (CKD), and is associated with increased mortality in this population.¹ CKD patients have several derangements of the metabolism of calcium and phosphate that are called metabolic bone disease (CKD-MBD).² These derangements lead ultimately to elevated blood levels of fibroblast growth factor (FGF-23). Several studies showed the association between elevation of FGF-23 and the development of heart failure.³,⁴ For example, Kestenbaum et al. demonstrated an increased risk of incident heart failure with higher concentrations of serum FGF-23 in a multi-ethnic middle aged population free of heart disease at baseline.⁵ The same relationship was shown to exist by Scilla et al. in a population with chronic kidney disease stage 2-4.⁴ We aim to study the mechanism in which FGF-23 affects the myocardium, specifically to confirm the findings of animal studies which link higher levels of FGF-23 with increased myocardial fibrosis.⁵

Myocardial fibrosis is a common endpoint in a variety of cardiac diseases.⁶ Diffuse myocardial fibrosis occurs as a part of normal aging;⁷ however, this process is accelerated in many diseases.⁸-¹⁰ This diffuse fibrosis is associated with worsening ventricular function and increased ventricular stiffness and has independent predictive value for major cardiac events and increased all-cause mortality.¹¹-¹⁵

FGF-23 is a hormone secreted by osteoblasts/osteoclasts. It functions as a phosphaturic factor in the renal tubules and a regulator of parathyroid hormone, thus regulating calcium and phosphate homeostasis. Higher serum levels of FGF-23 were
shown to be associated with a variety of cardiovascular diseases. For example, Kestenbaum et al. showed that higher serum FGF-23 concentrations are associated with incident heart failure, left ventricular hypertrophy and coronary disease events. Recently, FGF-23 has been shown to be associated with myocardial fibrosis and left ventricular hypertrophy in animal studies.\textsuperscript{16,17}

The best available non-invasive method of examining myocardial fibrosis is contrast-enhanced cardiac magnetic resonance (CMR) through T1 mapping. This technique facilitates the quantification of diffuse myocardial fibrosis measured as the extracellular volume fraction (ECV).

We propose to examine the association of serum FGF-23 with myocardial fibrosis in the Multi-Ethnic Study of Atherosclerosis (MESA) using baseline serum FGF-23 and CMR indices of myocardial fibrosis including ECV measurements and CMR-T1 mapping.

**Definition of CKD**

CKD is characterized by alterations in the kidney function for a minimum of 3 months. CKD is usually asymptomatic in the early stages.\textsuperscript{18} Symptoms generally are related to complications of CKD that usually occur in the late stages. These complications include cardiovascular disease (CVD),\textsuperscript{19} anemia,\textsuperscript{20} infectious complications, neuropathy and abnormalities related to mineral bone metabolism.\textsuperscript{2}

CKD was classified into five stages according to the National Kidney Foundation criteria\textsuperscript{18}. This classification is based on both kidney function and structure. The glomerular filtration rate (GFR) is used most commonly to assess function. For an individual to be diagnosed with CKD, he/she must have at least two separate
measurements of GFR >90 days apart that are in the abnormal range (GFR<60 mL/min/1.73m$^2$). Since many cases of kidney damage present with preserved GFR, other markers of kidney damage can be used such as albuminuria, proteinuria, hematuria or structural abnormalities. Thus, many patients with normal GFR with evidence of kidney damage are considered to have CKD$^{21}$.

CKD has major prognostic implications$^{22}$, which include increased all-cause mortality in patients with CKD compared with those without CKD. Most of the increased mortality is attributed to increased cardiovascular events. The increased risk of cardiovascular events is partly due to increased prevalence of traditional cardiovascular risk factors such as hypertension, diabetes mellitus and also due to direct increased cardiovascular risk from non-traditional risk factors such as anemia, malnutrition, inflammation, retention of toxins and CKD-MBD.$^{23,24}$ The advanced stages of CKD which is classified as end stage renal disease (ESRD) is associated with even higher risk of all-cause mortality compared with early stages of CKD.$^{19}$

**Cardiovascular disease in CKD population**

CKD is increasingly recognized as an important risk factor for cardiovascular (CV) mortality.$^{25,26}$ Recent registry data from the US Renal Data System show that >40% of ESRD deaths were due to CVD.$^{1}$

The evidence of the relationship between renal dysfunction and adverse CV events was first recognized in the dialysis population in whom incidence of CV mortality is strikingly high. Approximately 50% of individuals with ESRD die from a CV cause.$^{27,28}$ In fact, the prevalence of CV disease is already high by the time patients reach
ESRD. For example, 40% of patients who have started dialysis have evidence of coronary artery disease, and 85% of these patients have abnormal left ventricular structure and function.\textsuperscript{29} Furthermore, left ventricular hypertrophy (LVH) was found to be common in patients with CKD even before they progress to dialysis, and that the prevalence of LVH correlates with the degree of renal functional impairment.\textsuperscript{30,31}

This early link between CKD and CV events was further studied and was found to follow graded correlation with decreasing level of glomerular filtration rate (GFR). In a study by Go et al. showed a stepwise increase in the rate of mortality, CV events and increased hospitalization. With the reference cohort with (GFR>60 mL/min), the adjusted hazard ratio for death from any cause and any CV events increased to 1.2 and 1.4, respectively, for GFR between 45 to 59 mL/min; 1.8 and 2.0 for GFR between 30 to 44 mL/min; 3.2 and 2.8 for GFR 15 to 29 mL/min; and 5.9 and 3.4 for GFR <15 mL/min.\textsuperscript{19}

**The pathophysiology of CVD in CKD**

Even though patients with CKD have accelerated atherosclerosis and vascular calcification, more patients die from arrhythmias (both atrial and ventricular), sudden death, and congestive heart failure, than myocardial infarction\textsuperscript{1}. Indeed, 50% of ESRD patients suffer from heart failure (HF), thus forming the predominant cardiac abnormality observed in CKD. It is a common perception that HF in CKD is secondary to vascular comorbid conditions such as ischemic heart disease (IHD), hypertension and diabetes mellitus, but it is becoming apparent that primary CKD is an independent risk factor for incident HF even in non-diabetic and normotensive patients\textsuperscript{32}. 
LVH in CKD is a pathologic process and unlike physiologic adaptations to increased workload (e.g. “athletes heart”), is accompanied by fibrosis, which is attributed to conditions related to the uremic milieu, including increased levels of parathyroid hormone, endothelin, aldosterone, catecholamines, cardiotonic steroids and fibroblast growth factors. In addition to fibrosis and cardiomyocyte hypertrophy, histological changes of the heart in CKD also include myocyte apoptosis/necrosis resulting in myocyte number reduction, and microvascular abnormalities such as arteriolar wall thickening and decrease in the number of capillaries\textsuperscript{33-36}.

**Myocardial fibrosis in CKD:**

1- **Histologic evidence**

Diffuse myocardial interstitial widening and fibrosis is common in patients with CKD.\textsuperscript{37} Several histological studies have shown increased myocardial fibrosis that is not explained by other risk factors such as hypertension and/or diabetes. Experimental studies have shown that interstitial fibrosis occur early after subtotal nephrectomy in the absence of myocyte necrosis.\textsuperscript{38} Interstitial deposition of collagen type I was associated with swelling of interstitial cells – both cytoplasm and nuclei; in contrast, nuclear and cytoplasmic volumes of endothelial cells were unchanged (Figure 1).
Figure 1: Histological changes of the myocardial cells in CKD

A. Myocardial interstitial cells of a control animal (electron micrograph, magnification 12,100:1). Cytoplasm without evidence of cell activation. B. Activated interstitial cell of uremic rat three weeks after 5/6 nephrectomy (magnification 12,300:1). Note the increased cytoplasm. Note the abundant endoplasmic reticulum and Golgi complex and the numerous cytoskeletal filaments within the cytoplasm.


Furthermore, Aoki et al. demonstrated the pathologic characteristics of the heart of dialysis patients with dilated cardiomyopathy which includes diffuse interstitial fibrosis and severe myocyte hypertrophy with occasional disarray.\textsuperscript{36} In addition, the extent of the left ventricular fibrosis was shown to be a strong predictor of cardiovascular mortality (figure 2). The 3-year event-free cumulative survival rate for cardiac death was 42\% in dialysis patients with 30\% or more fibrosis, whereas it was 82\% in dialysis patients that had less than 30\% fibrosis (p=0.03).\textsuperscript{36}
2- Imaging evidence:

By far the easiest way to study uremic changes of the myocardium in CKD patients is echocardiography, which in studies, showed a graded relationship between the severity of CKD and the prevalence and severity of LVH. Further and more precise identification of CKD-related CVD can be demonstrated by CMR.

Mark et al. first used magnetic resonance imaging to described the patterns of myocardial fibrosis in end stage renal disease patients. Using late gadolinium enhancement (LGE) as a way to detect fibrosis, they demonstrated two patterns. The first pattern – subendocardial LGE, consistent with that described in myocardial infarction – followed a primarily subendocardial distribution. The second pattern was characterized as diffuse, less intense LGE reflecting diffuse myocardial fibrosis (figure 3). The latter
pattern was associated with greater LV mass compared with ESRD patients without LGE (p<0.01).

**Figure 3.** Patterns of myocardial fibrosis in CKD on cardiac magnetic resonance imaging

(a) Short axis view of the left ventricle of hemodialysis patient demonstrating a diffuse area of gadolinium enhancement in the inferior wall of the left ventricle (arrowed). Signal intensity of this area is 17.6 compared to the 6.9 for the LGE-negative area. (b) Short axis view of the left ventricle of another hemodialysis patient demonstrating a diffuse area of gadolinium enhancement in the lateral wall of the left ventricle. Signal intensity of the area of late gadolinium enhancement is 32.0 compared to 8.4 for the LGE-negative area. This patient had normal coronary arteries at angiography performed as transplant assessment.

*Adapted from Mark et al.; Kidney International (2006) 69, 1839-1845*

Even though CMR is the best modality to detect myocardial fibrosis, its use is currently restricted because of the risk of a fatal condition known as nephrogenic systemic fibrosis with gadolinium use in patients with CKD. However, in a recent cross-sectional study, 43 patients with non-diabetic CKD stage 2-4 (mean GFR 50±22 ml/min/1.73m²) and 43 age- and gender-matched controls with no history of cardiovascular disease underwent CMR imaging with T1 mapping. The patients with CKD had higher mean native T1 times (986±37 ms) and mean extracellular volume (28±4%) compared to controls (955±30 ms and 25±3%, each p<0.05). This study
demonstrated that the increase in diffuse myocardial fibrosis occurred even in early stages of CKD.  

**Fibroblast growth factor in individuals with CKD**

FGF-23 is a hormone secreted by osteoblasts and osteocytes and released into the circulation. The effects of FGF-23 are mediated by FGF receptors (FGFRs), which are located in the renal tubules (where FGF-23 induces urinary phosphate excretion), parathyroid gland (where FGF-23 regulates the production of parathormone [PTH]), and the blood vessels (Figure 4). Many studies show that the coreceptor Klotho is mandatory to induce FGF-23 specific signaling pathways.  

Furthermore, FGF-23 decreases dietary phosphate absorption through suppression of circulating levels of 1, 25 dihydroxyvitamin D. In turn, vitamin D controls production of FGF-23. It was shown that vitamin D3 stimulates FGF-23 generation. For example, vitamin D receptor null mice showed undetectable FGF-23 levels.

In addition, PTH is regulated by FGF-23, which suppresses both secretion and gene expression of PTH. However, patients with CKD have elevation of both PTH and FGF-23, which can be explained by FGF-23 resistant status associated with uremia. It was recently found that CKD is associated with Klotho deficiency which may explain FGF-23 resistance. For example, in experimental models of CKD, there is a downregulation of the FGF-23 receptor complex klotho-FGFR1 in the parathyroid, resulting in resistance of the parathyroid to FGF-23. This resistance contributes to the high levels of both PTH and FGF-23 in CKD. It was also shown that secondary hyperparathyroidism is essential for the high levels of FGF-23 in CKD, as
parathyroidectomy both prevents and corrects the high FGF-23 levels of experimental renal failure.\(^5^1\)

Serum levels of FGF-23 increase with the decline of kidney function. These levels can reach more than 200 times the normal levels in cases of ESRD (Figure 5).\(^5^2\) This rise in FGF-23 levels occurs before changes in levels of serum phosphate, PTH, or 1, 25(OH)\(_2\) vitamin D.

In summary, it has been observed that FGF-23 levels increase with the decline of kidney functioning through complex mechanisms that include involvement of bones, kidneys and the parathyroid gland.

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**Figure 4:** Regulation of FGF-23
Adapted from: Larsson et al. G Ital Nefrol 2014; 31(2)-ISSN 1724-5590

**Figure 5:** Expected concentration range of FGF-23 as a function of eGFR.
Adapted from: Larsson et al. G Ital Nefrol 2014; 31(2)-ISSN 1724-5590
The relationship between FGF-23 and all-cause and cardiovascular mortality

Increased FGF-23 levels in dialysis patients were associated with significantly increased risk of mortality during the first year on dialysis; individuals with C-terminal FGF-23 values above the median (1752 reference units (RU)/mL) were associated with an odds ratio of 4.5-5.7 for mortality, compared to those with C-terminal FGF-23 <1089 RU/ml. These results were confirmed even in predialysis CKD patients. Furthermore, other studies also showed an association between higher levels of FGF-23 and increased cardiovascular mortality and events.

The role of FGF-23 in the pathogenesis of CVD in individuals with CKD

Recent studies have implied a role for FGF-23 in the pathophysiology of CKD-mineral bone disease and in vascular calcification. It has been shown that FGF-23 levels are elevated in CKD patients both on dialysis and on conservative treatment, which showed association with increased mortality and left ventricular hypertrophy. Moreover, FGF-23 has been linked to increased arterial stiffness, endothelial dysfunction, vascular calcification, and major cardiovascular events. Furthermore, a recent study in animal models of renal failure showed reversal of uremic cardiomyopathy characterized by left ventricular hypertrophy and fibrosis- with fibroblast growth factor blockade.

Cardiac Magnetic Resonance indices of myocardial fibrosis

The myocardium is made of myocytes embedded in the myocardial interstitium, which represents the scaffolding tissue. An expansion of this interstitium due to increased collagen (focal and diffuse fibrosis) is the end result of many pathological processes such as acute myocardial infarction, chronic myocardial infarction, and hypertrophic
cardiomyopathy. Expansion of the interstitium is also evident in many infiltrative processes such as amyloidosis. The gold standard test to evaluate myocardial fibrosis is histological assessment with invasive myocardial biopsy. However, the clinical utility of this test is limited due to significant morbidity and sampling error.

Advancements in cardiac magnetic resonance (CMR) imaging have allowed for the characterization of specific patterns of myocardial fibrosis. For example, late gadolinium enhancement imaging (LGE) made it possible to identify focal areas of myocardial infarction and enabled a detailed phenotypic characterization of many nonischemic cardiomyopathies.

However, the LGE technique is limited in detecting diffuse myocardial fibrosis because it relies on qualitative detection of the signal intensity difference between fibrotic and normal myocardial tissue. This limits the ability to detect homogeneously distributed diffuse fibrosis, which is the main type in many forms of cardiomyopathy.

The recent development of T1 mapping has allowed the quantitative assessment of diffuse myocardial fibrosis. This technique measures the intrinsic magnetic resonance parameter T1 of the myocardium and maps its spatial distribution (T1 mapping). The measured differences in R1 (=1/T1) values pre- and post-gadolinium contrast allow the quantification of the myocardial extracellular volume fraction (ECV), which has been shown to be increased in the presence of diffuse myocardial fibrosis.

T1 relaxation time of a tissue indicates how rapidly protons recover after a radiofrequency pulse. Pre-contrast (native) T1 is affected by the water content and may represent edema or diffuse myocardial fibrosis. Bull et al. studied whether non-contrast
CMR-T1 mapping sequence could identify myocardial fibrosis in patients with moderate and severe aortic stenosis. They obtained biopsy samples for histologic assessment of collagen volume fraction (CVF%) in 19 patients undergoing aortic valve replacement. They showed a significant correlation between native T1 values and CVF% (r=0.65, p=0.002). Higher T1 values were found in participants with severe aortic stenosis compared to controls (972±33 ms vs. 944±16, p<0.05). However, this technique embodies composite signal from both myocytes and interstitium, and it varies with the magnetic resonance imaging field strength. Measurement of post-contrast T1 mapping provides a value linked to the interstitium, but it is also influenced by gadolinium dose, clearance rate, time post bolus, body composition, and hematocrit.

A newer, more accurate technique has been used recently to assess myocardial fibrosis called ECV mapping. In this technique, ECV is measured after accounting for the hematocrit level (ECV=100 X partition coefficient X [1-hematocrit]). The partition coefficient is determined by the slope of the linear relationship of ($1/T_{1\text{myo}}$ vs. $1/T_{1\text{blood}}$). A novel CMR technique called Modified Look-Locker Inversion (MOLLI) recovery was developed to allow the calculation of T1 mapping and ECV using a single breath-hold. This technique acquires a set of 11 source images in 17 heartbeats. It consists of 3 consecutively inversion recovery-prepared electrocardiography-synchronized Look-Locker trains.

As mentioned above, ECV is increased in conditions characterized with diffuse myocardial fibrosis. For example Broberg et al. found higher ECV in those with congenital heart disease compared to normal controls (31.9±5.8% versus 24.8±2%; p<0.001). However, LGE did not account for the differences seen. Furthermore,
Ugander et al. showed the ability of ECV measurement of quantitatively characterizing myocardial infarction (ECV infarct=51±8% vs ECV normal= 27±3%; p<0.001), atypical diffuse fibrosis (ECV=37±6), and subtle myocardial abnormalities not clinically apparent on LGE images. Recently in the Multi-Ethnic Study of Atherosclerosis, Liu et al. showed increased ECV with older age in men. In addition, they showed a higher ECV in females compared to males (28.1±2.8 vs. 25.8±2.9, p<0.001). Neilan et al. studied the distribution of ECV values in a healthy population and provided histological validation of ECV measurements in mice. The normal range they found from studying 32 well-defined healthy volunteers was (23 - 33%, mean 28±3%). In mice, they showed a significant association between ECV and histological extent of fibrosis (r=0.94, p <0.001).

CMR T1 mapping was shown to correlate with myocardial fibrosis in many different cardiac conditions such as acute myocardial infarction, chronic myocardial infarction, acute myocarditis, aortic stenosis, hypertrophic cardiomyopathy, congenital heart disease, idiopathic dilated cardiomyopathy, and infiltrative heart disease. These studies showed the ability of CMR-T1 mapping to detect diffuse myocardial fibrosis, validated by histological evidence in several forms of cardiomyopathy.

In summary, CMR imaging with T1 mapping and calculation of ECV is a novel and validated non-invasive method to quantify the extent of diffuse myocardial fibrosis even without the use of gadolinium. Furthermore, ECV measurement is superior to LGE which can only give a qualitative assessment of focal areas of fibrosis.
FGF-23 and CMR:

Higher serum FGF-23 concentrations were associated with progressively greater left ventricular mass and a greater prevalence of LVH. *Kestenbaum et al.* showed a linear relationship between FGF-23 and left ventricular mass even after adjustment for established LVH risk factors. For each 20-pg/mL higher serum FGF-23 concentration there was an increase of 1.2 g in left ventricular mass (95% confidence interval, 0.2-2.2 g greater) after full adjustment for common cardiovascular risk factors.³

**Conceptual Model**

Our understanding of the pathogenesis of heart failure and cardiovascular disease among CKD patients is summarized by the conceptual model in (Figure 6). Abnormally high levels of FGF-23 have several deleterious effects on the myocardium that ultimately lead to heart failure. The model includes different mechanisms by which FGF-23 increases. Several changes occur with declining kidney function. While serum calcium, and 1-25 (OH) vitamin D levels decline, PTH and phosphorus levels increase as part of CKD-MBD. As a response to these derangements, FGF-23 increases and Klotho decreases. Higher levels of FGF-23 are linked to a variety of clinical and pathological conditions that lead to heart failure. Myocardial fibrosis is one of these conditions that can be associated with higher levels of FGF-23. Furthermore, FGF-23 may lead to heart failure with preserved ejection fraction (HFpEF) or diastolic heart failure as well as heart failure with reduced ejection fraction (HFrEF) or systolic heart failure. Additionally, Figure 6 lists possible confounders that may potentially lead to myocardial fibrosis as
well as the modifiers that can modify the relationship between FGF-23 levels and myocardial fibrosis.

This conceptual model has several strengths, which include representation of the current understanding of the pathophysiologic changes of CKD-MBD, and linking that to the progression of heart failure through FGF-23 based on a comprehensive literature review. Also it acknowledges the complexity and the uncertainty of the current understanding of this association between CKD and heart failure. Furthermore, it considers the role of confounders and effect modifiers in the interactions between antecedent, independent and dependent variables.

However, this conceptual model should also be considered with several weaknesses, which include the inability to account for other mechanisms that interact with FGF-23 levels, for example, dietary phosphate contents, the use of phosphate binders or different vitamin D replacement preparations, aldosterone, estrogen, insulin resistance and other unknown factors that may confound the proposed pathologic mechanisms that link FGF-23 to heart failure.
Figure 6: Conceptual model of the role FGF-23 in the development of heart failure

**Knowledge gap:**

Though animal studies suggested a link between FGF-23 and myocardial fibrosis,\textsuperscript{57} it is unknown whether this relationship is applicable to human populations. Furthermore, the relationships between FGF-23 levels and CMR indices of myocardial fibrosis have not been studied before. Additionally, though higher levels of FGF-23 were shown to be associated with higher incidence of heart failure,\textsuperscript{3} it is yet to be determined whether this relationship applies to both HFrEF and HFrEF. This work will be helpful clinically and on a public health level by adding another tool of predicting myocardial fibrosis and heart failure and thus allocating resources to prevention of the population at risk at early stages.
Aims and Hypotheses

**Primary Aim:** To determine whether higher levels of fibroblast growth factor-23 (FGF-23) are associated with indices of myocardial fibrosis detected by cardiac magnetic resonance-T1 mapping in a multi-ethnic, middle aged community cohort free of heart disease at baseline.

**Secondary Aim:** To determine whether FGF-23 is associated with heart failure with preserved ejection fraction or with heart failure with reduced ejection fraction or both.

**Predictor:** baseline serum FGF-23

**Outcomes:** Extracellular volume fraction (ECV), pre-contrast T1 mapping, post-contrast T1 mapping after 12 min, post-contrast T1 mapping after 25 min, ejection fraction, incident HFpEF, incident HFrEF.

**Primary Hypothesis:** higher levels of FGF-23 are associated with higher levels of ECV

**Secondary Hypothesis:** FGF-23 levels are associated with both heart failure with preserved ejection fraction and heart failure with reduced ejection fraction.
Methods:

We will address the first aim using a large cohort study of 1183 participants free of cardiovascular disease at baseline with FGF-23 measured at exam 1 (between 2000-2002) and CMR T1 mapping performed at exam 5 (between 2010-2012). A subset of these participants (n=588) had ECV calculated at exam 5. Participants with myocardial scar (detected by visual assessment of any size using late gadolinium enhancement imaging) were excluded. We will use multiple linear regression models to examine the associations between FGF-23 and CMR indices of myocardial fibrosis (pre-contrast T1 mapping, 12-min and 25-min post-contrast T1 mapping and ECV %). ANOVA procedure will be used to compare SAS driven least square means of CMR indices of fibrosis between quartiles of FGF-23.

For the second aim we will use participants with baseline FGF-23 measured at baseline exam 1 (n=6549) to examine the associations of FGF-23 with incident heart failure, HFrEF and HFpEF. We will use Kaplan-Meier curves and log-rank procedure to compare cumulative incidence of Heart failure, HFrEF and HFpEF by quartiles of FGF-23. Cox proportional hazards regression models will be used to compute hazard ratios using multivariate models.
CHAPTER TWO: MANUSCRIPT

Fibroblast Growth Factor-23 and Cardiac-MRI Indices of Myocardial Fibrosis in the Multi-Ethnic Study of Atherosclerosis

Short Title: FGF-23 and CMR indices of myocardial fibrosis in MESA.

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Abstract

**Background:** Fibroblast growth factor-23 (FGF-23) is associated with myocardial fibrosis in animal studies. We examined the associations between FGF-23 and Cardiac Magnetic Resonance (CMR) indices of myocardial fibrosis using T1 mapping.

**Methods:** A total of 6547 study participants free of heart disease at baseline, had serum FGF-23 concentrations measured between 2000-2002. We included 1183 participants (49% Male; age 58±9 at baseline) with eGFR>45 mL/min/1.73m² who underwent CMR between 2010-2012 and had no evidence of focal scars on late gadolinium enhancement. ANOVA and multivariable linear regression models were performed to determine the relationships between baseline log₂ transformed FGF-23 levels and follow up myocardial T1 mapping (pre-contrast, 12- and 25-min post-contrast), and extracellular volume fraction (ECV %) (n=588). Models were adjusted for cardiovascular risk factors, left ventricular mass and stratified by gender.

**Results:** Overall, we found no significant relationships between FGF-23 and CMR indices of myocardial fibrosis. However, in gender stratified, a doubling of FGF-23 was associated with an absolute 1.2% higher ECV (95% CI: 0.33, 2.05%; p=0.007) and a near significant 7.1(ms) higher pre-contrast myocardial T1 mapping (95% CI: -0.1, 14.4; p=0.053) in men. Overall there was a trend for a positive relationship between FGF-23 quartiles and pre-contrast myocardial T1 mapping (p-trend =0.02). After stratification, men in the highest quartile (FGF-23 >46.1 pg/ml) had significantly higher pre-contrast myocardial T1 mapping and ECV values (973±3 (ms), 26.3±0.3%, respectively)
compared to those in the lowest quartile (FGF-23 <31 pg/ml) (961±4 (ms), 24.7±0.4%, respectively) (p value=0.002 and 0.01 respectively). In contrast, we found no significant association between FGF-23 and CMR indices of myocardial fibrosis in women.

**Conclusion:** In men FGF-23 is positively associated with CMR indices of myocardial fibrosis. This mechanism may explain the increased risk for heart failure associated with higher levels of FGF-23.
Introduction

Myocardial fibrosis is a common endpoint in a variety of cardiac diseases.\(^1\) Diffuse myocardial fibrosis occurs as a part of normal aging;\(^2\) however, this process is accelerated in many diseases.\(^3^\)\(^-\)\(^5\) This diffuse fibrosis is associated with worsening ventricular function and increased ventricular stiffness, and has independent predictive value for major cardiac events and increased all-cause mortality.\(^6^\)\(^-\)\(^10\) The pathogenesis of myocardial fibrosis, which leads to significant increase in collagen deposition in the myocardium, is yet not fully understood.

Recently, fibroblast growth factor-23 (FGF-23) and its coreceptor Klotho have been shown to be associated with myocardial fibrosis and left ventricular hypertrophy in animal studies.\(^1\)\(^1\)\(^2\) FGF-23 is a hormone secreted by osteoblasts/osteoclasts. It functions as a phosphaturic factor in the renal tubules and a regulator of parathyroid hormone, thus regulating calcium and phosphate homeostasis. Higher serum levels of FGF-23 were shown to be associated with variety of cardiovascular cardiac diseases.\(^1\)\(^3\)\(^,\)\(^14\) For example, Kestenbaum et al showed that higher serum FGF-23 concentrations are associated with incident heart failure, left ventricular hypertrophy and coronary disease events in a population free of heart disease at baseline\(^1\)\(^3\). However, it is unknown whether this increase in incident heart failure and left ventricular hypertrophy is associated with increased myocardial fibrosis.

The best available non-invasive method of examining myocardial fibrosis is contrast-enhanced cardiac magnetic resonance (CMR). The use of late gadolinium enhancement imaging (LGE) enabled the identification of focal myocardial fibrosis.\(^1\)\(^5\) However, this technique is limited in detecting diffuse myocardial fibrosis because it
depends on the signal intensity difference between fibrotic and nonfibrotic tissues which is reduced in homogeneously distributed fibrosis.\textsuperscript{16}

Recent development of T1 mapping allowed the quantitative assessment of diffuse myocardial fibrosis through the measurement of the intrinsic magnetic resonance relaxation parameter T1 of the myocardium.\textsuperscript{17} T1 mapping time (in milliseconds) represents protons recovery time after radiofrequency pulse. Longer pre-contrast (native) T1 mapping and shorter post-contrast T1 mapping were shown to be associated with diffuse myocardial fibrosis.\textsuperscript{18,19}

A newer more accurate technique allows quantification of the extracellular volume fraction (ECV) by measuring the ratio of change in myocardial T1 relative to blood-pool T1 pre and post-contrast after correcting for hematocrit.\textsuperscript{20} ECV is increased in conditions characterized with diffuse myocardial fibrosis.\textsuperscript{21,22}

In a large multi-ethnic cohort of men and women, we hypothesized that higher levels of FGF-23 would be associated with indices of myocardial fibrosis.

**Methods**

**Study population**

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study investigating risk factors and progression of subclinical cardiovascular disease among 6814 community-living individuals.\textsuperscript{23} Between 2000 and 2002, the MESA recruited participants free of cardiovascular disease aged 45 to 84 years from six sites from United States, Baltimore, MD; Chicago, IL; St. Paul, MN; Forsyth County, NC; New York, NY; and Los Angeles, CA. The MESA recruited a final population that was 38\% white, 28\% black, 22\% Hispanic, and 12\% Chinese-Americans. The study excluded any participants
with self-reported history of myocardial infarction, angina, stroke, transient ischemic attack, heart failure, atrial fibrillation, nitroglycerin use, angioplasty, coronary artery bypass grafting, valve replacement, pacemaker or defibrillator, or any surgery on the heart or arteries. All participants gave informed consent, and institutional review board approval was obtained for each site.

We evaluated all participants who had FGF-23 measurement at baseline and subsequently had cardiac magnetic resonance (CMR) with T1 mapping (n=1183) and ECV% measurement (n=588) on the 5th follow-up exam (between 2010-2012). Refer to (Figure 1) for explanation of participants’ enrollment in this study.

**Study procedures: CMR imaging.**

MESA participants without contraindications underwent CMR examinations by using 1.5-T scanners (Avanto and Espree, Siemens Medical Systems, Eelangen, Germany) with a 6-channel anterior phased array torso coil and corresponding posterior coil elements. Left ventricular (LV) function, dimensions, and myocardial mass were assessed by a cine steady-state free precession sequence. Twelve short axis slices, one 4-chamber view, and one 2-chamber view were acquired. Participants undergoing CMR scans were screened for gadolinium eligibility. The inclusion criteria were the estimated glomerular filtration rates (eGFRs). Participants with an eGFR ≥45 ml/min (60 ml/min for the site at Northwestern University) and with no history of allergic reaction to contrast agents were qualified to receive gadolinium. Late gadolinium enhancement images were obtained 15 min after an intravenous bolus injection of gadolinium-diethylene triamine pentaacetic acid (0.15 mmol/kg [Magnevist, Bayer Healthcare Pharmaceuticals, Montville, New Jersey]) to identify myocardial fibrosis. Twelve short-axis slices, 1
horizontal long axis, and 1 vertical long axis at the same positions as the LV function cine images were acquired.

For evaluation of diffuse fibrosis, 1 short axis MOLLI image at the mid-slice position was acquired pre-contrast (native), then repeated at 12 and 25 min post-contrast administration. 12-and 25 min post-contrast T1 maps are used to draw the inversion recovery curve. The timing was chosen to be comparable with previous studies and also to accommodate the design of the entire CMR protocol. The MOLLI sequence acquired a set of 11 source images in 17 heartbeats. It consisted of 3 consecutively inversion recovery-prepared electrocardiography-synchronized Look-Locker trains. Each of the 3 trains began with an inversion pulse at specific inversion time (T1= 100, 200, and 350 ms), after which multiple single shot, steady-state free precession images were acquired in consecutive heartbeats. All images were acquired with the same trigger delay time in end diastole. The exact scanning parameters were as follows: flip angle =35°; repetition time=2.2 ms; echo time=1.1 ms; field of view=360 X 360 mm; matrix=192 X 183; slice thickness = 8 mm; generalized autocalibrating partially parallel acquisitions factor=2.

We constructed T1 maps offline by using MASS research software (Department of Radiology, Leiden University Medical Center, Leiden, The Netherland). We performed a 3-parameter curve fit of the MOLLI source images according to the Levenberg-Marquardt algorithm to calculate T1 values for each pixel automatically. Then, we selected a region of interest around the core of myocardium to exclude the blood pool and epicardial fat and calculate the myocardial T1 time for each subject. T1 maps were calculated pre-contrast (native) and 12-min, 25 min post-contrast. The
extracellular volume fraction (ECV [%]) was determined using the following equation (ECV=100 X partition coefficient X [1- hematocrit]). To determine the partition coefficient, we used the slope of the linear relationship of (1/T$_{1\text{myo}}$ vs. 1/T$_{1\text{blood}}$) at three time points as described in previously published studies.²-²⁸ Because ECV calculation depends on both T1 maps and hematocrit level at the time of CMR performance, only a subset of the participants (n=608) with available hematocrit data at the time of CMR examination had ECV calculated. Of them 588 with available FGF-23 data were included in our analysis (Figure 1).

**Measurement of Serum FGF-23 Concentrations**

The University of Vermont Laboratory for Clinical Biochemistry stored fasting blood and urine samples collected by MESA study personnel using established methods.²⁹ These samples were shipped on dry ice to the University of Washington where serum FGF-23 concentrations were measured using the Kainos Immunoassay³⁰ which detects the full-length biologically intact FGF-23 molecule via mid-molecule and distal epitopes. Standarized FGF-23 controls within each run were used to monitor quality control. The coefficient of variation across 81 plates was 6.7% and 12.4% for high and low control samples respectively.¹³

**Statistical Analysis**

We examined the distribution of the variables and presented continuous variables as mean ± SD and categorical variables as frequencies and percentages. Participant characteristics were presented by FGF-23 quartiles. We used multiple linear regression models to estimate associations of baseline serum FGF-23 concentrations and follow-up CMR variables of myocardial fibrosis (native, 12- and 25-min post-contrast myocardial
T1 mapping in milliseconds (ms), and ECV %. We repeated multiple linear regression models after dividing subjects into quartiles based on serum FGF-23 concentrations. Due to skewed distributions, FGF-23 was explored as a continuous predictor variable after log base 2 transformations so the parameter coefficient can be interpreted as “per doubling of FGF-23.” Because of previous observations of gender differences in cardiac remodeling and myocardial fibrosis, we performed the analyses overall and stratified by gender. We performed linear regressions with multivariate adjustments for demographic variables and risk factors. The initial model for all analyses was unadjusted. Model 2 was adjusted for age, race, study site, highest education level completed (high school or less, some college but no degree, college degree or more), height and weight. Model 3 was further adjusted for systolic blood pressure (continuous), antihypertensive medications (yes versus no), LV mass, heart rate, LDL, HDL, diabetes mellitus status (yes versus no), C-reactive protein (CRP), smoking (current or former versus nonsmoker), urine albumin-creatinine ratio, and eGFR\textsubscript{CKD-Epi}. P<0.05 was considered significant for all analyses including interaction terms. All statistical analyses were performed with SAS software, version 9.3 (SAS Institute, Cary, NC).

Because we had 2 different datasets, one with native and post-contrast T1 mapping measurements (n=1183) and a subset with ECV measurements (n=588), we had mathematically but not clinically different cutoffs of FGF-23 quartiles. We used quartiles cutoffs from the main dataset (n=1183) for the analyses. However, when repeating the analysis of the association of FGF-23 with ECV based on FGF-23 quartiles specific for the ECV dataset (n=588), we found similar results. Supplemental table 2 shows the participant’s baseline characteristics by FGF-23 quartiles specific to the ECV dataset.
Results

A total of 1183 (49% men) participants with baseline FGF-23 and follow up T1 mapping free of focal scars on late gadolinium enhancement were included in the study. Of them 588 (47% men) participants had calculated ECV values (Figure 1). Baseline characteristics of the participants (n=1183) were presented according to quartiles of serum FGF-23 levels (Table 1). Overall, 52.4% of the participants were white, 22% blacks, 13.8% hispanics and 11.8% Chinese American. For the subset with calculated ECV, 58% were white, 28% blacks and 14% hispanics. Mean eGFR was 83.8 mL/min per 1.73 m$^2$. There was a gradual decline in mean eGFR with increasing serum FGF-23 quartiles (P <0.001). For doubling of FGF-23, eGFR decreased by 5.67 ml/min per 1.73 m$^2$ (95% CI 3.88, 7.47. p value <0.001). Participants in the 4th quartile had higher body mass index (BMI). The participant’s characteristics of the ECV dataset were presented in supplemental table 2.

Women had significantly higher native myocardial T1 mapping and ECV and lower 12-min and 25-min post-contrast myocardial T1 mapping compared to men (Table 2). The interaction between gender and log$_2$FGF-23, as measured by the regression coefficient for men and women, was near significant for native T1 mapping, and nonsignificant for 12-min post-contrast T1 mapping and ECV (p=0.07, p=0.4 and p=0.1, respectively) in the fully adjusted model.

Overall, there was no significant relationship between serum FGF-23 and CMR indices of myocardial fibrosis (Table 3). However, after stratification by gender, a doubling of FGF-23 was associated with an absolute 1.2% higher ECV (95% CI: 0.33,
and a near significant increase in native myocardial T1 mapping by 7.1 (ms) (95% CI: -0.1, 14.4 (ms); p=0.053) in men only.

When comparing adjusted mean values of CMR indices of fibrosis by quartiles of serum FGF-23 (Figure 2), overall there was a trend for higher native myocardial T1 mapping (p-trend=0.02). After stratification by gender, men had increasing native myocardial T1 mapping and ECV values with higher serum FGF-23 quartiles. The adjusted mean difference in native myocardial T1 mapping for men in the 4th quartile compared to the 1st quartile was 12 (ms) (95% CI: 3, 21 (ms), p-trend=0.002) and an absolute difference of 1.55% in ECV (95% CI: 0.6, 2.5%; p-trend =0.01). No statistically significant relationship between serum FGF-23 concentration and either native myocardial T1 mapping or ECV was seen in women.

We found no significant relationship between FGF-23 and 12-min, 25-min post-contrast myocardial T1 mapping. We found high correlation between the two post-contrast myocardial T1 mapping variables \[r^2\] =0.84, thus only 12-min post-contrast myocardial T1 mapping was presented in table (3) and figure (2).

**Discussion**

In the Multi-Ethnic Study of Atherosclerosis, we evaluated the relationship between serum FGF-23 concentrations and myocardial tissue composition by using CMR T1 mapping. Overall, we found no significant relationship between serum FGF-23 concentrations and indices of myocardial fibrosis. However, after stratification by gender, we found a significantly positive relationship between serum FGF-23 concentration and both native myocardial T1 mapping and extracellular volume in men. Additionally, we
found a significant trend between quartiles of FGF-23 and native myocardial T1 mapping overall and in men. These findings can be interpreted as an increase in the collagen composition of the myocardium and thus increased diffuse myocardial fibrosis. However, we did not find statistically significant associations between serum FGF-23 concentration and CMR indices of myocardial fibrosis in women.

Myocardial fibrosis is a pathological process observed as a response to myocardial injury. The gold standard method to detect diffuse myocardial fibrosis is endomyocardial biopsy. However, this method is invasive and is subject to sampling error. CMR T1 mapping has been shown to provide a non-invasive validated quantification of cardiac tissue composition and fibrosis. While focal fibrosis usually follows myocardial infarction, diffuse myocardial fibrosis has been detected in non-ischemic cardiomyopathy, hypertrophic cardiomyopathy, heart failure, diabetes, atrial fibrillation, aging, and infiltrative cardiomyopathies. It is commonly associated with LV hypertrophy, abnormal cardiac remodeling, and worsening ventricular systolic and diastolic function.

FGF-23 regulates calcium and phosphorus hemostasis through binding to FGF receptors and klotho, its coreceptor in the kidney and parathyroid glands. FGF-23 concentrations increase and soluble klotho concentrations decrease in chronic kidney disease with progressive decline of phosphorus excretion. Previous animal studies suggested a causal role for FGF-23 in the development of cardiovascular disease. In mice, intravenous injection of FGF-23 induced pathological hypertrophy of isolated cardiomyocytes by klotho-independent mechanism. Administration of FGF receptors blocker to a rat model of CKD attenuated the severity of LVH without reducing elevated
blood pressure. Furthermore, higher levels of FGF-23 concentrations were associated to increased myocardial fibrosis and remodeling in presence of moderate or severe klotho deficiency.

This pathologic elevation in FGF-23 provides a key mechanism of the abnormally high prevalence of LV hypertrophy, heart failure, and myocardial fibrosis, as well as the increased cardiovascular mortality noted in patients with CKD. This is important because FGF-23 levels start increasing at early stages of CKD (eGFR <75). In fact, it was found that CKD was associated with increased myocardial fibrosis even at early stages.

Our findings of differences in CMR indices of myocardial fibrosis between men and women are consistent with previous study of age-related effect on myocardial fibrosis by Liu et al., which demonstrated significantly higher ECV in women, and more pronounced increase in ECV with age in men. Our findings of different response of the myocardium to higher levels of FGF-23 are also consistent with previous studies of gender differences in myocardial response to different stimuli. For example, while women tend to preserve their myocardial mass, myocyte number and volume, men lose myocardial mass and myocyte number, but increase in myocyte cell volume. Furthermore, in a post-mortem study, Mallat et al. showed that the apoptotic index was found to be 3-fold higher in men compared to women free of cardiovascular disease. Compared to men, women have greater degree of LV hypertrophy and preserved LV function in response to pressure overload. Similar findings were seen as a response to volume overload. For example, Gardner et al. demonstrated that female rats developed concentric hypertrophy with no impairment of cardiac function and no changes in myocardial compliance after 8 weeks of infra-renal aortocaval fistula creation. In contrast
male rats developed a significant fistula-related dilation and increase ventricular compliance.\textsuperscript{56} Thus, women may be protected from fibrosis.

We found no significant relationship between FGF-23 and 12-min, 25-min post-contrast myocardial T1 mapping. However, measurement of post-contrast myocardial T1 mapping provides a value linked to the interstitium, but it is also influenced by gadolinium dose, clearance rate, time post bolus, body composition, and hematocrit. Because of these limitations, native T1 mapping and ECV measurement are preferred over post-contrast T1 mapping for the detection of myocardial fibrosis.\textsuperscript{57} Furthermore, ECV represent a newer, more accurate technique of myocardial fibrosis quantification that is intended to reduce the effects of these variables by using T1 maps of the myocardium and the blood at three time points (native, 12-and 25-min post-contrast T1 mapping) after correction for hematocrit.\textsuperscript{26}

Based on our findings, we believe there are fundamental differences in the myocardial response to higher serum FGF-23 concentrations between men and women consistent with the differences in cardiac remodeling present in response to aging,\textsuperscript{2} pressure overload,\textsuperscript{55} volume overload,\textsuperscript{56} and myocardial infarction.\textsuperscript{58}

\textbf{Limitations:}

ECV values were only measured for a subset of participants (n=588). Only those with available hematocrit data at the time of CMR examination were used to calculate ECV (Figure 1). Since only Participants with a GFR $\geq$45 ml/min (60 ml/min for the site at Northwestern University) underwent ECV calculation, this subset may represent a healthier group. Also, since different inclusion criteria were used at different sites as
mentioned above, this may introduce heterogeneity. To overcome this we used multivariate regression to adjust for study site and other potential confounders. We performed post-hoc stratification by gender. Finally, CMR with T1 mapping was performed only once at the 5th follow up exam 10 years after FGF-23 measurement. Because of this time gap we recognize the potential effect of attrition which may bias our results. For example, participants with higher FGF-23 are usually sicker and might be less likely to follow up.

**Conclusion:**

In a community-based, multi-ethnic cohort free of clinical cardiovascular disease at baseline, we observed significant associations between higher serum FGF-23 concentrations and higher native myocardial T1 mapping and ECV in men. Our findings suggest that FGF-23 effects on the myocardium varies by gender and are likely similar to the effects observed with cardiac remodeling noticed in aging. This mechanism may explain the accelerated myocardial aging and the higher prevalence of heart failure observed in patients with CKD. Further studies of FGF-23 effects on the myocardium may further explore these gender differences.
Figure 1. Participant enrollment in the study

Year 2000
N=6814 enrolled in baseline exam
N=6547 had FGF-23 measured at baseline exam

Year 2010-2012
N=1334 had T1 mapping
N=1183 with baseline FGF-23 and T1 mapping
N=113 excluded due to visible scar by late gadolinium enhancement

N=588 with baseline FGF-23 and ECV
### Table 1. Baseline characteristics by Fibroblast Growth Factor-23 Quartile

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FGF-23 Quartiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>overall</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>1183</td>
</tr>
<tr>
<td>Age, y</td>
<td>58±8.7</td>
</tr>
<tr>
<td>gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>603 (51%)</td>
</tr>
<tr>
<td>Male</td>
<td>580 (49%)</td>
</tr>
<tr>
<td>Race</td>
<td>620 (52%)</td>
</tr>
<tr>
<td>White %</td>
<td>140 (12%)</td>
</tr>
<tr>
<td>Chinese %</td>
<td>260 (22%)</td>
</tr>
<tr>
<td>Black %</td>
<td>163 (14%)</td>
</tr>
<tr>
<td>Hispanic %</td>
<td>176 (15%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>606 (51%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>303 (26%)</td>
</tr>
<tr>
<td>Education</td>
<td>677 (57%)</td>
</tr>
<tr>
<td>High school or less %</td>
<td>304 (26%)</td>
</tr>
<tr>
<td>Some college %</td>
<td>200 (17%)</td>
</tr>
<tr>
<td>College degree or more %</td>
<td>677 (57%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168±10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>175±37</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28±5</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>122±19</td>
</tr>
<tr>
<td>HTN medication</td>
<td>303 (26%)</td>
</tr>
<tr>
<td>Estimated GFR, mL/min per 1.73 m²</td>
<td>84±15</td>
</tr>
<tr>
<td>LVEDM (g)</td>
<td>122±32</td>
</tr>
</tbody>
</table>

Values are mean ±SD. SBP: Systolic blood pressure. Smoking: current and former versus never. HTN: Hypertension. GFR: glomerular filtration rate. LVEDM: left ventricular end-diastolic mass (g).
### Table 2. CMR indices of myocardial fibrosis by gender

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native T1 mapping (ms)</td>
<td>977±43</td>
<td>968±38</td>
<td>985±45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12 min post-contrast T1 mapping (ms)</td>
<td>457±40</td>
<td>472±33</td>
<td>442±42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25 min post-contrast T1 mapping (ms)</td>
<td>520±41</td>
<td>535±34</td>
<td>506±41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ECV%</td>
<td>26.9±3.0</td>
<td>25.8±2.8</td>
<td>28±2.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are mean ±SD. ECV: extracellular volume. P value from student t test comparing CMR indices between males and females. 1183 participants had pre- and post-contrast T1 mapping, while 588 participants had ECV measured.

### Table 3. Association of FGF-23 with native myocardial T1 mapping, post-contrast T1 mapping, and extracellular volume

<table>
<thead>
<tr>
<th>CMR indices of myocardial fibrosis</th>
<th>Gender</th>
<th>FGF-23 Quartiles</th>
<th>Linear Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;31</td>
<td>31-38.2</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>971±3</td>
<td>974±3</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td>961±4</td>
<td>966±3</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>978±4</td>
<td>983±4</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>458±2</td>
<td>459±2</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td>471±3</td>
<td>473±3</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>446±3</td>
<td>443±3</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>26.5±0.3</td>
<td>26.9±0.3</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td>24.7±0.4</td>
<td>25.4±0.3</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>27.7±0.4</td>
<td>28.2±0.4</td>
</tr>
</tbody>
</table>

Analysis of variance procedure was used to compare SAS derived least square means of CMR-indices of myocardial fibrosis between FGF-23 quartiles. For the linear model we used nested multivariate linear regression analysis to examine the association between log₂ FGF-23 and CMR indices of myocardial fibrosis. The values are regression coefficient values. For example, for every doubling of FGF-23 there is an absolute 1.2% increase in ECV. Values are presented as means ± SE. Models were stratified by gender and adjusted for age, race/ethnicity, study site, education, height and weight; systolic blood pressure, antihypertensive medications, left ventricular mass, heart rate, low density lipoprotein, high density lipoprotein, diabetes mellitus, smoking, C-reactive protein, urine albumin-creatinine ratio and eGFR<sub>CKD-EPI</sub>. P value <0.05 is considered significant. 1183 participants had pre- and post-contrast T1 mapping, while 588 participants had ECV measured.
Figure 2. Associations between serum FGF-23 quartiles and Native myocardial T1 mapping, 12-min post-contrast myocardial T1 mapping and ECV, overall (green color) and stratified by gender, male (blue color) and women (red color).
## Supplemental Table 1: Baseline participant’s characteristics by Fibroblast Growth Factor-23 Quartile for the participants with measured ECV

Values are mean ±SD. SBP: Systolic blood pressure. Smoking: current and former versus never. HTN: Hypertension. GFR: glomerular filtration rate.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FGF-23 Quartiles</th>
<th>Overall</th>
<th>&lt;31.8</th>
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<td>(94) 64%</td>
<td>(95) 65%</td>
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<td>39 (26%)</td>
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<td>College degree or more%</td>
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<td>29±5</td>
<td>29±5</td>
<td>29±6</td>
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<td>SBP, mm Hg</td>
<td>122±19</td>
<td>123±21</td>
<td>122±18</td>
<td>123±19</td>
<td>123±17</td>
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<td>80±14</td>
<td>79±15</td>
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CHAPTER THREE: ANCILLARY ANALYSES

Fibroblast Growth Factor-23 associations with Incident Heart Failure with Reduced versus Preserved Ejection Fraction in the Multi Ethnic Study of Atherosclerosis

Short Title: FGF-23 and HFrEF or HFpEF in MESA.

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Abstract

**Background:** Fibroblast Growth Factor (FGF)-23 is a hormone produced by osteoblast as a response to high serum phosphorus. High concentrations of serum FGF-23 are associated with incident heart failure (HF) in the community. We sought to determine the association between FGF-23 and incident heart failure with reduced (HFrEF) and with preserved (HFpEF) ejection fraction.

**Methods:** We studied 6549 study participants free of heart disease at baseline enrolled in Multi-Ethnic Study of Atherosclerosis (MESA). FGF-23 concentrations were measured at baseline between 2000-2002 using Kainos Immunoassay. Individuals were followed for a mean of 11.1 ± 3 years for incident HF. We classified HF events as HFrEF (ejection fraction (EF) <50%) or HFpEF (EF ≥50%) at the time of diagnosis. We used Kaplan-Meier curves and log-rank procedure to compare cumulative incidence of HFrEF and HFpEF by quartiles of FGF-23. Cox proportional hazard regression was used to compute hazard ratios (HR) and 95% confidence intervals (CI) for the association between serum FGF-23 and incident HFrEF and HFpEF. Models were adjusted for age, gender, race, education, study site, smoking, diabetes, systolic blood pressure, use of antihypertensive medications, left ventricular (LV) mass, HDL-C, LDL-C, C-reactive protein (CRP), eGFR, and log urine albumine creatinine ratio.

**Results:** Study participants had a total of 227 heart failure events. Of these 125 were classified as HFrEF and 102 as HFpEF. Higher FGF-23 levels were associated with higher risk for incident HF. For doubling of FGF-23 concentration the adjusted hazard ratio (HR) was 1.8 (95% CI 1.3-2.5). The association between FGF-23 and HF was
stronger with HFpEF compared with HFrEF. Corresponding HRs were 2.6 (95% CI: 2.4-4) for HFpEF and 1.5 (95% CI; 1-2.3) for HFrEF.

**Conclusion:** FGF-23 is associated with both HFpEF and HFrEF. We found a stronger association with HFpEF in the Multi-Ethnic Study of Atherosclerosis. Our findings may help explain the increase prevalence of HFpEF in patients with chronic kidney disease.
Introduction

Heart failure (HF) is a major cause of morbidity and mortality and associated high health-care related costs.\textsuperscript{1} It is estimated to affect almost 7 million Americans.\textsuperscript{2} Half of these patients considered to have normal left ventricular ejection fraction (EF), and are classified as heart failure with preserved ejection fraction (HFpEF).\textsuperscript{3} Current guidelines define HFpEF using an EF cut-off of ≥50%.\textsuperscript{4,5} While the morbidity and mortality of heart failure with reduced ejection fraction (HFrEF: defined as an EF<50%) is improving,\textsuperscript{6} HFpEF continues to have unchanged morbidity and mortality.\textsuperscript{7-9}

Although several effective evidence based therapies are now available for the treatment of patients with HFrEF,\textsuperscript{4} the same cannot be said about HFpEF.\textsuperscript{10} In fact, the exact pathophysiological processes that lead to HFpEF are not completely understood yet.\textsuperscript{11} These processes are diverse and the condition is considered to be multifactorial and heterogeneous.\textsuperscript{12,13} Due to this heterogeneity of HFpEF, additional research is needed to better characterize the phenotype of HFpEF, understand the pathophysiological processes, as well as identify the risk factors associated with this condition.

Fibroblast growth factor-23 (FGF-23), a hormone involved in phosphorus and vitamin D hemostasis,\textsuperscript{14-16} has been recently linked to the development of HF and left ventricular hypertrophy (LVH).\textsuperscript{17,18} In the Mult-Ethnic Study of Atherosclerosis, Kestenbaum et al. showed that higher serum FGF-23 concentrations are associated with incident HF, LVH and coronary disease events\textsuperscript{19}. However, it is unknown whether this increase in incident HF is of the preserved or reduced ejection fraction types. Even
though FGF-23 is strongly linked to LVH which is linked to both types of HF. FGF-23 is also associated with several other pathologies such as arterial stiffness, endothelial dysfunction, vascular calcifications as well as major cardiovascular events. All of these factors can predispose to both types of HF. We hypothesize that higher levels of FGF-23 are associated with both HFrEF and HFpEF in a Multi-Ethnic population free of heart failure at baseline.

Methods

Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study of cardiovascular disease among 6814 community-living individuals. Between 2000 and 2002, the MESA recruited participants free of cardiovascular disease aged 45 to 84 years from six sites from United States, Baltimore, MD; Chicago, IL; St. Paul, MN; Forsyth County, NC; New York, NY; and Los Angeles, CA. The MESA recruited a final population that was 38% white, 28% black, 22% Hispanic, and 12% Asian. The study excluded any participants with self-reported history of myocardial infarction, angina, stroke, transient ischemic attack, heart failure, atrial fibrillation, nitroglycerin use, angioplasty, coronary artery bypass grafting, valve replacement, pacemaker or defibrillator, or any surgery on the heart or arteries. All participants gave informed consent, and institutional review board approval was obtained for each site.

We evaluated all participants who had FGF-23 measurement at baseline with known ejection fraction at the time of developing incident heart failure (n=6549).
Study procedures: CMR imaging.

MESA participants without contraindications underwent CMR examinations by using 1.5-T scanners (Avanto and Espree, Siemens Medical Systems, Eelangen, Germany) with a 6-channel anterior phased array torso coil and corresponding posterior coil elements. LV function, dimensions, and myocardial mass were assessed by a cine steady-state free precession sequence. Twelve short axis slices, one 4-chamber view, and one 2-chamber view were acquired.

Measurement of Serum FGF-23 Concentrations

Blood and urine samples stored at the University of Vermont Laboratory for Clinical Biochemistry were shipped on dry ice to the University of Washington where serum FGF-23 concentrations were measured using the Kainos Immunoassay which detects the full-length biologically intact FGF-23 molecule via mid-molecule and distal epitopes. Standarized high- and low-value FGF-23 controls within each run were used to monitor quality control.

Ascertainment of heart failure events

MESA personnel screened participants for incident events through telephone contacts and scheduled follow up examinations. They abstracted any hospital records suggesting cardiovascular events. They recorded symptoms, history and biomarkers; scanned ECGs, echocardiograms, catheterization reports, outpatient records, and other relevant diagnostic reports; and transmitted these to the coordinating center.

Two study physicians blinded to other study data independently reviewed the medical records. Incident heart failure events were considered as probable or definite.
Definite or probable CHF required heart failure symptoms, such as shortness of breath or edema, as asymptomatic disease is not a MESA endpoint. In addition to symptoms, probable CHF required CHF diagnosed by a physician and patient receiving medical treatment for CHF.

Definite CHF required one or more other criteria, such as pulmonary edema/congestion by chest X-ray; dilated ventricle or poor LV function by echocardiography or ventriculography; or evidence of left ventricular diastolic dysfunction. We considered participants not meeting any criteria, including just a physician diagnosis of CHF without any other evidence, as having no CHF. The Events Committee classified heart failure as HFrEF (ejection fraction (EF) <50%) or HFpEF (EF≥50%) at the time of heart failure diagnosis.

Statistical Analysis

We examined the distribution of the variables and presented continuous variables as mean ± SD and categorical variables as frequencies and percentages. Basic characteristics were presented by FGF-23 quartiles. Due to skewed distributions FGF23 was explored as a continuous predictor variable after log base 2 transformations so the parameter coefficient can be interpreted as “per doubling of FGF23”. We used Kaplan-Meier curves and the log-rank procedure to compare cumulative incidence of HFrEF and HFpEF by quartiles of FGF-23. Cox proportional hazard regression was used to compute cause-specific hazard ratios (HR) and 95% confidence intervals (CI) for the association between log base 2 transformed FGF-23 and incident HFrEF and HFpEF first, then between quartiles of FGF-23 and incident HFrEF and HFpEF separately. The initial
model was unadjusted. Model 2 was adjusted for age, race, highest education level completed (high school or less, some college but no degree, college degree or more), study site and body mass index (BMI). Model 3 was further adjusted for systolic blood pressure (continuous), antihypertensive medications (yes versus no), LV mass, heart rate, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), diabetes mellitus status (yes versus no), C-reactive protein (CRP), smoking (Current versus former and never), urine albumin-creatinine ratio, and eGFR\textsubscript{CKD-Epi}. P<0.05 was considered significant for all analyses including interaction terms. All statistical analyses were performed with SAS software, version 9.3 (SAS Institute, Cary, NC). We tested for the proportional hazards assumption that the effect of an explanatory variable on the hazard is constant in time using SAS and we found no evidence of departure from this assumption.

Results:

Among the 6549 participants (mean age 62±5.5 years), there were 53% women, 39% white, 12% Chinese, 27% black and 22% hispanics. The mean FGF-23 was 40±15 pg/ml. The mean eGFR was 81±18 mL/min/1.73m\textsuperscript{2}.

Compared to participants in the lowest FGF-23 quartile (<31 pg/ml), those in the highest quartile were older, had higher BMI, systolic blood pressure, lower eGFR, and more likely to be diabetic (Table 1).

During a mean follow up of 11.1±3 years and a median follow up of 12.1(IQR: 11.6-12.7) years, 227 participants had incident heart failure events. Among these, 125 were classified as HFrEF and 102 were classified as HFpEF. When divided by FGF-23
quartiles, Q1 participants had 16 HFrEF and 16 HFpEF events, Q2 participants had 30 HFrEF and 18 HFpEF events, Q3 participants had 37 HFrEF and 24 HFpEF events, and Q4 had 42 HFrEF and 44 HFpEF events.

We found that higher FGF-23 levels are significantly predictive of future HF events. In adjusted models, there was an estimated 26% higher risk of HF for every 20 pg/mL increase in FGF-23 (HR 1.26; 95% CI, 1.1-1.5, p value=0.003). Each doubling of FGF-23 levels was associated with 80% higher risk of incident HF (HR 1.8; 95% CI: 1.3-2.5, p value <0.001) (Table 2A). We found stronger associations between higher FGF-23 levels and HFpEF events (HR 2.4; 95% CI: 1.4-4, p value <0.001) compared to HFrEF events (HR 1.5; 95 CI 1-2.3, p value =0.06) for each doubling of FGF-23.

When comparing quartiles of FGF-23, there was a graded significant increase of the risk of incident HF, incident HFrEF and incident HFpEF. Participants in the highest FGF-23 quartile had higher risk of HF (HR 2.6; 95% CI; 1.5-4.7), HFrEF (HR 2.3; 95% CI 1-5), and HFpEF (HR 2.9; 95% CI: 1.2-7.1) compared to participants in the lowest quartile (reference) (Table 2B, Figure 1). We presented Kaplan-Meier curves with 95% Hall-Wellner Bands and log-rank test for each outcome in Figure 2, Figure 3A and Figure 3B.

Compared to women, men had -statistically but not clinically significant- higher FGF-23 concentration (mean FGF-23=40.6 in men compared to 39.6 in women). Because of the observed difference we repeated the analysis with gender specific FGF-23 quartiles with minimal cutpoints changes. However, the results were similar.
As we found differences between men and women in myocardial response to higher levels of FGF-23 (in the previous chapter), we repeated the analysis after stratification by gender. Men had 134 incident HF events with an estimated 60% higher risk with doubling FGF-23 levels (HR 1.6; 95%CI: 1, 2.5, p value=0.05). In men, we found significant associations between FGF-23 and each HF types in unadjusted models; however these associations became non-significant in fully adjusted models. Women had 93 total heart failure events with stronger associations between FGF-23 levels and incident HF (HR 2.7; 95% CI: 1.5-4.7, p value <0.001) compared to men (supplemental Table 1). This association was especially stronger for HFpEF (HR 5.2; 95% CI: 2.4-11.7, p value<0.001).

In sensitivity analyses we performed the analyses without adjusting for LV mass (Supplemental Table 2A), and again after adjusting for LVH detected by electrocardiogram (LVH-ECG) instead of LV mass (Supplemental Table 2B) and found similar results. In additional sensitivity analyses, we adjusted for 24-hour urine phosphate excretion, serum calcium level, serum 1-25 hydroxy vitamin D level, and serum Parathyroid hormone level and had similar findings.

**Discussion:**

In a middle-aged multi-ethnic population free of cardiovascular disease at baseline (MESA), we demonstrated that higher levels of FGF-23 were associated with higher incidence of both HFrEF and HFpEF. We also confirmed a previous study done with the same population but with smaller number of events (n=183) due to less follow up time, which demonstrated an estimated 19% greater risk of incident HF with each 20 pg/mL
increase in FGF-23 (HR 1.19; 95% CI, 1.03-1.37).\textsuperscript{19} We found similar results. In our linear model there was an estimated 80% higher risk of incident heart failure with doubling of FGF-23 levels (Table 2A), and an estimated 26% higher risk for every 20 pg/mL increase in FGF-23 (HR 1.26; 95% CI, 1.1-1.5, p value=0.003).

When comparing quartiles we found a graded increase in the risk of all types of HF. Participants in the highest quartile had at least double the risk of developing HFrEF and almost 3 times the risk of HFpEF.

We believe the findings of a strong association between FGF-23 with HF can be explained by the previous observations that found a strong association between FGF-23 with higher LV mass.\textsuperscript{18,19} However, the reasons why we found stronger associations between FGF-23 and HFpEF are uncertain. Several previous reports demonstrated that higher LV mass is associated with greater risk of HF events.\textsuperscript{24-26} In a study by Velagaleti et al, eccentric LVH was associated with higher risk of HFrEF, while concentric LVH was associated with higher risk of HFpEF.\textsuperscript{24} Furthermore, Seliger et al, demonstrated that LVH was associated with higher risk of both types of HF especially HFrEF. Although we adjusted for LV mass in our fully adjusted model, other pathological mechanisms such as myocardial fibrosis (as we demonstrated in the previous chapter) should be recognize as another potential step that lead to the development of both HF types.

When comparing risk of heart failure by gender, we found high association between FGF-23 levels and risk of incident HF in both men and women. The risk for incident HF was stronger in women than in men. In women, the higher risk of HF was especially because of strong association with HFpEF. However, our results were limited
with wide confidence intervals in these strata because of small number of events. Further studies are needed to make more accurate conclusions.

To our knowledge this is the first study comparing the associations of FGF-23 with HFrEF and HFpEF. Previous studies examined the association between FGF-23 with ejection fraction as a continuous variable. For example, Kestenbaum et al, found no significant relationship between FGF-23 and ejection fraction. In previous community based studies, FGF-23 was associated with reduced ejection fraction. However, these studies were in participants with chronic kidney disease, and in a population undergoing elective coronary angiography. Both of these populations have sicker patients with more cardiovascular risk factors.

Based on our findings we believe that higher levels of baseline FGF-23 are more predictive of future HFpEF events. This is important because a significant proportion of patients with chronic kidney disease suffer from HF especially HFpEF. For example, 50% of ESRD patients have heart failure and up to 85% have abnormal left ventricular structure and function. Since patients with CKD have increasing FGF-23 levels even at early stages, it is important to estimate the risk and understand the underlying mechanism in order to develop new preventive and therapeutic techniques.

Furthermore, our findings of gender differences in the risk of incidence HF with higher levels of FGF-23 confirm our previous observations of different gender-related myocardial reaction to higher FGF-23 levels.
Limitations:

We only included participants with known EF at the time of HF diagnosis, thus it is unknown whether the relationship would change had we known the EF of these participants. Also we are limited by a small number of events to compare both types of HF so we used definite and probable events which could lead to misclassification error. However, even with close number of events the association was stronger for HFpEF. We adjusted for left ventricular mass in our fully adjusted models. This adjustment led to significant reduction in sample size and loss of power with wider confidence intervals. However, we found similar results when adjusting for LVH detected by ECG (available for majority of participants). Nevertheless, left ventricular hypertrophy could be a mediator and a pathologic mechanism by which higher levels of FGF-23 lead to heart failure.\textsuperscript{18} Finally, because of the complexity of FGF-23 physiology, residual confounding cannot be excluded. For example, we did not adjust for 24-hour urine phosphate excretion, serum calcium level, vitamin D level, or parathyroid hormone. However, in sensitivity analyses, adjusting for these covariates didn’t change our results.

Conclusion:

In the Multi-Ethnic Study of Atherosclerosis, higher levels of FGF-23 were associated with increased risk of incident HFrEF and HFpEF. We observed stronger association with HFpEF. When comparing the risk of HF by gender, women had stronger risk for HF, especially HFpEF.
Table 1. Baseline Characteristics by Fibroblast Growth Factor-23 1 quartile

<table>
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<th>FGF-23 Quartiles</th>
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<td>overall</td>
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<td>No. of subjects</td>
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<td>61±10</td>
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<td>Gender</td>
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<tr>
<td>Female</td>
<td>3487(53%)</td>
<td>959(58%)</td>
</tr>
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<td>Male</td>
<td>3062(47%)</td>
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<td>White %</td>
<td>2544(39%)</td>
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<td>Black %</td>
<td>1779(27%)</td>
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<td>Hispanic %</td>
<td>1432(22%)</td>
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<td>1063(16%)</td>
<td>268(16%)</td>
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<td>College degree or more %</td>
<td>3116(48%)</td>
<td>732(45%)</td>
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<td>SBP, mm Hg</td>
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<td>HTN medication</td>
<td>2403(37%)</td>
<td>511(31%)</td>
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<td>Estimated GFR, mL/min per 1.73 m²</td>
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<td>LV mass (g)</td>
<td>120±29</td>
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Values are mean ±SD. SBP: Systolic blood pressure. Smoking: current and former versus never. HTN: Hypertension. GFR: glomerular filtration rate. LV mass: left ventricular mass.
Table 2A. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF

<table>
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<th></th>
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<th>Incident HFrEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
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<td>125</td>
<td>1.8 (1.3-2.5)</td>
<td>0.001</td>
<td>102</td>
<td>2.6 (1.8-3.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td>226</td>
<td>1.7 (1.3-2.2)</td>
<td>&lt;0.001</td>
<td>124</td>
<td>1.5 (1-2)</td>
<td>0.04</td>
<td>102</td>
<td>2 (1.4-2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td>138</td>
<td>1.8 (1.3-2.5)</td>
<td>&lt;0.001</td>
<td>84</td>
<td>1.5 (1-2.3)</td>
<td>0.06</td>
<td>54</td>
<td>2.4 (1.4-4)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Cox proportional models were used to calculate the hazards ratios of each unit increase in log2 FGF-23 for the development of incident heart failure, HFrEF and HFpEF. Log2 FGF-23 is interpreted as doubling of FGF-23. For example, for each doubling of FGF-23 there is an increase of 2.4 of the risk of HFpEF.

Model 1; unadjusted, Model 2; adjusted for age, gender, race/ethnicity, education, study site and BMI, Model 3 adjusted for model 2 and systolic blood pressure, antihypertensive medications, LV mass, heart rate, low density lipoprotein, high density lipoprotein, diabetes mellitus, smoking, C-reactive protein, urine albumin-creatinine ratio and eGFRCKD-EPI. P value <0.05 is considered significant.

Table 2B. Association of FGF-23 and incident heart failure, HFrEF and HFpEF

(Hazards ratios per FGF-23 quartiles)

<table>
<thead>
<tr>
<th>Fibroblast Growth Factor-23 Quartiles</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident heart failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (ref)</td>
<td>1.5 (0.96-2.4)</td>
<td>1.9 (1.2-2.9)</td>
<td>2.7 (1.8-4.1)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (ref)</td>
<td>1.3 (0.9-2.1)</td>
<td>1.5 (1.2-4)</td>
<td>1.9 (1.3-2.9)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0 (ref)</td>
<td>1.8 (1-3.3)</td>
<td>2.1 (1.2-3.8)</td>
<td>2.6 (1.5-4.7)</td>
</tr>
<tr>
<td>Incident HFrEF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (ref)</td>
<td>1.9 (1-3.4)</td>
<td>2.2 (1-2-4)</td>
<td>2.7 (1.5-4.8)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (ref)</td>
<td>1.7 (0.9-3.1)</td>
<td>1.9 (1-3.4)</td>
<td>2 (1.2-3.6)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0 (ref)</td>
<td>2 (0.9-4.4)</td>
<td>2.8 (1-3.5-9)</td>
<td>2.3 (1-5)</td>
</tr>
<tr>
<td>Incident HFpEF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0 (ref)</td>
<td>1.1 (0.6-2.2)</td>
<td>1.5 (0.8-2.8)</td>
<td>2.8 (1.6-5)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0 (ref)</td>
<td>1 (0.5-2)</td>
<td>1.1 (0.6-2.2)</td>
<td>1.8 (1-3.3)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0 (ref)</td>
<td>1.6 (0.6-4.1)</td>
<td>1.3 (0.5-3.3)</td>
<td>2.9 (1.2-7.1)</td>
</tr>
</tbody>
</table>

Cox proportional model was used to calculate the hazards ratios. Values presented are hazards ratios and (95% confidence intervals) for each quartile of FGF-23 compared to reference group (Q1). Model 1; unadjusted, Model 2; adjusted for age, gender, race/ethnicity, education, study site and BMI, Model 3 adjusted for model 2 and systolic blood pressure, antihypertensive medications, LV mass, heart rate, low density lipoprotein, high density lipoprotein, diabetes mellitus, smoking, C-reactive protein, urine albumin-creatinine ratio and eGFRCKD-EPI. P value <0.05 is considered significant.
Figure 1. Hazard ratios of the associations of FGF-23 quartiles with incident heart failure, HFrEF and HFpEF

![Hazard Ratios of Heart Failure](image1.png)

Figure 2. Higher FGF-23 levels are associated with increased risk of incident heart failure. Participants were stratified into quartiles of FGF-23. (Kaplan-Meier curve with 95% Hall-Wellner Bands and log-rank test). Notice the Y axis start from 90% survival, X axis is the time to heart failure event (days).

![Kaplan-Meier Curve](image2.png)
Figure 3A. Higher FGF-23 levels are associated with increased risk of incident HFrEF. Participants were stratified into quartiles of FGF-23. (Kaplan-Meier curve with 95% Hall-Wellner Bands and log-rank test).

Figure 3B. Higher FGF-23 levels are associated with increased risk of incident HFpEF. Participants were stratified into quartiles of FGF-23. (Kaplan-Meier curve with 95% Hall-Wellner Bands and log-rank test).
## SUPPLEMENTAL MATERIAL

Supplemental Table 1. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Incident heart failure events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Incident HFrEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Incident HFpEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>134</td>
<td>1.9 (1.4-2.7)</td>
<td>&lt;0.001</td>
<td>86</td>
<td>1.8 (1.1-2.7)</td>
<td>0.01</td>
<td>48</td>
<td>2.3 (1.3-4)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>133</td>
<td>1.6 (1.1-2.2)</td>
<td>0.008</td>
<td>85</td>
<td>1.5 (1.2-3)</td>
<td>0.054</td>
<td>48</td>
<td>1.8 (1-3.2)</td>
<td>0.056</td>
</tr>
<tr>
<td>Model 3</td>
<td>86</td>
<td>1.6 (1-2.5)</td>
<td>0.05</td>
<td>59</td>
<td>1.6 (0.9-2.7)</td>
<td>0.1</td>
<td>27</td>
<td>1.7 (0.7-3.9)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>93</td>
<td>2.3 (1.6-3.2)</td>
<td>&lt;0.001</td>
<td>39</td>
<td>1.7 (0.9-3)</td>
<td>0.09</td>
<td>54</td>
<td>2.8 (1.8-4.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>93</td>
<td>1.8 (1.2-2.6)</td>
<td>&lt;0.001</td>
<td>39</td>
<td>1.3 (0.7-2.5)</td>
<td>0.4</td>
<td>54</td>
<td>2.2 (1.3-3.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 3</td>
<td>52</td>
<td>2.7 (1.5-4.7)</td>
<td>&lt;0.001</td>
<td>25</td>
<td>1.7 (0.7-4.2)</td>
<td>0.24</td>
<td>27</td>
<td>5.2 (2.4-11.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Cox proportional models were used to calculate the hazards ratios of each unit increase in log₂ FGF-23 for the development of incident heart failure, HFrEF and HFpEF. Log₂ FGF-23 is interpreted as doubling of FGF-23. For example, for each doubling of FGF-23 there is an increase of 5.2 of the risk of HFpEF in women. Model 1: unadjusted, Model 2: adjusted for age, race/ethnicity, education, study site and BMI, Model 3 adjusted for model 2 and systolic blood pressure, antihypertensive medications, LV mass, heart rate, low density lipoprotein, high density lipoprotein, diabetes mellitus, smoking, C-reactive protein, urine albumin-creatinine ratio and eGFR\textsubscript{CKD-EPI}. P value <0.05 is considered significant.

Supplemental Table 2A. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF overall and stratified by gender without adjustment for LV mass

<table>
<thead>
<tr>
<th>Model 3</th>
<th>Incident heart failure events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Incident HFrEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Incident HFpEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>217</td>
<td>1.6 (1.2-2.1)</td>
<td>&lt;0.001</td>
<td>121</td>
<td>1.3 (0.9-1.9)</td>
<td>0.13</td>
<td>96</td>
<td>2.1 (1.4-3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>129</td>
<td>1.5 (1-2.1)</td>
<td>0.03</td>
<td>82</td>
<td>1.5 (0.8-2.3)</td>
<td>0.11</td>
<td>47</td>
<td>1.5 (0.8-2.8)</td>
<td>0.17</td>
</tr>
<tr>
<td>Women</td>
<td>88</td>
<td>1.9 (1-3)</td>
<td>0.003</td>
<td>39</td>
<td>1.2 (0.6-2.2)</td>
<td>0.7</td>
<td>49</td>
<td>2.9 (1.6-5.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Cox proportional models were used to calculate the hazards ratios of each unit increase in log₂ FGF-23 for the development of incident heart failure, HFrEF and HFpEF. Log₂ FGF-23 is interpreted as doubling of FGF-23. For example, for each doubling of FGF-23 there is an increase of 2.9 of the risk of HFpEF in women. Model 3 adjusted for age, race/ethnicity, education, study site, BMI, systolic blood pressure, antihypertensive medications, heart rate, low density lipoprotein, high density lipoprotein, diabetes mellitus, smoking, C-reactive protein, urine albumin-creatinine ratio and eGFR\textsubscript{CKD-EPI}. P value <0.05 is considered significant.
Supplemental Table 2B. Associations of FGF-23 and incident heart failure, HFrEF and HFpEF overall and stratified by gender with adjustment for LVH-ECG

<table>
<thead>
<tr>
<th>Model 3</th>
<th>Incident heart failure events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Incident HFrEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
<th>Incident HFpEF events</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>217</td>
<td>1.6 (1.2-2.1)</td>
<td>&lt;0.001</td>
<td>121</td>
<td>1.3 (0.9-1.8)</td>
<td>0.17</td>
<td>96</td>
<td>2.1 (1.4-3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Men</td>
<td>129</td>
<td>1.5 (1-2.1)</td>
<td>0.045</td>
<td>82</td>
<td>1.4 (0.9-2.2)</td>
<td>0.13</td>
<td>47</td>
<td>1.5 (0.8-2.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Women</td>
<td>88</td>
<td>1.9 (1.3-3)</td>
<td>0.003</td>
<td>39</td>
<td>1.1 (0.6-2.2)</td>
<td>0.7</td>
<td>49</td>
<td>2.9 (1.6-5.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Cox proportional models were used to calculate the hazards ratios of each unit increase in log2 FGF-23 for the development of incident heart failure, HFrEF and HFpEF. Log2 FGF-23 is interpreted as doubling of FGF-23. For example, for each doubling of FGF-23 there is an increase of 2.9 of the risk of HFpEF in women. Model 3 adjusted for age, race/ethnicity, education, study site, BMI, systolic blood pressure, antihypertensive medications, heart rate, LVH-ECG (left ventricular hypertrophy detected by electrocardiogram), low density lipoprotein, high density lipoprotein, diabetes mellitus, smoking, C-reactive protein, urine albumin-creatinine ratio and eGFR<sub>CKD-EPI</sub>. P value <0.05 is considered significant.
4. McMurray JJ, Adamopoulos S, Anker SD, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. European journal of heart failure 2012;14:803-69.
CURRICULUM VITAE

Date Prepared: 04/01/2016

Name: Mohamed Faher Almahmoud

Office Address:
Medical Center Boulevard
Section on Hospital Medicine
Winston Salem, NC 27157-1021

Home Address:
6164 Chamberlain PL, Apt 301
Winston Salem, NC 27103

Work Phone: 336.716.2255

Cell Phone: 201-310-8484

Work Email: malmahmo@wakehealth.edu

Work FAX: 336.716.3202

Place of Birth: Aleppo, Syria

Education
2010 M.D. Medicine University of Aleppo

2014 Residency, Internal Medicine at St. John Hospital/Wayne State University

Professional Societies
2015 American Heart Association Member
2015 American College of Cardiology Member
2003 Syrian students union organization Member
2010 Syrian Ministry of Physicians Member
2011 American College of Physicians Member
**Honors and Prizes**

2013  
**Poster Presentation**  
American College of Physicians  
2nd place poster presentation at the associate day conference of ACP-Michigan chapter

2014  
Outstanding Resident Research Award, St. John Hospital Medical Center

**Report of Local Teaching and Training**

**Teaching of Students in Courses**

2011-2013  
Instructor of Internal Medicine  
Wayne State University Hospital

2014-2015  
Instructor of Internal Medicine  
Wake Forest Baptist Medical Center

2014-2016  
Cardiovascular research fellowship  
Wake Forest University

**Report of Regional, National and International Invited Teaching and Presentations**

**Invited Presentations and Courses**

**Regional**

2012  
Propionibacterium acne cerebral abscess following craniotomy  
Almahmoud, Mohamed Faher., Anilrudh, Venugopal., Leonard, Johnson.  
Oral presentation at the American College of Physicians-Michigan chapter associate meeting

2012  
Outcomes of correcting hyponatremia in patients with myocardial infarction  
Almahmoud, Mohamed Faher., Qureshi, Waqas., Alirhayim, Zaid., Khalid, Fatima  
Research presentation at Michigan state medical society annual scientific meeting

2013  
H1N1-induced encephalitis in an adult patient  
Poster presentation at the American College of Physicians-Michigan associate meeting

2013  
Isolated Mycobacterium kansasii Cellulitis and Osteomyelitis in an Immunocompetent Patient  
Poster presentation at the American College of Physicians-Michigan associate meeting
2013  Seizure induced stress cardiomyopathy in an epileptic patient
Almahmoud, Mohamed Faher., Qaqish, Nicole., Michael, Frederick.
Poster presentation at the American College of Physicians-Michigan
chapter annual scientific meeting

2013  Failure Rates After Arteriovenous Fistula Creations
Poster abstract presentation at the American College of Physicians-
Michigan chapter annual scientific meeting

2014  Outcomes of Laser-Assisted Balloon Angioplasty versus Balloon
Angioplasty Alone for Below Knee Peripheral Arterial Disease
Piyaskulkaew, Chatchawan., Parvataneni, Kesav., Ballout, Hussien.,
Sharma, Tarun., Almahmoud, Mohamed., Ketron, Lowell., Szpunar, Susan
., LaLonde, Thomas, Mehta, Rajendra., Yamasaki, Hiroshii.
Oral presentation at the American College of Physicians-Michigan
associate meeting

2014  Myxedema Coma in a Patient with End Stage Renal Disease on Thyroid
Replacement Therapy
Almahmoud, Mohamed Faher., Alamiri, Khaled., Russel, Sherily.
Poster presentation at the American College of Physicians-Michigan
associate meeting

2014  Moyamoya Syndrome in a 28-year old female with Systemic Lupus
Erythematous
Hassanali, Alia., Almahmoud, Mohamed Faher., Qaqish, Nicole.. 
Poster presentation at the American College of Physicians-Michigan
associate meeting

2014  Implications of Transesophageal Echocardiography on the Diagnosis and
Management of Implantable Cardiac Device Infections
Almahmoud, Mohamed Faher., Fishbain, Joel., Othman, Hussein.,
Sabbagh, Salah., Szpunar, Susan
Poster Presentation at St.John hospital Annual research day

National

2012  Hemophagocytic lymphohistocytosis syndrome
Almahmoud, Mohamed Faher., Andreeff, Michael.
Oral presentation at the weekly meeting of the leukemia
department at MD Anderson Hospital, University of Texas
in Houston, Texas
<table>
<thead>
<tr>
<th>Year</th>
<th>Title</th>
<th>Authors</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>The Role of Transesophageal Echocardiography in Extraction of Infected Cardiac Implantable Electrophysiological Devices</td>
<td>Almahmoud, Mohamed Faher., Fishbain, Joel., Othman, Hussein., Sabbagh, Salah., Szpunar, Susan..</td>
<td>American College of Cardiology 64th Annual Scientific Session and TCT@ACC-i2, San Diego, CA, USA</td>
</tr>
</tbody>
</table>
2016  Fibroblast Growth Factor-23 and Cardiac MRI indices of Myocardial Fibrosis in the Multi-Ethnic Study of Atherosclerosis
   American College of Cardiology 65th Annual scientific session & Expo, 2016, Chicago, IL, USA

International
2008  Updates in Rheumatoid Arthritis management
   Almahmoud, Mohamed Faher.
   Oral presentation at the University of Aleppo 50th Anniversary Meeting.

Report of Clinical Activities and Innovations

Current Licensure and Certification

2010  Educational Commission for Foreign Medical Graduates
2014  Controlled Substance Registration Certificate
2014  North Carolina Medical Board, Physician License Certificate
2014  Council on Epidemiology and Prevention, Participation as a Fellow in the 40th Ten-Day Seminar on the Epidemiology and Prevention of Cardiovascular Disease
   July 28-August 8, 2014
2015  American Heart Association Basic Life Support (BLS) for healthcare providers (CPR and AED) program.
   May 20th, 2017
2015  American Heart Association Advanced Cardiovascular Life Support (ACLS) program.
   May 20th, 2017
2015  West Virginia Medical Board, Physician License Certificate
Practice Activities

2014  Cardiovascular Research Fellow at Wake Forest University, School of Medicine

2014  Master of Science in Clinical and Population Translational Sciences at Wake Forest University, School of Medicine

2014  Instructor of Internal Medicine at Wake Forest Baptist Hospital

Report of Education of Patients and Service to the Community Activities

2012  Diabetic Educations at St. John Hospital Diabetes Clinic
Role: educating patients about current management of diabetes

2015  Triad Health free community clinic
Role: volunteering primary physician

Report of Scholarship Publications

1- Resting heart rate and incident atrial fibrillation in the elderly
O’neal, Wesley T., Almahmoud, Mohamed Faher., Soliman, Elsayed Z...

2- The Role of Transesophageal Echocardiography in Extraction of Infected Cardiac Implantable Electrophysiological Devices
Almahmoud, Mohamed Faher., Fishbain, Joel., Othman, Hussein., Sabbagh, Salah., Szpunar, Susan.

3- Outcomes of Laser-Assisted Balloon Angioplasty versus Balloon Angioplasty Alone for Below Knee Peripheral Arterial Disease
4- Electrocardiographic and Echocardiographic Left Ventricular Hypertrophy in the Prediction of Stroke in the Elderly

5- Electrocardiographic versus Echocardiographic Left Ventricular Hypertrophy in Prediction of Congestive Heart Failure in the Elderly
Almahmoud, Mohamed Faher., O’neal, Wesley T., Qureshi, Waqas T., Soliman, Elsayed Z..

6- Outcomes of Correcting Hyponatremia in Patients with Myocardial Infarction

7- Incidence of Arterial and Venous Thrombosis in Vonwillebrand Disease
322. Disorder of Coagulation or Fibrinolysis I, Blood (ASH annual Meeting Abstracts) 2012 120: Abstract 101

8- Laser in infrapopliteal and popliteal stenosis 2 study (LIPS2): Long-term outcomes of laser-assisted balloon angioplasty versus balloon angioplasty for below knee peripheral arterial disease

9- Left Ventricular Hypertrophy by Electrocardiogram versus Cardiac Magnetic Resonance Imaging as a Predictor for Heart Failure: The MESA study

Moderated poster presentation
American Heart Association, Epi/lifestyle Scientific Sessions, Phoenix, AZ, USA
Circulation.2016;133: AMP31

10- Assisted Reproductive Techniques
Book, Aleppo Publications, Pub Status: Published.

Professional educational materials or reports, in print or other media

1- Heparin Induced Thrombocytopenia in Patients with Cancer
Clinical Research

2- The Role of Transesophageal Echocardiography in Extraction of Infected Cardiac Implantable Electrophysiological Devices
Clinical Research

3- Failure rates after Arteriovenous Fistula creations
Clinical Research

4- Changes in Plasma Volume as a Prognostic Marker in Myocardial Infarction
Clinical Research

5- Manuscript Reviewer for Frontiers in Medicine Journal 2014:
Cardiovascular epidemiology

6- Fibroblast Growth Factor 23 and Myocardial Fibrosis in Multi-Ethnic Study of Atherosclerosis using Cardiac Magnetic Resonance T1-Mapping
Accepted proposal

7- Relationship Between Cardiac Structure, Function and Mechanics with Neurocognition in the Hisopanic Community Health Study/Study of Lations (SOL) Echocardiographic Study of Lations (Echo-SOL) Ancillary Study
Accepted proposal

8- Electrocardiographic versus Cardiac Magnetic Resonance Left Ventricular Hypertrophy in Prediction of Congestive Heart Failure in the Mutli-Ethnic Study of Atherosclerosis
Accepted proposal
9- I was an Ad hoc reviewer for Catheterization and Cardiovascular Interventions Journal. 2015

**Work Experience**

11/2012 - 12/2012  **Average Hours/Week: 60**

**MD Anderson Hospital/University of Texas, Texas**
Rotating Resident, with Dr. Michael Andreeff
I worked as a rotating resident at the leukemia department at MD Anderson Hospital. During this rotation, I had exposure to inpatient and outpatient clinical cases of leukemia and attended conferences and lectures about emerging researches in the field of oncology and leukemia at MD Anderson hospital; also I did a presentation about hemophagocytic lymphohistocytosis syndrome at the leukemia department.

03/2011 - 06/2011

**Al Fatih Hospital, Syrian Arab Republic**
ER Physician, with Dr. Mahmoud, Haj Mohamed
I worked in the ER Department as an assistant physician. During this period, I managed medical, surgical and pediatric emergencies.

12/2009 - 01/2010

**Suny Downstate University, New York**
Medical Student, with Dr. Nicholas Shorter
I rotated as a medical student in the Pediatric Surgery Department with Dr.Shorter. I helped in all pediatric operations and interventions at King's County Hospital and Downstate Hospital. This was an elective that I did during my medical education.

11/2009 - 12/2009

**Suny Downstate University, New York**
Medical Student, with Dr. JEFFREY WEISS
I rotated as a medical student in the department of Urology with Dr.Weiss. I participated in inpatient and outpatient urological services. I also helped in many different Urological operations and interventions.

10/2008 - 11/2008

**American University of Beirut, Lebanon**
Medical student, with Dr. Mahmoud Choucair
I rotated as a medical student elective at the department of Internal Medicine at the American University of Beirut Hospital, where I had exposure to inpatient and outpatient general medicine, cardiology and neurology patients.
Volunteer Experience
12/2010 - 01/2011
Wayne state University, Michigan
Rotating Physician, with Dr. Theodore Jones
I worked at Hutzel Women's Hospital in the department of OB/GYN as a rotating physician.
I rotated mainly in the Department of Labor and delivery, and also helped in the OB/GYN operations.

10/2014 - 01/2015
Wake Forest University School of Medicine
Participated in the WFU School of Medicine admission interviews.

Current/Prior Training
07/2015
Cardiovascular research fellowship, NIH T32 training grant (Mentor- David Herrington), Wake Forest School of Medicine, July 2015-June 2016

07/28-08/08, 2014
Council on Epidemiology and Prevention, Participation as a Fellow in the 40th Ten-Day Seminar on the Epidemiology and Prevention of Cardiovascular Disease
Lake Tahoe, CA, USA

07/2011 – 06/2014
Internal Medicine residency
St John Hospital and Medical Center Program, Grosse pointe Woods, Michigan
Internal Medicine
Raymond C Hilu
Rozzell, Donald

11/2012 - 12/2012
Internal Medicine residency
Md Anderson Cancer center/university of Texas, Houston, Texas
Internal Medicine
Philip R Orlander
Michael Andreeff

Other Awards/Accomplishments
1-Syrian Youth organization award and medal for first place in Arabesque art 1994
2-Aleppo Youth organization award and medal for first place in Arabesque and Arabic calligraphy 1994-1995-1996
3-University of Aleppo soccer championship 2006 second place
4-University of Aleppo soccer championship 2007 first place
5-University of Aleppo soccer championship 2008 second place
6-University of Aleppo soccer championship 2009 first place
7-Medical school soccer championship 2010 First place
8-Medical school soccer championship top scorer 2010
9-Top 10 Medical students honor