INTAKE OF OMEGA-3 POLYUNSATURATED FATTY ACIDS AND ASSOCIATIONS WITH CARDIOMETABOLIC RISK FACTORS

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Abstract

Background: The American Heart Association (AHA) and the American Diabetes Association (ADA) recommend at least two servings of oily fish a week to promote cardiovascular health. Oily fish is rich in the long-chain omega-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These, along with the vegetable-derived omega-3 PUFA alpha-linolenic acid (ALA), play major roles in normal physiological processes. The aim of this dissertation was to consider associations of fish, fish-derived omega-3 PUFAs DHA and EPA, and vegetable-derived omega-3 PUFA ALA with cardiovascular and glycemia outcomes, presented in three related manuscripts.

Methods: All analyses utilized data from the Atherosclerosis Risk in Communities (ARIC) Study, a multi-center prospective study designed to investigate the etiology and natural history of cardiovascular disease. There have been five visits: the baseline in 1987–89 (visit 1) and four follow-up visits in 1990–92, 1993–95, 1996–98 and 2011–13. Data from visits 1 through 4 were used in this dissertation. Dietary data were collected at visits 1 and 3 via food frequency questionnaire (FFQ). *Paper 1*: We studied the association of consumption of seafood, EPA, DHA, and ALA with fasting blood glucose (FBG) (n=13,173), HbA1c (n=11,575), and incident type 2 diabetes (T2D) (n=11,874). FBG and HbA1c were obtained using blood samples collected during study visits and diabetes status was identified through self-report and lab values. To estimate differences across exposure categories, linear regression was used for continuous

outcomes (FBG, HbA1c), adjusting for repeated measures as appropriate; Cox proportional hazards regression with time varying covariates was used for the incident T2D outcome. *Paper 2*: We studied the association of consumption of seafood, EPA, DHA, and ALA with J-point height and heart rate-corrected (QTc) interval (n = 12,611). QTc interval and J-point height were measured using ECGs obtained during study visits. To estimate differences across exposure categories, generalized estimating equations were used to estimate odds ratios of prolonged QTc and J-point elevation and differences in continuous measures of QTc interval and J-point height. *Paper 3*: One ARIC field center collected plasma biomarker values from participants at visit 1, and these data were used to augment self-report dietary data obtained via FFQ. We imputed biomarker values for other participants using multiple imputation for chained equations and investigated the associations of plasma phospholipid measures of ALA, DHA, and EPA with prolonged QTc, HbA1c, and incident T2D.

Results: *Paper 1*: In multivariable analyses, intake of seafood and DHA+EPA was favorably associated with FBG and HbA1c in non-diabetic participants, although the magnitude of the associations was small. ALA was not associated with FBG or HbA1c in non-diabetic participants. Among diabetic participants, intake of seafood, DHA+EPA, and ALA were adversely associated with FBG and HbA1c, with differential effects for seafood by sex and race. Finally, higher intake of ALA was associated with higher risk of incident T2D in normoglycemics, while seafood and DHA+EPA were not. *Paper 2*: Higher intakes of ALA+DHA+EPA and ALA were associated with a shorter QTc interval.

None of the exposures were associated with prolonged QTc, J-point elevation, or J-point height. *Paper 3*: In the full cohort (imputed) and the Minnesota (observed) populations, none of the exposures was significantly associated with prolonged QTc, HbA1c, or incident T2D. Point estimates in both populations were similar across different covariate adjustments, and confidence intervals were narrower in the full cohort population than in the observed plasma population.

Conclusions: Considering the dietary recommendations of the ADA and AHA, this dissertation examined the associations of dietary omega-3 PUFAs with cardiovascular and glycemia outcomes while also considering the implications of measurement error in the exposure of interest. Taken together, these results suggest that consumption of omega-3 PUFAs are not associated with certain cardiovascular outcomes in healthy individuals, and may be associated with deleterious glucose homeostasis in those with diabetes.

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1 BACKGROUND

1.1 INTRODUCTION

Cardiovascular disease (CVD) is a major public health problem. The American Heart Association (AHA) estimates that, in 2014, more than 30% of all deaths in the United States were due to CVD [1]. Similarly, diabetes, a major risk factor for CVD, is highly prevalent, with approximately 9% of the adult population being diagnosed with this condition [1]. Lifestyle factors constitute an important component of preventive strategies to improve cardiovascular health in the population – key among those factors is diet [1, 2].

Fish consumption has been frequently included in dietary guidelines for the prevention of CVD and its risk factors. Both the AHA and the American Diabetes Association (ADA) recommend at least two servings of oily fish a week to promote cardiovascular health [3, 4]. Oily fish is rich in the long-chain omega-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These, along with the vegetable-derived omega-3 PUFA alpha-linolenic acid (ALA), play major roles in normal physiological processes [5].

With respect to studies considering nutrient intakes and CVD outcomes, few nutrients have been studied more than omega-3 PUFAs [6]. Consumption of fish and the fish-derived omega-3 PUFAs DHA and EPA has been associated with lower risk of coronary heart disease (CHD) mortality [6-8], particularly sudden cardiac death (SCD) [6, 9]. Additionally, intake has been found to be associated with decreased levels of triglycerides in both non-diabetics [10-12] and persons with type 2 diabetes (T2D) [13-15] – a finding of great importance given the propensity for elevated triglycerides in those with T2D.

Even with all this evidence, the scientific picture is far from complete. For example, many different studies have evaluated the association between omega-3

PUFAs and measures of glucose metabolism, as a major cardiovascular risk factor, but their results have been mixed.

Additionally, there is scant literature regarding the mechanism by which omega-3 PUFAs could prevent SCD.

Thus, the existing recommendations from two large professional health associations to "eat more oily fish" and the existing gaps in the literature mentioned in the prior paragraph motivated the following three questions:

1. Given that the ADA recommends intake of oily fish, what is the association between dietary intake of DHA, EPA, and ALA and various measures of glucose metabolism?
2. Given that fish-derived omega-3 PUFAs are associated with lower incidence of SCD, can the association be elucidated by showing DHA, EPA and/or ALA are associated with electrocardiographic predictors of SCD?
3. Since assessment of dietary exposures is subject to measurement error, does calibrating reported omega-3 PUFA exposure values using measurement error correction techniques alter the associations observed in questions 1 and 2?

The rest of the background section will provide greater detail on the following: (1) the omega-3 PUFAs DHA, EPA, and ALA; (2) the physiological effects of omega-3 PUFAs; (3) description of disorders of glucose metabolism; (4) description of SCD and electrocardiogram (ECG) predictors of SCD; (6) literature on the relationship between omega-3 PUFAs and CVD – specifically glucose metabolism and ECG predictors of SCD; and (7) how dietary measurement error can affect measures of association for outcomes where dietary data are used as the exposures (e.g., omega-3 PUFAs).

1.2 TYPES OF OMEGA-3 POLYUNSATURATED FATTY ACIDS

The three types of long-chain omega-3 PUFAs most abundant in our diet and commonly described in epidemiological literature are ALA, EPA, and DHA. Those PUFAs along with the EPA metabolite docosapentaenoic acid (DPA) can be seen in Figure 1-1.

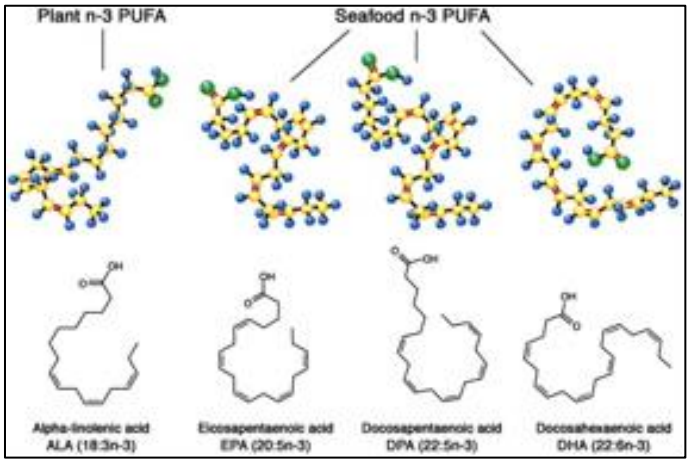

Figure 1-1. Structure of Omega-3 PUFAs. Adapted from Mozaffarian and Wu. [6]

ALA is a plant-derived omega-3 PUFA while DHA and EPA are fish-derived. ALA is an essential fatty acid – that is, the body cannot synthesize it – and dietary sources include flaxseed oil and walnuts. Biochemical pathways exist that allow for the conversion of ALA to EPA and ALA to DHA, however these metabolic conversions contribute very little to the body's concentration of these fatty acids [5]. Only 0.2-0.8% of ALA is converted to EPA and 0-4% of ALA is converted to DHA. Thus the primary dietary source of DHA and EPA are fish and shellfish [6]. Oily fish like sardines, swordfish, anchovies and salmon provide the richest concentration of DHA and EPA while white fish such as cod, haddock, tilapia, and catfish contain lower amounts of these fatty acids [6].

1.3 PHYSIOLOGICAL EFFECTS OF OMEGA-3 PUFAS

Figure 1-2 summarizes the effects of omega-3 PUFAs on various organs and tissues suggested through human (in vitro and in vivo) and animal research. The rest of this section will provide details on these effects. Greater detail regarding the biochemical mechanism for the potential effect of omega-3 PUFAs on glucose metabolism and SCD will be provided in Sections 3 and 4 (Manuscripts 1 and 2).

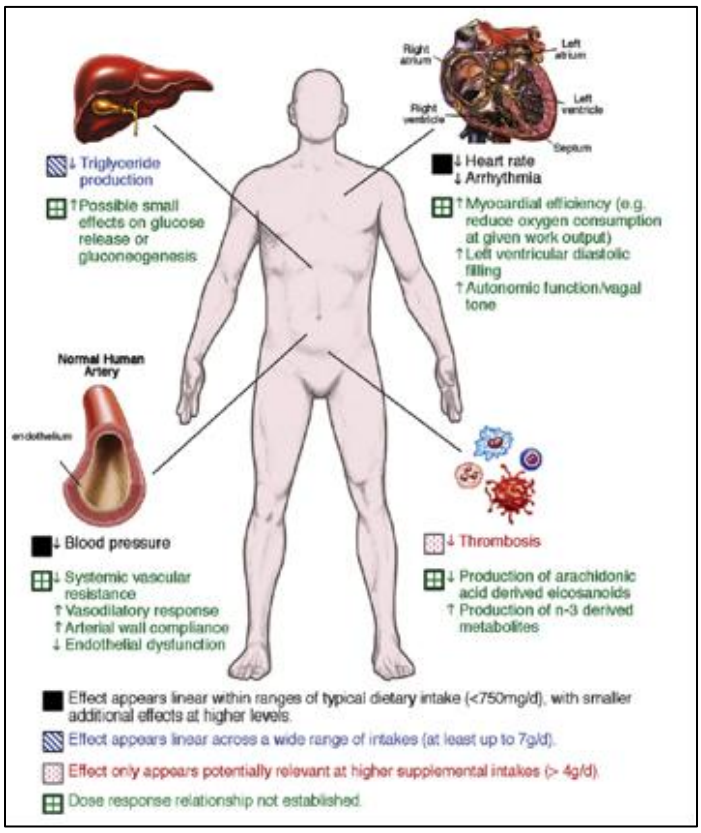

Figure 1-2. Physiological Effects of Omega-3 PUFAs. Adapted from Mozaffarian and Wu. [6]

1.3.1 OMEGA-3 PUFAS AND THE LIVER

Studies have suggested two effects of omega-3 PUFAs on the liver: (1) triglyceride lowering and (2) glucose release via gluconeogenesis. The triglyceride lowering properties of fish-derived omega-3 PUFAs has been well-established [16].

Studies have suggested that omega-3 PUFAs directly regulate hepatic genes decreasing de novo lipogenesis [16-21]. Results from randomized clinical trials (RCTs) and observational studies have shown that reductions are modest with typical dietary intake versus the more clinically significant impact of fish oil supplements [6].

This triglyceride lowering effect is hypothesized to affect gluconeogenesis as well. The carbohydrates no longer being used for triglyceride production could instead be used for glucose production which would raise fasting blood glucose (FBG) [6].

1.3.2 OMEGA-3 PUFAS AND THE HEART

Studies have also investigated the effect of omega-3 PUFAs on the heart. Results suggest that omega-3 PUFAs are associated with (1) heart rate; (2) arrhythmia risk; (3) myocardial efficiency; (4) left ventricular diastolic filling; and (5) autonomic function/vagal tone.

Omega-3 PUFA consumption is associated with reduced heart rate [22] and this reduction is hypothesized to be a result of several mechanisms including the effect of omega-3 PUFAs on cardiac electrophysiological pathways [6] such as the accumulation of DHA in myocardial cell membranes [23], slowing of the heart rate, shortening of the QT-interval, reduction of left ventricular systolic pressure, and prolongation of the electrocardiographic atrial-ventricular conduction time (P-R interval) [24].

It is these same pathways that are hypothesized to effect risk of arrhythmias, although studies in animals and humans have been inconsistent [6]. Animal experiments and human studies show that fish oil increased myocardial efficiency. Hearts from rats fed a diet high in omega-3 PUFAs did not need as much oxygen during high cardiac demand compared to rats fed other types of fat [25]. Fish oil supplementation in human cyclists reduced amount of oxygen needed during exercise [26]. Omega-3 PUFA consumption has also been shown to be associated with more

favorable measures of left ventricular cardiac filling [27-29], left ventricular ejection fraction [27, 30], and vagal tone [31].

1.3.3 Omega-3 PUFAs and Coagulation

Studies investigating the effect of omega-3 PUFAs on thrombolytic particles found no association with platelet aggregation or coagulation factors [12, 32-34] and no excess bleeding risk in RCTs investigating fish or fish oil supplementation – even in patients taking aspirin or warfarin [35-37]. There were associations, however, with decreased production of the pro-inflammatory, pro-thrombotic arachidonic acid-derived eicosanoids and increased production of potentially beneficial omega-3 PUFA metabolites such as plasma oxylipins [38].

1.3.4 Omega-3 PUFAs and Human Vasculature

Studies have shown that omega-3 PUFA consumption is associated with lower blood pressure [34, 39]. This may be due to improved flow-mediated arterial dilation [40-44], a marker of endothelial health. ALA may lower blood pressure through the creation of eicosanoids, leading to the production of prostaglandins and leukotrienes that can reduce vascular tone [45].

1.4 OMEGA-3 PUFAS AND CARDIOVASCULAR DISEASE

As mentioned above, both observational studies and RCTs have shown fish, fish oil supplements, and intake of ALA are associated with more favorable measures of some cardiovascular risk factors including lower plasma triglycerides [6, 16, 34], reduced heart rate and blood pressure [6, 34, 46], improved endothelial and autonomic functions [40-44, 47-49], improved cardiac filling [6, 27-29, 50], greater myocardial efficiency [6, 25, 26], reduced inflammation [6, 34], and anti-arrhythmic effects [6].

As for fatal endpoints, observational studies have demonstrated that higher consumption of fish, fish-derived omega-3 PUFAs, and ALA (compared to lower) is associated with lower CHD mortality [6-8], particularly SCD [6, 51, 52]. ALA has been shown to be associated with lower incidence of CHD and stroke [34]. In 2004, a meta-analysis of 11 published manuscripts utilizing 13 cohort studies found that eating fish at least once a week was associated with lower CHD mortality [7]. Additionally, a report from the US Physicians Health Study showed that a fish meal at least once per week was associated with lower incidence of SCD compared to men who consumed less than one fish meal a month [52].

1.4.1 Gaps in Data Regarding the Relationship of Omega-3 PUFAs and CVD

Evidence has been mixed regarding the association between omega-3 fatty acid consumption (ALA, DHA, and EPA) and insulin resistance and diabetes, which some consider CHD risk equivalents [53], although evidence has been mixed [54-56]. Section 3 (Manuscript 1) will provide greater detail.

1.5 CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF GLUCOSE METABOLISM AND TYPE 2 DIABETES

1.5.1 Measures of Glucose Metabolism

Glucose metabolism indicators can be measured in several ways. The following are measures that will be used in the proposed studies.

1.5.1.1 Glucose Concentration in Blood

Measuring glucose levels in the blood are done internationally in terms of the glucose molar concentration and are measured in mmol/L (millimoles per liter). In the United States, measurements are done using glucose concentration mass and are

measured in mg/dL (milligrams per deciliter). FBG is a measurement of blood glucose in plasma and is performed after the subject has been fasting, generally for 8-10 hours. In contrast, a random blood glucose measurement is a measurement of blood glucose in plasma and is performed at any time and not based on postprandial timeframes.

1.5.1.2 Hemoglobin A1c

Hemoglobin A1c (HbA1c) is a measurement of type A hemoglobin subtype and does not vary based on time since food was last consumed. Hemoglobin is found in red blood cells, and the cells' exposure to glucose in blood plasma causes the hemoglobin to glycate. The extent of the glycation gives an estimate of the average blood glucose levels over the past 6-8 weeks.

1.5.2 NORMAL GLUCOSE METABOLISM

Normal levels of blood glucose are between 70 and 110 mg/dL. When blood glucose is low the pancreas decreases its secretion of insulin and releases glucagon. Glucagon causes the liver to convert stored glycogen into glucose [57].

Postprandially, the digestive system converts food into glucose, amino acids, fatty acids, and other nutrients that are released into the bloodstream. This results in blood glucose levels that are higher than the typical range. Insulin is released allowing the glucose to leave the bloodstream and fuel the body's cells [57].

Glucose homeostasis is dependent on normal insulin production and normal insulin sensitivity. If the body does not make enough insulin then there are not sufficient amounts to get blood glucose into cells. But, even if insulin production is not compromised, if the body's cells are not sensitive to insulin (i.e., there is insulin resistance) the glucose still cannot be utilized by cells, sometimes even if higher than normal amounts of insulin are secreted. These metabolic defects result in elevated blood glucose. It is thought that nutrient overload (e.g., eating too much) can lead to insulin resistance [58].

1.5.3 Definition of Diabetes

The generic term “diabetes mellitus” encompasses a group of metabolic diseases characterized by hyperglycemia. This elevated glucose state can result from defects in insulin secretion, insulin action, or both [59].

Several pathogenic processes are involved in the development of diabetes. Type 1 diabetes mellitus (T1D) is characterized by autoimmune destruction of the beta-cells of the pancreas, which produce insulin, and generally onset is in childhood or adolescence, although it can manifest at any age [59]. T2D represents 90-95% of all cases of diabetes mellitus and characterized by insulin deficiency and/or insulin resistance [59].

Diagnosis with diabetes, as defined by ADA, requires one of the following four criteria to be met: fasting plasma (blood) glucose (FPG) ≥ 126 mg/l (7.0 mmol/L) after a minimum of eight hours with no caloric intake; symptoms of hyperglycemia and a casual (random) plasma glucose ≥ 200 mg/dL (11.1 mmol/L); or two-hour plasma glucose ≥ 200 mg/dl (11.1 mmol/L) during a 75-gram oral glucose tolerance test (OGTT) [60]. In 2015 the ADA added HbA1c criteria similar to those introduced in 2011 by the World Health Organization (WHO) where an HbA1c cut-point 6.5% was recommended [61]. The ADA noted that age, race, and other clinical factors should be considered before making an HbA1c-related diagnosis [60].

1.5.4 Descriptive and Clinical Epidemiology of Diabetes

1.5.4.1 Prevalence

According to the National Health and Nutrition Examination Survey (NHANES) data from 2011-2014, diabetes affects 31.0 million Americans – 9% of the U.S. Population. This number includes 23.4 million with diagnosed diabetes and 7.6 million with undiagnosed diabetes [59, 62].

Using data from NHANES and National Center for Health Statistics (NCHS) collected between 1984 and 2004, the total prevalence of diabetes is expected to more than double in the United States between 2005 and 2050 (from 5.6% to 12.0%) [1]. This increase will occur in all age, sex, and race/ ethnicity groups, but are projected to be largest in the oldest (e.g., a four-fold increase among those 75 years of age and older) [1]. The group with the largest increase in prevalence is projected to be blacks aged 75 or older – a 606% increase [1].

1.5.4.2 Incidence

In 2012 there were approximately 1.7 million Americans aged 20 and older that were newly diagnosed with diabetes [63]. Incidence varies by race, with rates higher in blacks (9.5%) than whites (6.3%) [1].

1.5.4.3 Mortality

Diabetes is the seventh leading cause of death in the United States [62]. Furthermore, the risk for death among people with diabetes is about twice that of people of similar age but without diabetes [62]. In 2014 there were 245,016 deaths in the United States where diabetes was the primary or secondary cause of death [1]. Death rates per 100,000 people were 23.4 for white males, 43.9 for black males, 14.6 for white females, and 34.0 for black females [1].

1.5.5 Populations at Increased Risk for Type 2 Diabetes

Prediabetes (Pre-T2D) is a condition in which individuals have abnormal glucose metabolism not yet severe enough to be classified as diabetes [1, 62]. An individual is considered prediabetic if at least one of the following are present: (1) impaired fasting glucose (IFG), (2) impaired glucose tolerance (IGT), or (3) elevated HbA1c.

IFG is a condition in which an individual's fasting (>8 hours) blood glucose value is greater than 100 mg/dl but less than 126 mg/dl. IGT is diagnosed when an individual

fails at least two OGTTs – i.e., the 2-hour blood glucose value is greater than 140 mg/dL (but below 200 mg/dl). Finally, Pre-T2D can be established using HbA1c values. An HbA1c value between 6 and 6.5% is considered prediabetic.

People with Pre-T2D have an increased risk of developing T2D, heart disease, and stroke [59, 62, 64] and approximately 33.9% percent of US adults (81.6 Million) have prediabetes [1]. NHANES data from 2005-2008 showed 35% of U.S. adults aged 20 years or older had Pre-T2D, with half of identified individuals aged 65 years or older [62].

These prediabetic populations are of interest as studying individuals at higher risk may provide insight into methods to delay or prevent transition to clinical diabetes and to better understand the pathophysiology of the disease.

1.5.6 OMEGA-3 PUFAS AND TYPE 2 DIABETES

Intake of the vegetable-derived omega-3 fatty acid ALA and the fish-derived omega-3 PUFAs DHA and EPA may also affect risk of T2D and markers of glucose homeostasis such as fasting blood glucose levels and HbA1c.

1.5.6.1 Biological Mechanisms

There are data to support the biological plausibility of fish-derived omega-3 PUFAs DHA and EPA affecting glucose homeostasis in those with T2D.

Omega-3 PUFA may favorably affect glucose homeostasis. A pro-inflammatory state interferes with insulin signaling and inhibits insulin action on adipocytes [58], Fish-derived omega-3 PUFAs have anti-inflammatory properties, and inhibit the production of pro-inflammatory cytokines [65] and increase insulin sensitivity [66]. Furthermore, omega-3 PUFAs are incorporated into cell membranes where they may modify the activities of membrane-associated enzymes and receptors [5] and increase cell-sensitivity to insulin [65].

There are also mechanisms through which omega-3 PUFA may adversely affect glucose homeostasis. A previous study showed that diabetics who took a fish-derived omega-3 PUFA supplement had lower glucose utilization (insulin sensitivity) and increased glucagon-stimulated C-peptide [67]. In another trial, diabetic subjects taking fish oil supplements showed reduced hepatic gluconeogenesis [68]. Obese subjects with T2D who took fish oil supplements had increased uptake and oxidation of non-esterified fatty acids in the liver [69]. In another study of obese subjects with T2D, fish oil supplementation increased glycerol gluconeogenesis, and the authors hypothesized it could cause the deterioration of glycemic control during long-term treatment with high doses of fish-oil supplements [69]. Finally, the consumption of seafood may affect glucose homeostasis via contaminants in seafood. Mouse models have shown that elevated blood mercury levels may interrupt insulin signaling pathways, and decrease plasma insulin and elevate blood glucose levels [70].

1.5.6.2 Prior Clinical and Epidemiologic Research

Contradictory epidemiologic and clinical data exist on the association of omega-3 PUFAs and the risk of diabetes and markers of glucose homeostasis. A Cochrane review considering fish oil supplement trials in diabetics showed that, overall, the omega-3 fatty acids did not affect FBG levels, HbA1c, or fasting insulin [14]. Fish oil trials in nondiabetic patients have been similarly null [71, 72]. In diabetics, a meta-analyses of fish oil supplementation studies found that supplementation was associated with more favorable – but non-significant – associations with HbA1c and insulin [73], although the AHA no longer recommends fish oil supplementation for diabetics to prevent cardiovascular disease [74].

Observational studies have provided inconsistent results. Fish and EPA/DHA intake have been positively associated with risk of T2D [75-78]; associated with a

reduction in risk of T2D [79, 80] and lower FBG [81]; or have had null associations [78, 82-84].

Recent reviews and meta-analyses have reported that dietary intake of fatty fish and the fish-derived omega-3 PUFAs DHA and EPA are associated with higher risk of T2D in American and European populations [85-88] but not Asians [86], but not lean fish or shellfish [86], or plasma measures of DHA and EPA [85].

Studies investigating intake of the vegetable-derived ALA have been similarly mixed. A meta-analysis showed lower risk of incident T2D with higher levels of intake [85] but a subsequent analysis two years later showed favorable associations in Asians only, although Americans and Europeans had a nonsignificant favorable trend [86].

Data from randomized and observational studies may conflict for several reasons: (1) study duration: trials were short term compared to observational trials and there may not been enough time for the effect of fish oil supplements to be fully realized; (2) dose: trials used DHA and EPA doses that far exceed typical dietary intake; (3) study populations: different study populations with different food preparation methods and cultural differences may not be comparable; and (4) different exposure levels: some studies had high levels of ALA, DHA, EPA intake (i.e., fish oil or flaxseed supplementation) while others only investigated typical dietary consumption.

1.6 CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF SUDDEN CARDIAC DEATH

1.6.1 DEFINITION OF SUDDEN CARDIAC DEATH

Definitions of SCD are varied and no agreement has been reached on an official definition [89].

Definitions differ on their inclusions of temporality, geography, disease attribution, whether the event was witnessed, age range, and whether sudden cardiac

arrest is included [89]. The most widely cited definition was published in 2001 and used U.S. vital statistics and death certificate data from 1989 to 1998 [89, 90]. SCD was defined as a witnessed or un-witnessed, cardiac disease-related death within one hour of symptom onset, taking place out of hospital or in the ER in individuals aged 35 years of age or more [89, 90]. The most recent definition in the published literature was published in 2008 and used data collected between May 1, 2006, and April 30, 2007 [89, 91]. That definition was very broad – it included sudden cardiac arrest, required the death be from a cardiac cause and occur out of hospital, but had no other restraints [89, 91]. The most common cause of SCD is a sustained ventricular tachyarrhythmia [92].

1.6.2 Descriptive Epidemiology

A systematic review in 2011 [89] found only six peer-reviewed articles regarding SCD incidence. The estimated U.S. annual incidence of SCD varied widely from 180,000 to >450,000 among those 6 studies. Differences were due to differences in data sources, year of data collection differences in SCD case definition and case ascertainment, and methods of extrapolating to the US population [89]. The most commonly cited source [90] standardized rates to the 2000 US population and found an estimated annual incidence of 456,076 cases of SCD – 63% of all cardiac deaths. The most recent source (2008) [91] in the review was also the most conservative, with an estimated annual incidence of 294,851 cases of SCD. A 2014 study cited in the AHA Heart Disease and Stroke Statistics Report [1] used data from the Oregon Sudden Unexpected Death Study and extrapolated a risk-adjusted incidence rate of 69 per 100,000 per year, or approximately 210,000 annual cases of SCD in the United States each year [93].

1.6.3 Modifiable Risk Factors

Studies have shown a strong concordance between risk factors for CHD and SCD, but no clear modifiable risk factors that are specific for SCD have been identified once CHD is established [92, 94, 95]. This is most likely due to risks for SCD are linked to risks

for CVD that create the structural damage linked to sustained arrhythmia [92]. In fact, the strongest predictor of SCD once CHD is established is the degree of cardiac damage sustained [92].

1.6.4 Electrocardiographic Predictors of Sudden Cardiac Death

The ECG is a graphic recording of the electrical activity of the heart, and it is a noninvasive and inexpensive test in the study of cardiac function. The ECG is useful to detect arrhythmias, conduction disturbances, and myocardial ischemia. Also, the ECG can provide information on susceptibility to SCD. The two ECG variables consistently associated with a higher risk of SCD are long QT interval [96, 97] and J-point elevation [98, 99] – both are important markers of abnormal ventricular repolarization. In an ECG, the QT interval represents electrical depolarization and repolarization of the left and right ventricles and the J-point – the junction of the QRS complex and the ST segment – marks the end of depolarization and the beginning of repolarization [100, 101].

1.6.4.1 Biological Plausibility

Studies suggest that fish-derived PUFAs could have anti-arrhythmic effects, thus reducing SCD risk. Specifically, fish-derived omega-3 PUFAs may inhibit the fast, voltage-dependent sodium current and the L-type calcium currents [102, 103] that allow pre-SCD arrhythmias to be sustained [46]. ALA may favorably influence arrhythmias through modification of the eicosanoid system or modulation of L-type calcium channels in the sarcolemma of cardiac myocytes [104].

1.6.4.2 Prior Clinical and Epidemiologic Research

There have been a limited number of studies evaluating whether omega-3 PUFAs are associated with repolarization abnormalities detected in the surface ECG – prolonged repolarization (prolonged QT interval) [104-107] and early repolarization (J-

point elevation, JPE) [104]. With respect to prolonged QT interval, intake of the fish-derived omega-3 PUFAs DHA and EPA have been shown to be associated with shorter QT intervals in Greek adults [107] and predominately white Americans aged >65 years [106]. A study of white, middle-aged American adults found higher intakes of the vegetable-derived omega-3 PUFA ALA were associated with lower risk of prolonged QT [104]. With respect to JPE, a study of Japanese men found that higher intake of the fish-derived omega-3 PUFA DHA and EPA attenuated the association between JPE and cardiac death [105]. To our knowledge, no studies have investigated the association of ALA with JPE.

Although omega-3 PUFAs are inversely associated with SCD, and prolonged QT interval and JPE are positively associated with SCD, further details regarding the association between omega-3 PUFAs, QT interval, and JPE in a biracial cohort of middle-aged American populations may help elucidate the mechanisms relating omega-3 fatty acid consumptions and SCD.

1.7 MEASUREMENT ERROR

One difficulty in investigating exposure-disease associations is measurement error. While epidemiologists' goal is to find the true association (causal effect) between exposure and outcome in a population of interest, they are limited to determining the association between measured exposure and outcome in a sample of the population of interest. Mis-measured – or unmeasured – covariates and confounders can distort the desired association further. This section will focus on measurement error issues unique to dietary exposures and covariates. It will start a brief description of measurement error of the exposure variable, consequences of measurement error with potential solutions, and recommendations.

1.7.1 TYPES OF MEASUREMENT ERROR

There are several ways in which dietary measurement error can occur including: (1) subject forgets actual consumption; (2) subject purposely misreports due to social desirability or other psychological factors; (3) subject accidentally misreports due to differences in serving size; and (4) a correctly reported food can be incorrectly translated into its nutrient components due to differences in food preparation [108, 109]. The type of measurement instrument (e.g., 24-hour dietary recall versus food frequency questionnaire (FFQ)) can also affect the frequency and magnitude of measurement error. Food records and 24-hour diet recalls are generally considered the gold standard for self-reported diet intake [109].

Measurement error can be classified as differential or non-differential. Differential measurement error of the exposure is one where the error in measuring the exposure is dependent on other variables in the analysis. A classic example is recall bias in case-control studies. Those with the disease are more likely to remember exposures of interest than those who do not have the disease. Non-differential classification bias of the exposure is measurement error that is not dependent on other study variables. An example is where a technician always rounds a patient's systolic blood pressure to the nearest 5 (e.g, 138 mg Hg becomes 140, 112 mm Hg becomes 100). If exposures are dichotomous and misclassification is perfectly non-differential and errors are independent then associations will be biased towards the null [110]. In cohort studies measurement error of the exposure is generally considered to be non-differential as exposure is measured before onset of disease [108] but errors are not independent as dietary measurement error generally has bias related to true intake with subjects with high intake under-reporting and subjects with low intake over-reporting [108]. This "flattened slope phenomenon" can bias results away from the null [111] but random variation usually overwhelms the flattened slope phenomenon resulting in an overall relative risk estimate biased towards the null [112].

1.7.2 Consequences of Measurement Error

There are three main consequences of exposure measurement error: biased measures of association, loss of power, and invalidation of statistical tests.

1.7.2.1 Biased Measures of Association

Section 1.7.1 mentioned non-differential measurement error with a dichotomous exposure and independent errors will bias towards the null and that over- and under-reporting of intake based on actual intake is generally not sufficient to alter that trend.

Two analytic approaches to decrease or eliminate the bias are (1) adjust for energy intake and (2) correct the measured exposure values. Adjusting for energy intake [109, 113] allows for exposures to be assessed as part of overall diet composition – that is, it addresses the problem that an individual may have consumed more fish than another individual simply because he is taller and has more muscle mass. This type of energy adjustment can improve attenuation [108] but there can still be significant bias towards the null. Another potential solution is use of regression calibration [108] or multiple imputation [114] to correct the mis-measured exposures using an appropriate reference instrument. Reference instruments need to have measurement errors that are not correlated with the original exposure measurements [112, 115], so 24-hour recalls and food diaries are imperfect solutions [108]. Other reference instrument options, however, are recovery biomarkers and concentration biomarkers [116]. These are objective measures of intake that indicate how much of the nutrient was absorbed (bioavailability), is may be a good measure of usual intake provided between-season variability in an individual's intake is not large [109]. Unfortunately biomarkers can be affected by potential confounders – and if these confounders are dietary in nature they may be subject to the measurement error inherent in self-report measures resulting in additional bias [109].

1.7.2.2 Loss of Power

All exposure measures are subject to random variation, and the greater the deviation from the true value the greater the loss of power [117]. There are two sample-size related techniques researchers use to increase power: (1) increase sample size of individual studies; and (2) increase sample size by performing meta-analyses.

The first technique researchers use to increase power is to increase sample size, oftentimes by establishing large cohort studies such as the Atherosclerosis Risk in Communities (ARIC) Study [118]. Unfortunately, increasing sample size may not be sufficient to completely address power lost through FFQ measurement error [108]. One study found that to maintain desired power calculated assuming no exposure measurement error, the measurement error inherent in a FFQ resulted in a needed sample size 25-100 times larger for a total energy exposure [108].

Another technique to increase power is to perform meta-analyses of several studies – generally observational – thus harnessing the power of each component study [108]. Unmeasured confounders, however, can distort results from observational studies and meta-analyses can generate very precise but equally distorted measures of association [119]. Additionally, heterogeneity of study methods can be an obstacle to meta-analyses [120].

1.7.2.3 Invalidation of Statistical Tests

If there is only one mis-measured dietary exposure in the disease model then, theoretically, the standard exposure-disease regression (null-hypothesis: no association) is statistically appropriate although measures of association may be attenuated [108]. In multivariable models with multiple (mis-measured) dietary exposures, the standard exposure-disease regression may no longer be statistically valid and the direction of the bias is unpredictable [108]. This phenomenon is due to residual confounding resulting from the correlated nature of the dietary variables [108].

1.7.2.4 Recommendations

At a minimum, most dietary exposure models should include total energy as a covariate [108, 113]. Another recommendation for addressing dietary measurement error is combining dietary data with biomarker data [108]. This technique not only reduces exposure measurement error [108, 121], but also allows for an increase in the power to detect measures of association [108, 114, 121]. This approach will be described further in Section 5 (Manuscript 3).

Another method for addressing dietary measurement error is regression calibration [122, 123] using data from a validation study [108]. Unfortunately the most common validated study instrument is a more detailed self-report (e.g., food diary, 24-hour recall) [108] – these instruments are limited as their measurement errors are correlated with true intake and the FFQ [112, 115]. An alternative is to use biomarker validation data as a reference instrument to compute attenuation and contamination factors [108, 123]. While some studies have shown that regression calibration with self-report validation data and regression calibration using biomarker data can yield similar results [108], this cannot be guaranteed. Finally, multiple imputation is an approach for calibrating mis-measured exposure data [114] and will be discussed in greater detail in Section 5 (Manuscript 3).

1.8 SUMMARY OF THE INTRODUCTION

In this section, we have described the public health importance of T2D and SCD in the United States. We reviewed biological and epidemiological evidence for long-chain omega-3 PUFAs, focusing on the potential association of long-chain omega-3 PUFA intake with glucose homeostasis and the risk of diabetes, with the risk of CVD and, particularly, SCD. Additionally, we provided an overview of the issues created by measurement error in nutritional epidemiology, which could influence the associations found when dietary omega-3 PUFAs are the exposure of interest. Overall, we highlight

the need for studies that clarify the impact of omega-3 PUFA intake on diabetes risk, that assess electrophysiological mechanisms responsible for the association between omega-3 PUFA intake and SCD, and that evaluate methods for correction of measurement error using biomarker data. In the next section, we provide a description of the Atherosclerosis Risk in Communities (ARIC) cohort, which we used to address the aims of this dissertation.

2 THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY – DATA AND DATA COLLECTION

2.1 STUDY OVERVIEW

This dissertation utilized data from the Atherosclerosis Risk in Communities (ARIC) Study. The three aims were to investigate (1) association of seafood and omega-3 PUFA intake with measures of glucose metabolism; (2) association of seafood and omega-3 PUFA intake with ECG predictors of SCD; and (3) if addressing potential measurement error in our dietary exposures modifies select outcomes from aims 1 and 2. In this section, we describe the ARIC study and how data were obtained for the exposures, outcomes, potential confounders, and other covariates of interest.

2.2 THE ATHEROSCLEROSIS RISK IN COMMUNITIES STUDY

The ARIC study is a multi-center prospective study designed to investigate the etiology and natural history of cardiovascular disease. The health of each participant – including a comprehensive physical exam, medical history, interview, and measurement of traditional and novel risk factors for CVD, diabetes, and other important health outcomes – was assessed at baseline and during follow-up exams using standardized protocols. The study design and methods have been described previously [118]. This section summarizes the study population and timeline.

ARIC is a cohort of 15,792 subjects from four communities. ARIC participants were chosen via probability sampling from four economically and socially diverse US communities: Forsyth county, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland. The Jackson sample includes African Americans only. The other field center samples are representative of the populations in their respective communities: mostly white in suburban Minneapolis and Washington County, white and African American in Forsyth county. There were

15,792 subjects (8,710 women, 7,082 men; 11,478 whites and 4,314 nonwhites) aged 45-64 at baseline (visit 1).

There have been five visits, although this dissertation only utilizes data from visits 1 through 4. The first four visits were approximately 3 years apart: visit 1 (1987-89), visit 2 (1990-92), visit 3 (1993-95), and visit 4 (1996-98); visit 5 was fifteen years later: 2011-13. Baseline response rates were 46% of the target population in Jackson and 65% of the target population in the other communities. Additionally, survivor retention proportions were 93% for visit 2, 86% for visit 3, 80% for visit 4, and 65% for visit 5. Participants were contacted yearly by phone – twice a year starting in 2012 – to obtain information about hospital admissions and ascertain vital status.

The same data were not collected at every visit. The details of the data collection procedures are covered in the next section, focusing on exposures, outcomes, potential confounders, and other covariates of interest.

2.3 EXPOSURE VARIABLE MEASUREMENT

For our exposures, we focused on dietary consumption of fish and shellfish, the fish-derived omega-3 fatty acids DHA and EPA, and the vegetable-derived omega-3 PUFA ALA. Biomarker values for circulating concentrations of DHA, EPA, and ALA were available for a subset of participants. Detailed descriptions appear below.

2.3.1 THE FOOD FREQUENCY QUESTIONNAIRE

Participants' usual dietary intake was assessed by using an interviewer-administered, 66-item FFQ administered to all subjects at visit 1 (1987-1989) and visit 3 (1993-1991). The FFQ was based on the 61-item instrument developed by Willett et al. [124]. Three modifications were made for the ARIC version: (1) separation of some items into detailed subcategories (notably, the one question on fish intake was broken down into three fish categories of dark meat fish, other fish, and shellfish); (2) addition

of several food items such as biscuits and donuts; (3) detailed questions were added to assess the consumption of beer, wine, and hard liquor.

The ARIC questionnaire was also validated in a sample (n=419) of black and white ARIC participants who repeated the FFQ after three years [125]. The study found that, after adjusting for total caloric intake, the median reliability coefficient for blacks was 0.42 and the reliability for white ARIC participants was 0.49 – a value similar to that of other studies of white subjects. The study found no difference in the median reliability coefficients of men and women after adjusting for total calorie intake.

Another study investigated the validity of the ARIC FFQ by comparing Minnesota field center participants' dietary fat FFQ data against their plasma fatty acid concentrations [126]. Plasma measures reflect the types of fats proportionally consumed over the past several weeks to months [127] and the proportionate composition in plasma was moderately correlated with dietary intake, with highest correlations in the fish-derived omega-3 fatty acids DHA and EPA (r=0.42 and r=0.20 for plasma phospholipid measures of DHA and EPA, respectively) [126].

2.3.1.1 Fish/Shellfish Servings

Fish and other seafood intake was assessed through four FFQ questions with nine response categories. The questions asked how often they consumed: 3–4 ounces of canned tuna fish; 3–5 ounces of dark meat fish such as salmon, mackerel, swordfish, sardines, and bluefish; 3–5 ounces of other fish such as cod, perch, catfish, etc.; and shrimp, lobster, scallops as a main dish. Interviewers used food models to help participants with portion size estimation. Subjects could provide answers to each question ranging from ''never or less than once per month'' to ''6 times per day.''

Applying the same methodology used in another study of seafood intake in the ARIC cohort [128], each of the participants' seafood-related FFQ responses were grouped into three exposure categories – (1) omega-3 rich fish (tuna + dark); (2) total

fish (tuna + dark+ other); and (3) total seafood (tuna + dark + other + shellfish) – and further translated into four weekly serving categories: none, less than one, one to two, and more than two.

2.3.1.2 Intake of ALA, DHA, and EPA

Data from the FFQ were coded for nutrients and food groups by Harvard University. Nutrient values for each food were obtained from the Harvard database [124], which was predominately based on the US Department of Agriculture publications [129]. Daily intake of nutrients was calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and then summing the results. This calculation yielded consumption of ALA, EPA, and DHA in grams/day. Three different classifications of omega-3 fatty acids were investigated: (1) vegetable-derived ALA, (2) fish-derived DHA+EPA; and (3) ALA+DHA+EPA.

2.3.2 Biomarker Measures of ALA, DHA, and EPA

Blood samples were obtained from Minnesota field participants at visit 1 (n= 3,757) and plasma fatty acids were measured in cholesterol esters and phospholipids using gas chromatography [126]. Wang et al. contains a very detailed description of the chemical processes used to extract and measure the plasma fatty acids [83]. Since cholesterol esters reflect medium-term dietary intake of fatty acids (weeks) and phospholipids reflect intake over a slightly longer duration (weeks to months) [127], we used phospholipid measurements in our analyses. Circulating concentrations of individual fatty acids were expressed as a percentage of total fatty acids – we investigated concentrations of ALA, DHA+EPA, and ALA+DHA+EPA grouped into quartiles.

It should be noted that there are certain instances where plasma measures may not perfectly reflect dietary intake. Things to consider are: (1) sensitivity to intake (do

plasma measures change with changes in diet); (2) if the body attempts to maintain plasma fatty acid homeostasis; (3) if the measure is sensitive to consumption; and (4) usual intake over a year versus the seasonality of plasma levels [109]. Thus, when using plasma measures of omega-3 PUFAs as an exposure, the association may not describe usual dietary intake but bioavailability.

Keeping this in mind, previous analyses have shown that plasma measurements of omega-3 PUFAs correlate with dietary intake (as measured via a FFQ) in ARIC [126] and similar heterogeneous European populations [130]. However, as previously mentioned, plasma measurements of fatty acids are not a perfect measurement of dietary intake as many factors (e.g., alcohol intake, obesity, chronic diseases) affect fatty acid metabolism [126].

2.4 OUTCOME MEASURES

We had two main classifications of outcome variables: glycemia and ECG markers of ventricular repolarization associated with SCD. In these next sections, we describe how outcome data were obtained.

2.4.1 MEASURES OF GLYCEMIA

Three measures of glycemia were used in this study: FBG, HbA1c, and incident T2D. Detailed descriptions of blood draw [131] and chemistry analyses [132] can be found in the ARIC Manual of Operations.

2.4.1.1 Fasting Blood Glucose

Data on FBG were obtained during visits 1-4. Fasting blood samples were drawn from an antecubital vein with minimal trauma. FBG was then measured by a hexokinase method on a Coulter DACOS (Coulter Instruments). In a small validation study blood samples from volunteers (n=40) were taken two weeks apart. For serum glucose the intraclass correlation was 0.84, the within-person coefficient of variation (CV) was 4% , and the laboratory CV was 2% [133].

2.4.1.2 Hemoglobin A1C

HbA1c was measured from whole blood samples using high-performance liquid chromatography. HbA1c values capture the average blood glucose concentration over approximately the past three months. The blood was collected during visit 2 (1990-92) and stored at -70 Cº for 14-18 years until HbA1c measurements could be obtained. Selvin et al. give a detailed description of the measurement process [134].

Briefly, measurements were obtained in the same laboratory during two separate time periods using two different instruments: n=4,918 subjects in 2003-04 using Tosoh 2.2 Plus HPLC (Tosoh Bioscience, South San Francisco, CA) and n=9,151 subjects in 2007-08 using Tosoh G7 HPLC. Both instruments were certified by the National Glycohemoglobin Standardization Program (NGSP). This calibration provides stable results despite changes in HbA1c methodologies over time.

A convenience sample (n=383) was analyzed using both instruments. Pearson's correlation coefficient between the two samples was high (r=0.99) but there was a slight bias with 2007-08 values showing a 0.29 higher %HbA1c ($p<0.0001$). The intraclass correlation coefficient was 0.99 (95% CI: 0.97-0.99) and within-sample CV of 3.9% (95% CI: 3.6% to 4.2%). CVs of 5% or less generally reflect good method performance.

2.4.1.3 Incident T2D

Diabetes status was defined based on information collected at visits 1-4. Specifically, diabetes was defined as (1) self-report of physician-diagnosed diabetes; (2) self-reported use of diabetes medication in the past two weeks; (3) fasting glucose level ≥ 7.0 mmol/liter (126 mg/dl); or (4) non-fasting glucose level > 11.1 mmol/liter (200 mg/dl). For incident T2D, prevalent cases will be excluded at visit 1 and cases newly identified at subsequent visits will be considered incident.

Although this methodology does not distinguish between type 1 diabetes (T1D) and T2D, T2D is the most commonly occurring variant of diabetes and rarely occurred before age 30 in individuals who were middle-aged in the 1980's [135]. Other

researchers have defined T1D in ARIC as diabetic subjects with age of onset before age 30 [136].

2.4.2 ECG Predictors of Sudden Cardiac Death

For each visit in ARIC, a standard, resting, supine 12-lead ECG was obtained for each subject a minimum of 1 h after any smoking or caffeine ingestion using MAC PC personal cardiography equipment (Marquette Electronics, Inc., Milwaukee, WI). An electrode locator was used to determine and standardize the positioning of chest electrodes. Tracings were sent to be computer coded at the ARIC ECG Reading Center. All records with significant Minnesota Code [137] findings as determined by the computer, as well as a random sample of tracings, were sent to the ECG coding center to be visually coded. Discrepancies between the computer code and visual code were adjudicated by a senior coder. Subsequent processing of the ECGs took place at EPICARE (Epidemiological Cardiology Research Center at Wake Forest University, Winston-Salem, NC, USA). We considered four ECG-derived outcomes: QT interval, prolonged QT, J-point height, and JPE.

2.4.2.1 QT Interval and Prolonged QT

The QT interval represents electrical depolarization and repolarization of the ventricles. A participant's visit 1 QT interval was derived by the Dalhousie ECG analysis program using the digital 12-lead ECG. Subsequent visits used the GE Marquette 12-SL analysis program, which generated an average waveform derived from all 12 simultaneously measured leads.

We used a heart rate-corrected QT interval (QTc) – as recommended by the AHA, the American College of Cardiology, and the Heart Rhythm Society for the Standardization and Interpretation of the Electrocardiogram [138]. The most appropriate formula for correction is the one resulting in the least amount of correlation

between heart rate and the calculated rate-corrected QT [139]. We tested Framingham [140] and Hodges [141] and found that the Framingham formula had the least correlation with heart rate in our study population (r= -0.23 and r=-0.38, respectively). Thus $QTc = QT + 154(1 - \frac{60}{Heart\ Rate})$; where heart rate is in beats per minute.

In addition to the continuous measure of QT interval, we defined prolonged QTc as QTc values of 460 ms or longer in women and 450 ms or longer in men [138].

2.4.2.2 J-Point Height and J-Point Elevation

The ST amplitude at the J-point was determined the 2001 version of the GE Marquette 12-SL program. We calculated a continuous measure of the J-point (J-point height) as the maximum amplitude of the 12 STJ leads. As has been done in other ARIC studies, JPE was defined as a ST amplitude greater than 100 microvolts in at least two contiguous leads [142].

2.5 POTENTIAL CONFOUNDERS

We selected potential confounders a priori based on their hypothesized relationship with exposure and outcome. While most variables were measured at multiple visits, potential confounders were measured contemporaneously with exposure values (visits 1 and 3) to avoid adjusting for confounders measured after our exposure of interest [143]. Potential confounders were grouped into four main categories: sociodemographic, lifestyle, dietary, and clinical variables.

2.5.1 SOCIODEMOGRAPHIC VARIABLES

Sociodemographic variables included age, sex, race, field center, and education level. Age, sex, self-reported race, and field center were obtained at visit 1 and confirmed at subsequent visits. For race, participants were handed a card and asked to tell the interviewer which best described his or her race. Choices offered were: white,

black, American Indian/ Alaskan native, Asian/Pacific Islander, other: specify. Over 99% identified as either white or black race. Education level was measured at visit 1 via self-report and categorized based on years of education: basic (no high school degree), intermediate (completed high school), and advanced (at least some college).

2.5.2 Lifestyle Variables

Lifestyle variables included body mass index (BMI), physical activity, smoking status, and drinking status and amount. Technicians measured height and weight, and BMI was calculated as weight (kilograms) divided by height squared (meters^2). Physical activity was measured at visits 1 and 3 using the Baecke questionnaire [144]. The questionnaire included 16 items about usual exertion, and three indexes ranging from 1 (low) to 5 (high) were derived for physical activity at work, during leisure time, and in sports. The reliability and validity of the Baecke questionnaire are good for both male and female subjects, and equal to many other physical activity instruments [145]. The three physical activity scores were summed and then translated into tertiles of physical activity (low, medium, and high). Smoking status was assessed via self-report and participants classified as current smokers, former smokers (more than 100 cigarettes in the past), and never smokers. Alcohol intake status (current, former, never) and amount (grams/day) were measured at visits 1 and 3.

2.5.3 Dietary Variables

Dietary variables included *trans* fatty acids, saturated fatty acids, and dietary fiber. Intake of *trans* fatty acids, saturated fatty acids, and dietary fiber were measured at visits 1 and 3 via FFQ and translated into nutrient values as described in Section 2.3.1.2.

2.5.4 Clinical Variables

Clinical variables included hypertension, LDL, HDL, and triglycerides. During each visit, three blood pressure measurements were taken with a random-zero sphygmomanometer after 5 minutes of the participant in the sitting position; the mean of the last two measurements was used. Hypertension was defined as a systolic blood pressure above 140 mmHg, a diastolic above 90 mmHg, or self-reported use of antihypertensive medication. For metrics requiring phlebotomy, blood was drawn after a minimum 8-hour fasting period with minimal trauma from an antecubital vein [146]. Plasma total cholesterol and triglycerides were measured by enzymatic methods [132], and LDL cholesterol was calculated using the Friedewald formula [147]. HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins [132].

2.6 OTHER COVARIATES OF INTEREST

Other variables of interest include self-reported medication use, variables used for inclusion/exclusion criteria that had not yet been defined, and variables used to stratify participants into sub-populations of interest.

2.6.1 Prescription and Non-Prescription Medication

Prior to each visit, participants were asked to bring all prescription and non-prescription medications used in the two weeks leading up to the visit. Trained interviewers collected information on the medications that participants reported taking, and these were coded according to drug category.

2.6.2 Inclusion and Exclusion Criteria

For all analyses, participants with prevalent or incident CVD – defined as coronary heart disease (CHD), heart failure (HF) or stroke – were excluded from analysis.

Prevalent disease was established at visit 1. Incident disease was identified at subsequent visits, or through three surveillance methods: (1) ARIC Study participants were contacted annually by phone (twice a year after 2012) and all hospitalizations and deaths during the previous year were identified; (2) local hospitals provided lists of cardiovascular disease discharges, which were examined for participant hospitalizations and qualifying CVD outcomes; and (3) death certificates.

Prevalent CHD was defined by a positive history of angina or intermittent claudication by the Rose questionnaire [148, 149], a self-reported physician-diagnosed history of a heart attack, evidence of old myocardial infarction by electrocardiogram, or a self-reported history of cardiovascular surgery or angioplasty [150]. Incident CHD was defined as fatal CHD, definite or probable MI, and/or coronary revascularization.

HF prevalence criteria (at visit 1) included current medication use for HF and/or having manifest HF as defined by Gothenburg criteria stage 3, which requires the presence of specific cardiac and pulmonary symptoms as well as medical treatment for HF [151]. Incident HF was defined as either (1) a hospitalization which included an International Classification of Diseases, 9th revision (ICD-9), discharge code of 428 in any position; or (2) a death certificate with a ICD-9 code of 428 or an ICD-10 code of I50 in any position. Non-hospitalized, non-fatal HF was not captured.

Prevalent stroke (visit 1) was defined as a self-reported history of physician-diagnosed stroke [152]. Incident stroke (definite or probable) was defined as evidence of sudden or rapid onset of neurological symptoms lasting for >24 hours or leading to death. Furthermore, these neurological symptoms could not have been attributable to non-stroke causes including major brain trauma, neoplasm, coma due to metabolic disorders or disorders of fluid or electrolyte balance, vasculitis involving the brain, peripheral neuropathy, hematologic abnormalities, or central nervous system infections.

2.6.3 GLYCEMIA STATUS

Manuscript 1 (omega-3 PUFAs and glycemia) will involve analyzing different populations of subjects, specifically non-diabetic participants, diabetic participants, and those with pre-diabetic conditions. As such, the ARIC population will need to be divided into subgroups defined below. Variables involved in classifying participants were collected at each visit.

2.6.3.1 Type 2 Diabetes (T2D)

As previously mentioned, diabetes status was updated at each visit and defined as (1) self-report of physician-diagnosed diabetes; (2) self-reported use of diabetes medication in the past two weeks; (3) fasting glucose level ≥ 7.0 mmol/liter (126 mg/dl); or (4) non-fasting glucose level ≥ 11.1 mmol/liter (200 mg/dl).

2.6.3.2 Pre-Diabetics (Pre-T2D)

FBG was tested at each visit. Those with FBG between 100-125 mg/dL were classified as having pre-diabetes (Pre-T2D).

2.6.3.3 Normoglycemics (NGT)

The normoglycemic (NGT) population was defined as participants without T2D or Pre-T2D.

2.7 SUMMARY

This dissertation used data from the ARIC study collected over four visits from 1987 through 1998. Use of these data offer many strengths. It is a population-based, biracial cohort who were followed over several years with repeated measures of exposures, outcomes, and covariates.

3 MANUSCRIPT 1: INTAKE OF LONG-CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS, INCIDENCE OF DIABETES, AND MARKERS OF GLUCOSE HOMEOSTASIS IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

3.1 SYNOPSIS

Background: The incidence and prevalence rates of type 2 diabetes are high in the United States, with blacks disproportionately affected. The evidence regarding the association of dietary intake of fish, fish-derived omega-3 fatty acids, and vegetable-derived omega-3 fatty acids has been mixed.

Methods: We studied the association of consumption of seafood, the fish-derived omega-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the vegetable-derived omega-3 PUFA alpha-linoleic acid (ALA) with fasting blood glucose (FBG) (n=13,173), HbA1c (n=11,575), and incident type 2 diabetes (T2D) (n=11,874) in a bi-racial cohort of individuals aged 45–64 participating in the Atherosclerosis Risk in Communities (ARIC) study. Intake of seafood, DHA, EPA, and ALA were measured via food frequency questionnaire. FBG and HbA1c were obtained using blood samples collected during study visits and diabetes status was identified through self-report and lab values. To estimate differences across exposure categories, linear regression was used for continuous outcomes (FBG, HbA1c) adjusting for repeated measures as appropriate. Cox proportional hazards regression with time varying covariates was used for the incident T2D outcome.

Results: In multivariable analyses, intake of seafood and DHA+EPA was favorably associated with FBG and HbA1c in non-diabetic participants, although the magnitude of the associations were very small ranging between a decrease of 0.35 to 1.35 mg/dL for FBG and a decrease of 0.01 to 0.10 percentage points for HbA1c when comparing extreme categories. ALA was not associated with FBG or HbA1c in non-diabetic

participants. Among diabetic participants, intake of seafood, fish-derived DHA+EPA, and the vegetable derived ALA was adversely associated with FBG and HbA1c, with differential effects for seafood in men (favorable) versus women (adverse) and whites (favorable) versus blacks (adverse). Finally, higher intake of the vegetable derived ALA was associated with higher risk of incident T2D in normoglycemics (HR=4.0, 95% CI: 1.7, 9.6, comparing extreme quartiles), while seafood and the fish-derived DHA+EPA were not.

Conclusions: In this population based cohort, dietary intake of seafood and all omega-3 nutrients – including ALA – were adversely associated with FBG and HbA1c in diabetic participants, although there were differential effects by sex and race for seafood. In contrast, neither seafood nor DHA+EPA was associated with time to incident T2D, but higher intake of ALA was associated with higher risk.

3.2 INTRODUCTION

Approximately 9% of the adult population in the United States aged 20+ years has been diagnosed with type 2 diabetes (T2D), with the highest age-adjusted prevalence among non-Hispanic black adults with less than a high school education (16.0%) and the lowest among non-Hispanic white adults with more than a high school education (6.6%) [1]. Having diabetes is associated with a greater clustering of cardiovascular disease (CVD) risk factors compared to those without diabetes [1, 153]. Lifestyle factors are an important component of preventive strategies to improve cardiovascular health, and key among those factors is diet [2].

Fish consumption has been frequently included in dietary guidelines for the prevention of CVD and its risk factors. Both the American Heart Association and the American Diabetes Association recommend at least two servings of oily fish a week to promote cardiovascular health [3, 154]. Oily fish is rich in the long-chain omega-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic

acid (EPA). These, along with the vegetable-derived omega-3 PUFA alpha-linolenic acid (ALA) play major roles in physiological processes [5].

Studies investigating seafood intake and intake of fish-derived and vegetable-derived omega-3 PUFAs with markers of glucose homeostasis have been mixed. Recent reviews and meta-analyses have reported that dietary intake of fatty fish and the fish-derived omega-3 PUFAs DHA and EPA are associated with higher risk of T2D in American and European populations [85-88] but not Asians [86]. There was no association found between dietary intake of lean fish or shellfish with T2D [86]; and plasma measures of DHA and EPA were also not associated with a higher risk of T2D [85]. In diabetics, a meta-analyses of fish oil supplementation studies found that supplementation had favorable – but non-significant – associations with HbA1c and insulin [73], although the AHA no longer recommends fish oil supplementation for diabetics for the prevention of cardiovascular disease [74].

Studies investigating intake of the vegetable-derived ALA have been similarly mixed. A meta-analysis found higher intake of ALA was associated with lower risk of incident T2D [85] but a subsequent analysis two years later showed favorable associations in Asians only, although Americans and Europeans had a nonsignificant favorable trend [86].

Given the inconsistent nature of the previous literature, we tested the associations among dietary intakes of seafood, the fish-derived omega-3 PUFAs DHA and EPA, and the vegetable derived omega-3 PUFA ALA with glycemia outcomes in the Atherosclerosis Risk in Communities (ARIC) study – a population-based, biracial cohort, with twelve years of follow-up data and repeated measures for the exposures and outcomes of interest.

3.3 METHODS

3.3.1 STUDY POPULATION

The ARIC study has been described previously [155]. Briefly, ARIC is a prospective study of cardiovascular disease including 15,792 men and women 45–64 years of age at baseline (visit 1). Participants were recruited from four US communities using probability sampling techniques. The communities and racial composition were: predominately white subjects from suburbs of Minneapolis, Minnesota, and Washington County, Maryland; black subjects from Jackson, Mississippi; and white and black subjects from Forsyth County, North Carolina.

Visit 1 data were collected in 1987–89 and three additional exams were performed at approximately 3-year intervals (1990–92, 1993–95, 1996–98). A fifth exam was conducted in 2011-13 (visit 5), but those data were not utilized in this study as outcomes would have occurred more than 20 years after our exposure assessment.

Our exclusion criteria were as follows (see Figure 3-1). We excluded participants with missing values for exposures, outcomes, or covariates. Those whose race was neither black nor white (n = 48) were excluded, and we further excluded black participants at the Minneapolis and Washington County sites (n = 55) due to small n. We excluded those participants who had prevalent or incident coronary heart disease, heart failure, or stroke as (1) prevalent conditions influence how patients have their comorbidities managed, diagnosed and treated; and (2) diagnoses may result in changes to previously reported dietary and lifestyle behaviors. Finally, participants who reported implausible caloric intakes were excluded for potentially unreliable exposure data. Implausible was defined as less than 500 kcal/day for women and 700 kcal/day for men or more than 3500 kcal/day for women and 4500 kcal/day for men. These ranges represent the sex-specific first and 99th percentiles for ARIC energy intake distributions – see Tell et al. for the initial description of the exclusion methodology and justification [156] and Steffen et al. for first use of current ranges [157].

3.3.2 Fasting Status

All participants self-reported 8-hour and 12-hour fasting status. Compliance was high in non-diabetics, with approximately 98% reporting having fasted for at least 8 hours and 96% reporting fasting for at least 12 hours. Compliance was lower in those with diabetes, with approximately 88% reporting 8 or more hours and 84% reporting 12 or more hours fasting.

3.3.3 Glycemia Status Population Assignment

At each visit, participants who met the inclusion/exclusion criteria were categorized by glycemia status: diabetic participants, those with pre-diabetic conditions, and normoglycemic participants.

Diabetic participants were identified at each visit based on the following criteria (1) self-report of physician-diagnosed diabetes; (2) self-reported use of diabetes medication in the past two weeks; (3) fasting glucose level ≥ 7.0 mmol/liter (126 mg/dl); or (4) non-fasting glucose level > 11.1 mmol/liter (200 mg/dl). If a participant was not diabetic, then the participant was defined as having pre-diabetes (Pre-T2D) if his/her fasting blood glucose value was between 100 mg/dl and 125 mg/dl. The normoglycemic (NGT) population was defined as those who had neither Pre-T2D nor diabetes.

3.3.4 Outcome Assessment

Three measures of glucose metabolism are used in this study: Fasting Blood Glucose (FBG), HbA1c, and incident T2D. Detailed descriptions of blood draw [146] and chemistry analyses [132] can be found in the ARIC Manual of Operations. For outcomes that could be influenced by use of anti-hyperglycemic medications (i.e., FBG, HbA1c), a correction factor was applied (see the *Correction Factor* section 3.3.4.4).

3.3.4.1 Fasting Blood Glucose

FBG was measured at visits 1-4. Fasting blood samples were drawn from an antecubital vein with minimal trauma. FBG was then measured by a hexokinase method on a Coulter DACOS (Coulter Instruments). In a small validation study blood samples from volunteers (n=40) were taken two weeks apart. For serum glucose the intraclass correlation was 0.84, the within-person coefficient of variation (CV) was 4%, and the laboratory CV was 2% [133].

3.3.4.2 Hemoglobin A1c

HbA1c was measured from whole blood samples using high-performance liquid chromatography. The blood was collected during visit 2 (1990-92) and stored at -70 Cº for 14-18 years until HbA1c measurements could be obtained.

Selvin et al. give a detailed description of the HbA1c measurement process [158], but briefly, measurements were obtained in the same laboratory during two separate time periods using two different instruments: n=4,918 subjects in 2003-04 using Tosoh 2.2 Plus HPLC (Tosoh Bioscience, South San Francisco, CA) and n=9,151 subjects in 2007-08 using Tosoh G7 HPLC. Both instruments were certified by the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes Control and Complications Trial assay. This calibration provides stable results despite changes in HbA1c methodologies over time. A convenience sample (n=383) was analyzed using both instruments. Pearson's correlation coefficient between the two samples was high (r=0.99) but there was a slight bias with 2007-08 values showing a 0.29 higher %HbA1c ($p<0.0001$). The intraclass correlation coefficient was 0.99 (95% CI: 0.97-0.99) and within-sample coefficient of variation (CV) of 3.9% (95% CI: 3.6% to 4.2%).

3.3.4.3 Incident Type 2 Diabetes

As previously mentioned, diabetes was defined as (1) self-report of physician-diagnosed diabetes; (2) self-reported use of diabetes medication in the past two weeks; (3) fasting glucose level ≥ 7.0 mmol/liter (126 mg/dl); or (4) non-fasting glucose level > 11.1 mmol/liter (200 mg/dl).

Although investigators did not distinguish type 1 diabetes mellitus from T2D, T2D is the most commonly occurring variant of diabetes and rarely occurs before age 30 in individuals who were middle-aged in the 1980's [135]. Defining participants who met these criteria as having T2D is consistent with other ARIC studies where type 1 diabetes was defined as subjects with age of onset before age 30 [136].

Analyses with incident T2D as the outcome of interest excluded participants who had prevalent diabetes at visit 1. Participants who met the diabetes criteria at subsequent visits were considered to have incident T2D and date of onset was defined as the visit date.

3.3.4.4 Correction Factor

For those participants who reported taking anti-hyperglycemic medications – insulin (mixed, beef, pork, human, or unspecified), sulfonylureas or sulfonylurea combinations, biguanides, meglitinides, aldose reductase inhibitors, alpha-glucosidase inhibitors, thiazolidinediones, or other – we applied a correction factor using the approach described in Tobin et al. [159] and other studies with glycemia outcomes [160, 161]. Specifically, for medicated participants, we added a constant of 1 mmol/dl (18 mg/dl) to fasting blood glucose measures and 1 percentage point to HbA1c values. These constants were based on pharmaceutical studies, systematic reviews, and meta-analyses of the effect of medication on glycemia biomarkers [162-166].

3.3.5 EXPOSURE ASSESSMENT

In this study, we focused on dietary consumption of fish and shellfish, the fish-derived omega-3 fatty acids DHA and EPA, and the vegetable-derived ALA.

Participants' usual dietary intake was assessed by an interviewer-administered, 66-item food frequency questionnaire (FFQ). The FFQ was based on the instrument developed by Willett et al. [167], with three principal modifications: (1) Data regarding alcohol consumption were obtained using a separate, more detailed instrument; (2) Several food items were added (e.g., donuts, biscuits, and cornbread); and (3) Some items were split into detailed subcategories – notably a single item on fish consumption was separated into three specific fish items.

The 61-item Willett version has been validated against 28-day food record, but the validation took place in a population of educated, predominately white women [124, 167]. The ARIC questionnaire was also validated in a sample (n=419) of black and white ARIC participants who repeated the FFQ after three years [125]. The study found that, after adjusting for total caloric intake, the median reliability coefficient for blacks was 0.42 and the reliability for white ARIC participants was 0.49 – a value similar to that of other studies of white subjects. The study found no difference in the median reliability coefficients of men and women after adjusting for total calorie intake.

Another study investigated the validity of the ARIC FFQ by comparing Minnesota field center participants' intake of dietary fat as measured via FFQ against their plasma fatty acid concentrations [126]. Plasma measures reflect the types of fats proportionally consumed over the past several weeks to months [127] and the proportionate composition in plasma was moderately correlated with dietary intake, with highest correlations in the fish-derived omega-3 fatty acids DHA and EPA ($r=0.42$ and $r=0.20$ for plasma phospholipid measures of DHA and EPA, respectively) [126].

3.3.5.1 Fish/Shellfish Servings

Fish and other seafood intake was assessed through four FFQ questions with nine response categories. Participants were asked how often they consumed: 3–4 ounces of canned tuna fish; 3–5 ounces of dark meat fish such as salmon, mackerel, swordfish, sardines, and bluefish; 3–5 ounces of other fish such as cod, perch, catfish, etc.; and shrimp, lobster, scallops as a main dish. Interviewers used food models to help participants with portion size estimation. Subjects could provide answers to each question ranging from ‘‘never or less than once per month’’ to ‘‘6 times per day.’’

Applying the same methodology used in another study of seafood intake in the ARIC cohort [128], each of the participants’ seafood-related FFQ responses were grouped into three exposure categories: (1) omega-3 rich fish (tuna + dark); (2) total fish (tuna + dark+ other); and (3) total seafood (tuna + dark + other + shellfish). Exposure categories were categorized into four weekly serving categories: none, less than one, one to two, and more than two.

3.3.5.2 Quartiles of Omega-3 PUFA

Daily intake of macro- and micronutrients was calculated via the FFQ by multiplying the nutrient content of each food by the frequency of daily consumption and then summing the results [124]. This process yielded daily intake of nutrients expressed as grams per day. Three different classifications of omega-3 fatty acids were investigated: (1) vegetable-derived ALA, (2) fish-derived DHA+EPA; and (3) ALA+DHA+EPA.

Intake of nutrients was adjusted using the residual method [168, 169]. In this method, nutrient residuals (observed intake – predicted intake) are obtained from the regression of total nutrient intake on total energy intake. The nutrient residuals are then rescaled by adding the overall mean nutrient intake to each participant’s residual. For this manuscript, we created rescaled residuals for the three nutrient classifications – ALA, DHA+EPA, and ALA+DHA+EPA – and categorized these into quartiles.

We selected the residual method rather than the standard multivariable method (quartiles of raw nutrient values as the exposure with total energy intake as a covariate) because (1) with the residual method, differences in exposure values amongst participants are due to differences in nutrient intake from the nutrient composition of the diet (versus overall variation in nutrient intake, which is due to diet composition and calorie amount) [169]; (2) when dietary exposure variables are categorized, the residual and the standard multivariable models are no longer mathematically equivalent [168-170]; and (3) the residual model allows for greater precision [168]. All regression models where residual-adjusted nutrients were the exposure of interest included total energy intake (kcal/day) as a covariate.

3.3.6 POTENTIAL CONFOUNDERS

We selected potential confounders a priori based on their hypothesized relationship with exposure and outcome. While most variables were measured at multiple visits, potential confounders were measured contemporaneously with exposure values (visits 1 and 3) to avoid adjusting for confounders measured after our exposure of interest [143]. Potential confounders were grouped into four main categories: sociodemographic, lifestyle, dietary, and clinical variables.

3.3.6.1 Sociodemographic Variables

Sociodemographic variables included age, sex, race, field center, and education level. Age, sex, and self-reported race were obtained at visit 1 and confirmed at subsequent visits. Education level was measured at visit 1 via self-report and categorized based on years of education. We grouped education level as basic (no high school degree), intermediate (completed high school), and advanced (at least some college).

3.3.6.2 Lifestyle Variables

Lifestyle variables include body mass index (BMI), physical activity, smoking status, and drinking status and amount. Technicians measured height and weight, and BMI was calculated as weight (kilograms) divided by height squared (meters^2). Physical activity was measured at visits 1 and 3 using the Baecke questionnaire [144]. The questionnaire included 16 items about usual exertion, and three indexes ranging from 1 (low) to 5 (high) were derived for physical activity at work, during leisure time, and in sports. The reliability and validity of the Baecke questionnaire are good for both male and female subjects, and equal to many other physical activity instruments [145]. The three physical activity scores were summed and then translated into tertiles of physical activity (low, medium, and high). Smoking status was assessed via self-report and participants were classified as current smokers, former smokers (more than 100 cigarettes in the past), and never smokers. Alcohol intake status (current, former, never) and amount (grams/day) were measured at visits 1 and 3.

3.3.6.3 Dietary Variables

Dietary variables included *trans* fatty acids, saturated fatty acids, and dietary fiber. Intake of *trans* fatty acids, saturated fatty acids, and total dietary fiber from all plant sources (fruits, legumes, cereals, and vegetables) were measured at visits 1 and 3 via FFQ and translated into nutrient values as described in the exposure section (Section 3.3.5).

3.3.6.4 Clinical Variables

Clinical variables included hypertension, LDL, HDL, and triglycerides. During each visit, three blood pressure measurements were taken with a random-zero sphygmomanometer and the mean of the last two measurements was used. Hypertension was defined as a systolic blood pressure above 140 mmHg, a diastolic above 90 mmHg, or self-reported use of antihypertensive medication. Participants with

missing hypertension values (n=62 visit 1; n=56 visit 3) were imputed as not having hypertension (no disease). For metrics requiring phlebotomy, blood was drawn after a minimum 8-hour fasting period with minimal trauma from an antecubital vein [146]. Plasma total cholesterol and triglycerides were measured by enzymatic methods [132], and LDL cholesterol was calculated using the Friedewald formula [147]. HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins [132].

3.3.7 STATISTICAL ANALYSES

The statistical methods used varied by the study design used to investigate the relationship between the exposure and outcome of interest. Outcome-specific study populations, study designs, and statistical methods are described in this section.

For clarity, Figure 3-2 depicts the study design for all three outcomes of interest including the temporality of the exposure, outcomes, covariates, and subpopulations based on glycemia status.

The green ovals represent exposures and covariates obtained at visit 1 and visit 3. Visit 1 values were used in regressions involving outcomes obtained at visit 1 and visit 2, whereas visit 3 values were used for outcomes obtained at visits 3 and 4. The blue arrow gives the timeline for each of the ARIC visits. The grey boxes represent that participants' glycemia status (T2D, Pre-T2D, NGT) and was updated at each of the four ARIC visits (where applicable). Similarly, the pink box demonstrates that inclusion/exclusion criteria were updated and applied at each visit (where applicable).

All statistical analyses were performed with SAS (version 9.4, Enterprise guide 7.1, SAS Institute Inc., Cary, NC, USA).

3.3.7.1 Fasting Blood Glucose

For this outcome, we used a quasi-repeated cross-sectional design where each participant who met the inclusion/exclusion criteria at visit 1 could be included in the analysis up to four times (visit 1, plus once for each subsequent visit where he/she met

the inclusion/exclusion criteria). For each visit meeting the inclusion/exclusion criteria, we noted the participant's glycemia status and outcome (FBG). As previously mentioned, exposure and confounder values from visit 1 were used in observations for visits 1 and 2, and observations for visits 3 and 4 used exposure and covariate values from visit 3. Associations between fish/shellfish servings, quartiles of omega-3 PUFA intake, and FBG were estimated using generalized estimating equations to account for repeated measures, using a normal distribution and an identify link, and assuming an independent working correlation structure. A sensitivity analysis was conducted evaluating the impact of assuming an unstructured working correlation structure and this modification did not appreciably alter our results. Because our sample size was large, we did not test for violations of the normality assumptions [171].

3.3.7.2 Hemoglobin A1c

For this outcome, we used a modified cross-sectional study design. Exposures and covariates were measured at visit 1, glycemia status and HbA1c were measured at visit 2, and inclusion/exclusion criteria were applied at both visits 1 and 2. Associations between fish/shellfish servings, quartiles of omega-3 PUFA intake, and HbA1c were estimated using linear regression. Because our sample size was large, we did not test for violations of the normality assumptions [171].

3.3.7.3 Incident Type 2 Diabetes

For this outcome, we used a prospective study design. Those free of diabetes at visit 1 were followed through each of the subsequent visits until either diagnosis or censoring. We used Cox proportional hazards regression models to estimate hazard ratios (HRs) for incident T2D by level of fish/shellfish consumption and quartiles of omega-3 PUFA intake. Exposure status and potential confounders were modeled as time-dependent covariates with visit 1 data used for the period between visit 1 and visit

3, and visit 3 data afterwards. Because we used time-varying covariates, we did not test for violations of the proportional hazards assumption.

3.3.8 Covariate Adjustment Models

Three models were used to adjust for potential confounders measured contemporaneously with exposure values: Model 1 adjusted for sociodemographic variables (age, sex, race, center, education); Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount) and dietary variables (*trans* fatty acids, saturated fatty acids, and dietary fiber); Model 3 further adjusted for clinical variables (hypertension, HDL, LDL, triglycerides). All models included total energy intake (kcal/day) as a covariate.

3.3.9 Glycemia Status as an Effect Measure Modifier

For all outcomes, we evaluated whether glycemia status (NGT, Pre-T2D, and, if applicable, T2D) was an effect measure modifier on the multiplicative scale by including the interaction term (*glycemia status*exposure*) in the regression models and considering the term's p-value. If the interaction term was significant ($p<0.1$), the term was kept in the model and results were presented for each sub-population (i.e., one set of results for those with NGT, another for those with Pre-T2D, and if applicable, another for those with T2D). If the interaction term was not significant, then we presented one set of results for the full cohort. In either instance, we tested for a linear trend in the association between our exposure and outcome by modeling the exposure category medians as a continuous variable.

3.3.10 Race and Sex as Effect Measure Modifiers

Where appropriate, we tested for effect modification by race and sex by considering *race*exposure* and *sex*exposure* interaction terms. For HbA1c and FBG

outcomes, if we had previously found that glycemia status was an effect modifier, race and sex interaction terms were tested in glycemia status-stratified subsets of data to avoid testing a three-way interaction (*race*exposure*glycemia status*; or *sex*exposure*glycemia status*).

For Incident T2D, if we had previously found that glycemia status (NGT vs. Pre-T2D) was an effect modifier, we could not subset the data as we did for continuous outcomes without introducing an overly complicated person time model. Consider a participant who was NGT at visit 1 and transitioned to Pre-T2D at visit 2 and then was diagnosed with T2D at visit 3. If we were to subset the data based on diabetes status, that participant would contribute three years of person time to the NGT time-to-event dataset and three years of person time to the Pre-T2D dataset. For simplicity, we used a single dataset and tested the interaction term *–exposure*race* (or sex)**glycemia status* (NGT or Pre-T2D).

Finally, to avoid being overly reliant on p-values across multiple exposure/outcome/glycemia status models, we qualitatively compared the measures of association by race and by sex. That is, we reviewed exposure/outcome results for white participants and compared them to results for black participants. We performed the same qualitative analysis for male- and female-specific results.

3.3.11 SENSITIVITY ANALYSES

We performed several sets of sensitivity analyses. In Section 3.3.7 we described the working correlation structure sensitivity analyses (independent vs. unstructured). We also examined if including protein (grams/day) as a covariate altered our associations, as seafood is high in protein and protein has been shown to be associated with incident T2D [172, 173]. For FBG outcomes, we investigated if limiting our analysis to those who self-reported compliance with the 12-hour fasting request appreciably

altered our results. Finally, among those with T2D, we considered results stratified based on antihyperglycemic medication use.

3.4 RESULTS

The characteristics of the 13,173 participants who met the least restrictive inclusion/exclusion criteria (FBG analyses) by categories of total seafood intake (total fish + shellfish) are shown in Table 3-1.

The greatest proportion of participants consumed one to two servings of seafood per week (45%). The fewest number of participants fell into the lowest exposure category with zero servings of seafood per week (8%). Those who consumed more seafood tended to be younger, female, and more educated. Additionally, there was a greater proportion of black participants at higher levels of total seafood compared consumption compared to lower levels of consumption. Clinically, cardiovascular risk profiles varied across seafood consumption categories. Prevalence of hypertension and BMI were higher with greater amounts of seafood consumption; smoking rates were lower with greater seafood consumption.

3.4.1 ASSOCIATIONS WITH FASTING BLOOD GLUCOSE

For each of the three food exposures (total seafood, total fish, total omega-3 rich fish) and the three omega-3 PUFA exposures (quartiles of ALA, DHA+EPA, and ALA+ DHA+EPA) the *exposure*glycemia* status interaction term was significant. P-values for the interactions along with glycemia status-specific results are reported in tables 3-2 through 3-7. Including protein as a covariate in the regression models did not substantively change our results (data not shown).

3.4.1.1 Normoglycemic and Pre-Diabetic Populations

3.4.1.1.1 Fish/Shellfish Servings and Quartiles of Fish-Derived Omega-3 PUFAs (DHA+EPA)

In most models, intake of seafood and DHA+EPA was associated with lower FBG in both NGT and pre-T2D populations, though these associations were of small magnitude and possibly not clinically relevant (tables 3-2 through 3-5). With zero servings/week as the reference category for the food exposures, differences in FBG for those consuming less than one, one to two, or more than 2 servings were all less than 1.26 mg/dl. With the first quartile as the reference category for DHA+EPA, all differences in FBG were less than 1.35 mg/dl.

3.4.1.1.2 Quartiles of Vegetable-Derived Omega-3 PUFAs (ALA)

In both NGT and pre-T2D populations, intake of ALA was not associated with FBG (tables 3-3 and 3-5).

3.4.1.2 Diabetic Populations

3.4.1.2.1 Fish/Shellfish Servings

In those with T2D, intake of fish/shellfish was associated with higher FBG (Table 3-6). Total fish had the largest association with FBG, with those consuming two or more servings per week having 16.1 mg/dl higher FBG compared to those consuming no fish (Model 3, 95% CI: 6.5, 25.6, p=0.003). Results for total seafood were similar to those for total fish (Figure 3-3). Differences in FBG among categories of omega-3 rich fish were smaller than the differences in FBG for total fish or total seafood, but also statistically significant. Participants who consumed two or more servings of omega-3 rich fish per week had 7.8 mg/dl higher FBG values on average compared to those who consumed none (Model 3, 95% CI: -0.9, 16.5, p=0.003). Figure 3-3 shows all three exposures and their association with FBG. Limiting the populations to those who self-reported fasting

for 12 or more hours did not substantively change our results; and stratifying by antihyperglycemic medication use also yielded similar results (data not shown).

3.4.1.2.2 Omega-3 PUFA Intake

Among participants with T2D, intake of vegetable-derived ALA, fish-derived DHA+EPA, and total omega-3 PUFA (ALA+DHA+EPA) was associated with higher FBG values (Table 3-7). The largest difference in FBG for the highest quartile of intake (compared to quartile 1) was in DHA+EPA+ALA (Model 3, Q4 vs. Q1 = 23.4 mg/dl, 95% CI: 16.3, 30.5). When considered separately, ALA and DHA+EPA were also statistically significantly associated with FBG, with differences across quartiles of ALA (compared to the first quartile) slightly larger than those for DHA+EPA (Model 3, Q4 vs. Q1 (95% CI): ALA = 18.1 mg/dL, 95% CI: 11.2, 25.0; DHA+EPA = 16.6 mg/dL 95% CI: 9.4, 23.7). Figure 3-4 shows all three omega-3 PUFA exposures and their associations with FBG. Limiting the populations to those who self-reported fasting for 12 or more hours did not substantively change our results; and stratifying by antihyperglycemic medication use also yielded similar results (data not shown).

3.4.1.3 FBG Race and Sex Effect Modifier Analyses

Among those with NGT and Pre-T2D, and where the *race*exposure* or *sex*exposure* interaction terms were statistically significant, the differences in associations amongst race- and sex-stratified groups were so small as to be clinically irrelevant.

For those with T2D, there was no evidence of effect modification by race or sex for the three omega-3 PUFA quartile exposures (ALA, DHA+EPA, ALA+DHA+EPA). However, there was evidence of effect modification by race and sex for total seafood intake and total fish intake. The association between total seafood and FBG in diabetic women suggested an adverse effect whereas there was no association in diabetic males.

Intake of total fish had similar point estimates, but overall the interaction term was non-significant (figures 3-5 and 3-6). The association between total seafood and FBG and total fish and FBG in blacks and whites suggested there may be effect modification by race, although the interaction term was not statistically significant (figures 3-7 and 3-8).

3.4.2 ASSOCIATIONS WITH HBA1C

As seen in Figure 3-1, there were n=11,575 participants who met the inclusion/exclusion criteria for the HbA1c analysis. For each of the three food exposures (total seafood, total fish, total omega-3 rich fish) and the three omega-3 PUFA exposures (quartiles of ALA, DHA+EPA, and ALA+ DHA+EPA) the *exposure*glycemia* status interaction term was significant. Glycemia status-specific results are reported in tables 3-8 through 3-13. Including protein as a covariate in the regression models did not substantively change our results (data not shown).

3.4.2.1 Normoglycemic Populations

3.4.2.1.1 Fish/Shellfish Servings

As seen in Table 3-8, intake of total seafood (Model 3, p=0.07) and total fish (Model 3, p=0.06) had borderline significant p-values, with higher intake of fish/seafood associated with lower HbA1c values, but the associations were of small magnitude; the largest point estimate was 0.11 percentage points (Model 3, 2+ servings total seafood versus none, 95% CI: -0.19, -0.02). Omega-3 rich fish was not associated with HbA1c (Model 3, p=0.33).

3.4.2.1.2 Omega-3 PUFA Intake

Intake of DHA+EPA was significantly associated with lower HbA1c values in NGT populations (Table 3-9). Compared to those in the lowest quartile of DHA+EPA intake, those in the fourth quartile of intake had 0.09 percentage points lower HbA1c (Model 3,

95% CI: -0.16, -0.03). Neither ALA nor ALA+DHA+EPA intake was significantly associated with HbA1c (Table 3-9).

3.4.2.2 Pre-Diabetic Populations

Neither fish/shellfish servings nor quartiles of fish-derived omega-3 PUFAs DHA+EPA were associated with HbA1c among participants with Pre-T2D (tables 3-10 and 3-11). Associations resulting from Model 1 showed a significant positive association between intake of ALA and HbA1c (Q4 - Q1 = 0.09 percentage points, 95% CI: 0.01, 0.16), the association disappeared after additional adjustment (p=0.32 Model 2; p=0.29 Model 3).

3.4.2.3 Diabetic Populations

3.4.2.3.1 Fish/Shellfish Servings

In those with T2D, higher intakes of total seafood and of total fish (compared to lower) were associated with higher HbA1c. There was no association between omega-3 rich fish intake and HbA1c. (Table 3-12; Figure 3-9)

Of the three seafood exposures, total fish had the largest point estimate among the fully adjusted model results, with those consuming two or more servings per week having 0.28 percentage points higher HbA1c compared to those consuming no fish (Model 3, p=<0.0001, 95% CI: 0.05, 0.51). Results for total seafood followed a similar pattern. Stratifying by antihyperglycemic medication use also yielded similar results (data not shown).

3.4.2.3.2 Omega-3 PUFA Intake

In those with T2D, intake of DHA+EPA and ALA was associated with higher HbA1c values (Table 3-13). The largest difference in HbA1c, when comparing quartile 4 to quartile 1, was in DHA+EPA+ALA (Model 3, Q4 vs. Q1 = 1.06 percentage points, 95% CI: 0.89, 1.22). The fish-derived omega-3 PUFAs DHA+EPA and the vegetable-derived

omega-3 PUFA ALA were also statistically significant, with differences in HbA1c across quartiles of ALA slightly larger than those for DHA+EPA (Model 3, Q4 vs. Q1 (95% CI): ALA = 0.88 percentage points (0.71, 1.04), DHA+EPA = 0.50 percentage points (0.34, 0.66). (Figure 3-10.) Stratifying by antihyperglycemic medication use also yielded similar results (data not shown).

3.4.2.4 HbA1c Race and Sex Effect Modifier Analyses

Among those with NGT and Pre-T2D, in instances where the *race*exposure* or *sex*exposure* interaction terms were significant, the differences in the exposure/outcome relationship by race and sex categories were so small as to be clinically irrelevant (data not shown).

For those with T2D, there was no evidence of effect modification by race or sex for the three PUFA exposures (DHA+EPA+ALA, DHA+EPA, ALA) but there was evidence of effect modification by race and sex for total seafood intake and total fish intake.

The association between total seafood and HbA1c in diabetic women suggested an adverse effect whereas results for diabetic males showed a non-significant beneficial association. Intake of total fish had similar point estimates, but the interaction term was not significant (figures 3-11 and 3-12).

The association between total seafood and HbA1c and total fish and HbA1c in blacks and whites suggested there may be effect modification by race, although the interaction term was not statistically significant (figures 3-13 and 3-14).

3.4.3 ASSOCIATIONS WITH INCIDENT T2D

As seen in Figure 3-1, there were n=11,874 participants who met the inclusion/exclusion criteria for the incident T2D analysis. Results are reported in tables 3-14 through 3-16. Including protein as a covariate in the regression models did not substantively change our results (data not shown).

3.4.3.1 Normoglycemic and Pre-Diabetic

3.4.3.1.1 Fish/Shellfish Servings and Fish-Derived Omega-3

As shown in tables 3-14 and 3-15, neither the whole food exposures (total seafood, total fish, omega-3 rich fish) nor the fish-derived omega-3 fatty acids DHA+EPA were significantly associated with incident T2D. There was no evidence of effect modification by diabetes status (NGT and Pre-T2D).

3.4.3.1.2 Vegetable-Derived Omega-3 PUFA (ALA) Intake

As seen in Table 3-16, ALA intake was adversely associated with incident T2D. The population*exposure interaction was significant, likely because associations in NGT were strong (Model 3, Q4 vs. Q1, HR=4.0, 95% CI: 1.65, 9.64) and those in Pre-T2D were mostly null (Model 3, Q4 vs. Q1, HR=1.1, 95% CI: 0.75, 1.6).

3.4.3.2 Incident T2D Race and Sex Effect Modifier Analyses

There was no evidence of effect modification by race or sex in the incident T2D analyses.

3.5 DISCUSSION

In this population-based study of middle aged adults, our investigation of the relationship among seafood, omega-3 PUFA, and markers of glycemia is summarized in Table 3-18.

Among those participants who were diabetic, we found consistent evidence that higher intake of fish and shellfish, the fish-derived omega-3 PUFAs DHA+EPA, and the vegetable derived omega-3 PUFA ALA was adversely associated with both FBG and HbA1c. Moreover, there was evidence of effect modification, with adverse associations in blacks and females and non-significant favorable associations in whites and males – although the interaction terms often failed to reach statistical significance.

Among those participants who were not diabetic (NGT and Pre-T2D), we found that while intake of fish and shellfish and of the fish-derived omega-3 PUFA DHA+EPA was associated with lower values of FBG and HbA1c in those with NGT and Pre-T2D, these differences were quite small and of uncertain clinical relevance. Furthermore, while intake of the vegetable-derived omega-3 PUFA ALA was not associated with FBG or HbA1c in those with NGT or Pre-T2D, intake of ALA was associated with higher risk of incident T2D.

3.5.1 DIABETIC POPULATIONS

3.5.1.1 Fish/Shellfish Servings and Fish-Derived Omega-3 PUFA (DHA+EPA) Intake

Our results for seafood intake and intake of fish-derived omega-3 PUFA in diabetics are consistent with other studies. A recent systematic review and meta-analysis evaluated the associations of DHA, EPA, and seafood with incident type 2 diabetes (T2D) and found that consumption of fish-derived omega-3 PUFAs higher risk of T2D in Americans; although risk was reduced in Asians and Australians [86]. Another meta-analysis of cohort studies found that intake of fish and fish-derived omega-3 PUFAs might be weakly positively associated with T2D, especially in American (versus Asian) populations [174].

There are data to support the biological plausibility of fish-derived omega-3 PUFAs DHA and EPA adversely affecting glucose homeostasis in those with T2D. A previous study showed that diabetics who took a FDn3FA supplement had lower glucose utilization (insulin sensitivity) and increased glucagon-stimulated C-peptide [67]. In another trial, diabetic subjects taking fish oil supplements showed reduced hepatic gluconeogenesis [68]. Obese subjects with T2D who took fish oil supplements had increased uptake and oxidation of non-esterified fatty acids in the liver [69]. In another study of obese subjects with T2D, fish oil supplementation increased glycerol

gluconeogenesis, and the authors hypothesized it could cause the deterioration of glycemic control during long-term treatment with high doses of fish-oil supplements [69]. Finally, the associations may be due to contaminants in seafood. Mouse models have shown that elevated blood mercury levels may interrupt insulin signaling pathways, and decrease plasma insulin and elevate blood glucose levels [70].

The relationship between fish and omega-3 PUFAs with FBG and HbA1c in those with T2D was similar but not exact. While higher intakes of omega-3 PUFA (in quartiles) was associated with higher values of both FBG and HbA1c, the relationship across servings of seafood was less consistent (figures 3-15 and 3-16). These differences may be an artifact of residual confounding by total energy intake as, consistent with other researchers, we did not apply the residual method to whole foods [175, 176], although we did adjust for total energy intake. It may also be a result of protein in seafood, as protein has been found to be associated with T2D [172]. However, when we did a sensitivity analysis including total protein (grams/day) as a covariate, the measures of association did not appreciably change. It could be that these differences offer insight into omega-3 PUFAs influence on biological pathways. FBG is a marker for impaired fasting glucose (IFG) while HbA1c can be used to diagnose impaired glucose tolerance (IGT). The progression from NGT to IFG results from a decrease in insulin secretion followed by changes in hepatic insulin sensitivity. In contrast, progression from NGT to IGT is a consequence of low whole-body insulin sensitivity followed by beta cell compensation and exhaustion [177, 178]. The mechanism through which omega-3 PUFA acts on FBG may be similar to the mechanism through which NGT transitions to IFG (decreases in insulin secretion and sensitivity) whereas the mechanism through which omega-3 PUFA acts on HbA1c may be similar to that of NGT to IGT (beta cell exhaustion). Further studies may help determine if differences in association are due to bias, biological mechanism, or due to chance.

3.5.1.2 Effect Modification by Race and Sex in Type 2 Diabetics (Fish/Shellfish Servings)

There was a significant interaction by sex for the associations of servings per week of total seafood (shellfish + total fish) with FBG, with females showing adverse associations and males non-significant favorable associations. This finding was mirrored in the association of total fish intake with FBG and in the associations of HbA1c with total seafood and total fish, although none of these last three findings reached statistical significance.

A recent meta-analysis found evidence of sex-specific differences of omega-3 PUFAs effect insulin resistance, but the relationship was reversed with favorable results in women but not men [179]. Lack of consistency of our results with previous studies should make us cautious in the interpretation of the sex interaction found in the ARIC study.

Results also suggested that there may be effect modification by race for servings per week of total fish and total seafood with FBG and HbA1c – while associations were null for whites and adverse for blacks, all interaction terms were non-significant. Regardless, this finding suggests that the relationship between dietary intake of fish/shellfish and glycemic control may differ by race or region. Because data were not collected on food preparation methods, differences could be due to regional/cultural differences in food preparation.

3.5.1.3 Vegetable-Derived Omega-3 PUFA (ALA) Intake

Previous studies have found null [180-182] or favorable associations with ALA intake and glycemic control in diabetics [183] or those glucose intolerance [184, 185]. Our finding that ALA intake is associated with reductions in glycemic control in diabetics is novel. A potential mechanism to explain our findings is that while ALA is not

efficiently converted to DHA and EPA – only 0.2-0.8% of ALA is converted to EPA and 0-4% of ALA is converted to DHA [186] – this conversion could explain the adverse associations of ALA with glycemic control in diabetics. There is also the potential for a Type 1 error given the number of analyses we performed.

3.5.1.4 Limitations in Diabetic Population Sub-Studies

The results in the diabetic populations should be interpreted with caution. Individuals with diabetes lack the feedback mechanisms that regulate insulin and glucose levels, and daily variance in glucose measures can be influenced by medication type and adherence, lifestyle habits like diet, exercise, sleep hygiene, and stress management, and clinician protocols. We attempted to adjust for confounding by including physical activity and education level (proxy for socioeconomic status) in our regression models, but there may still be residual confounding. Additionally, there is likely unmeasured confounding by sleep quality, stress level, provider-specific treatment plans, non-diabetic mediation use, diet quality, and other variables.

FBG is also influenced by fasting status. Among diabetics that met the FBG inclusion/exclusion criteria, self-reported 8-hour fasting compliance ranged from 86.2% (visit 3) to 91.2% (visit 2), with an overall average of 88.3%. Self-reported 12-hour fasting compliance was similar, with a minimum of 81.2% compliance (visit 4) and a maximum of 85.6 (visit 2), with an overall average of 83.7%. When we limited our analyses to those who reported fasting for at least 12 hours, results were not substantively different.

We also could not account for intra-individual variability in glucose measurements, and studies have shown daily variation in FBG levels can be 15% or more [187]. Even if we were to assume that any single FBG measure falls within an individual's range, and that the distribution of measurements in the lower, medium, and

upper parts of individuals' ranges were nondifferential across exposure categories, that quasi-misclassification could bias our estimates away from the null [188, 189].

Despite these limitations, our results for FBG and HbA1c were relatively consistent across exposures, with diabetics consuming greater amounts of omega-3 PUFAs having higher FBG and HbA1c values than diabetics who consumed less. We also performed sensitivity analyses where diabetic participants were stratified based on antihyperglycemic medication use. Results in the subpopulations were not different than those found in the full diabetic population. Further studies designed to address the limitations described could provide additional insight into the relationship between seafood and omega-3 PUFA intake and glycemic control in those with diabetes.

3.5.2 NORMOGLYCEMIC AND PRE-DIABETIC POPULATIONS

3.5.2.1 Fish/Shellfish Servings and Fish-Derived Omega-3 PUFA (DHA+EPA) Intake

While the effect of fish, shellfish, and DHA+EPA was favorable but modest in non-diabetics, this is consistent with other studies that have shown fish oil improves insulin secretion, resistance, and sensitivity in non-diabetics [190-192].

3.5.2.2 Vegetable-Derived Omega-3 PUFA (ALA) Intake

Our findings regarding ALA and incident T2D are novel. With respect to its association with incident diabetes, previous meta-analysis studies have found that higher intake of ALA was not associated with incident T2D [85, 174] or that it was associated with reduced risk of T2D in Asian populations but not in Americans [86]. There is also the potential for a Type 1 error given the number of analyses we performed.

3.6 STRENGTHS AND LIMITATIONS

This study has many strengths. It is a population-based, biracial cohort of participants who were followed over several years with multiple measures of exposures, outcomes, and covariates. Furthermore, we could study the same individuals over time as some moved among NGT, Pre-T2D, and T2D, provided they met our inclusion/exclusion criteria. Finally, we had multiple markers of glucose homeostasis.

However, our study is not without limitations. There are the limitations we listed in section 3.5.1.4 that are specific to our diabetes sub-population analyses. Furthermore, with dietary data, there is always the potential for misclassification bias. FFQs have been shown to underestimate total caloric intake when compared to doubly labeled water, and our dietary data were based on FFQ [193]. Additionally, data were not available on fish preparation technique. Analysis in the Cardiovascular Health Study have shown that fish preparation method differentially effects the association between fish-derived omega-3 PUFAs and CHD, with only intake of tuna and other baked or broiled fish associated with cardiovascular benefits, with no or deleterious associations for fried fish or fish sandwiches [8]. Fish preparation technique may also effect the relationship among seafood, fish-derived and vegetable-derived omega-3 PUFAs, and markers of glucose homeostasis. A similar hypothesis was suggested by Muley et al. [86] – that different preparation methods may results in differential effects; and while that study was focused on country-specific differences, there are differences in preparation techniques among different regions of the United States as well. Our FFQ rolled up fish into very broad categories – different types of fatty fish may have different composition of fish-derived omega-3 FA which may significantly alter the overall effect of fish. As previously mentioned, the range of seafood intake in the ARIC population was limited. Finally, there is potential for unmeasured confounding.

3.7 CONCLUSION

In summary, our results suggest that higher dietary intake of omega-3 fatty acids is associated with higher values of FBG and HbA1c amongst diabetics, and with greater risk of incident T2D amongst non-diabetics.

3.8 FIGURES

Exclusion Criteria	Visit 1	Visit 2	Visit 3	Visit 4
Cohort	15,792	15,792	15,792	15,792
Not at Visit	-	1,444	2,905	4,136
Race Criteria	103	91	80	69
Covariate Exclusions	1,042	888	2,238	2,248
Prevalent CVD	1,443	962	845	1,963
Outcome Exclusions	31	67	14	68
Final Visit N (FBG)	13,173	12,340	9,710	7,308

HbA1c Outcome	N
Met FBG criteria @ Visit 1	13,173
Not at Visit 2	833
No HbA1c value	765
Final HbA1c dataset	**11,575**

Incident T2D Outcome	N
Met FBG criteria @ Visit 1	13,173
Prevalent T2D	1,299
Final Incident T2D Dataset	**11,874**

Figure 3-1. Inclusion Exclusion Criteria, ARIC, 1987-1998.

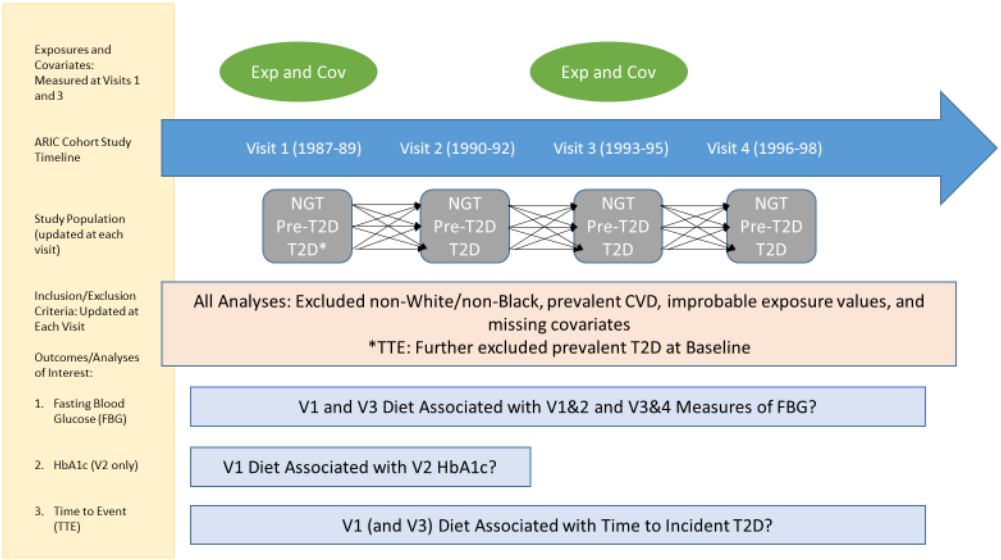

Figure 3-2. Outcome-specific study design depiction with variable temporality, ARIC, 1987-1998.

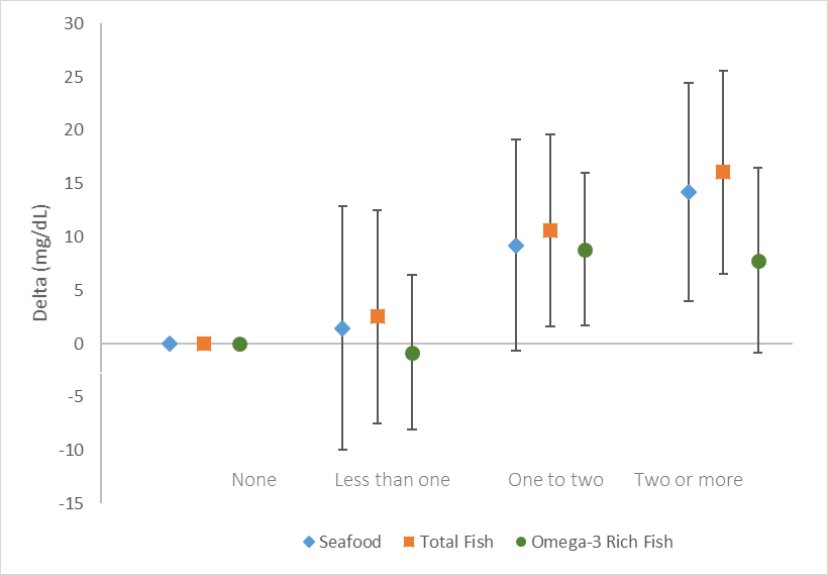

Figure 3-3. Associations among seafood types (servings/week) and FBG (mg/dL) in those with diabetes (Model 3), ARIC, 1987-1998.

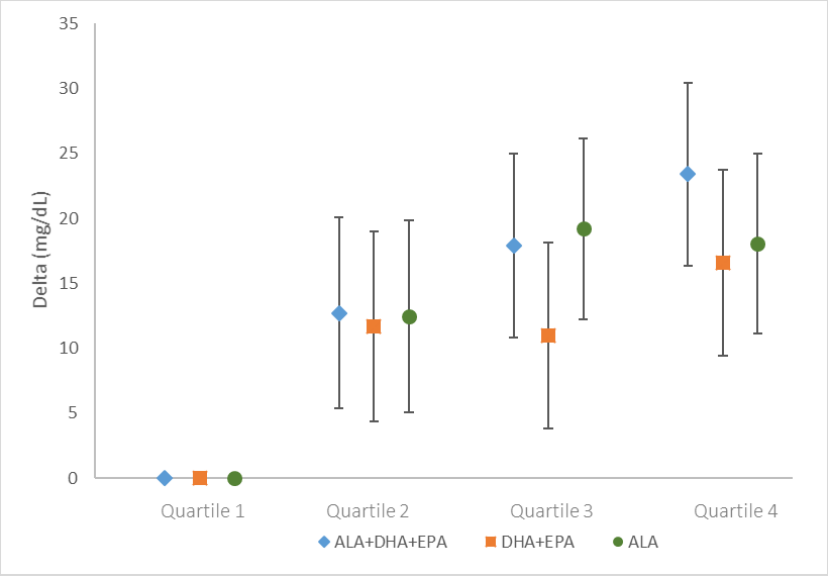

Figure 3-4. Associations among omega-3 PUFA (quartiles) and FBG (mg/dL) in those with diabetes (Model 3), ARIC, 1987-1998.

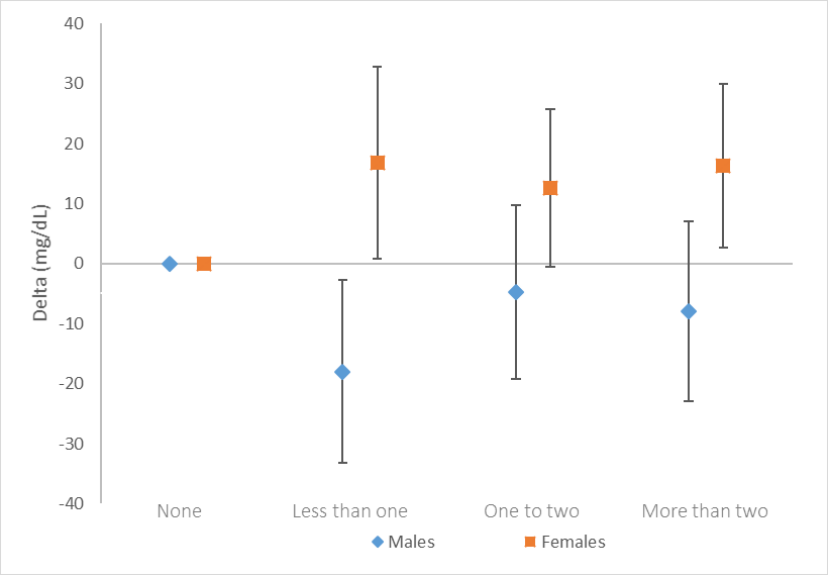

Figure 3-5. Sex modifies the associations between total seafood (servings/week) and FBG (mg/dL) in those with diabetes (Model 3), p for interaction = 0.01, ARIC, 1987-1998.

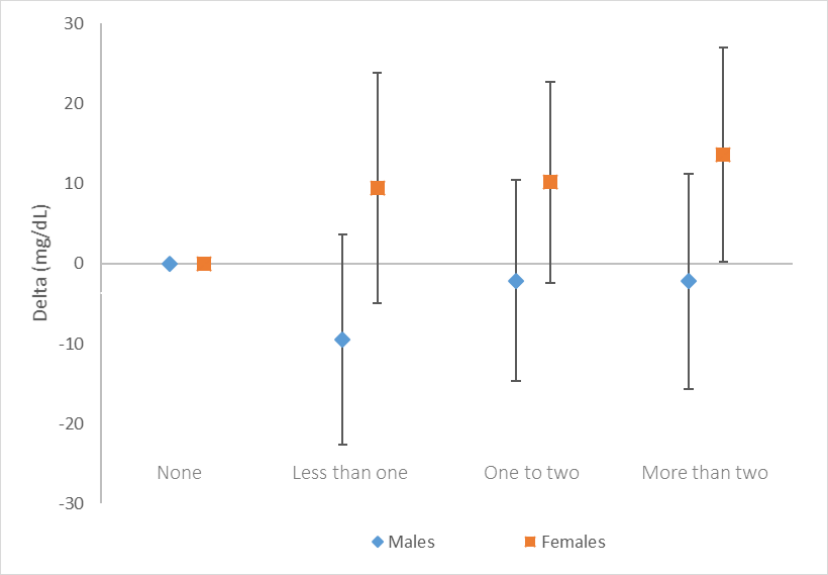

Figure 3-6. Sex modifies the associations between total fish (servings/week) and FBG (mg/dL) in those with diabetes (Model 3), p for interaction = 0.26, ARIC, 1987-1998.

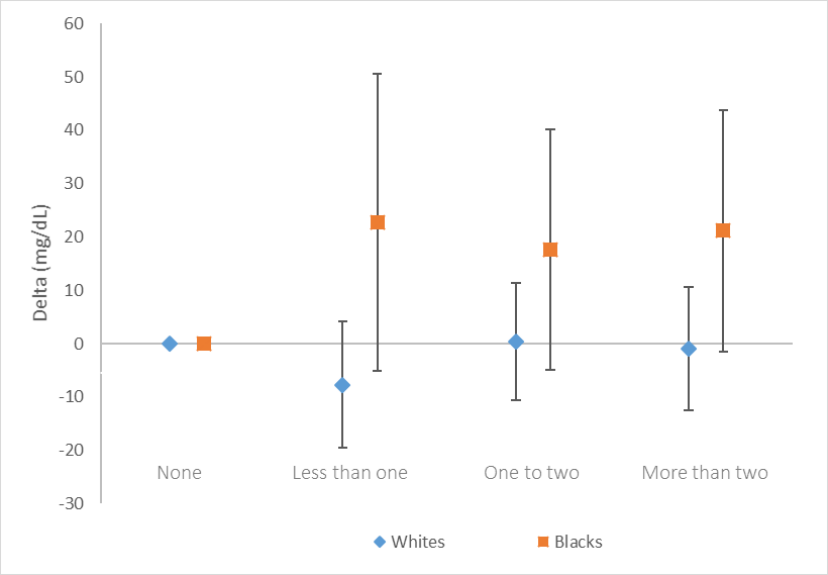

Figure 3-7. Race modifies the associations between total seafood (servings/week) and FBG (mg/dL) in those with diabetes (Model 3), p for interaction = 0.20, ARIC, 1987-1998.

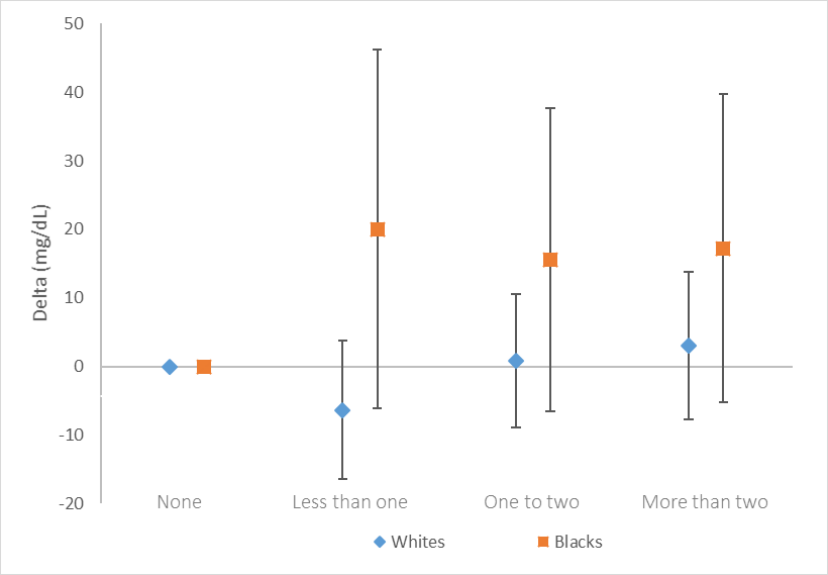

Figure 3-8. Race modifies the associations between total fish (servings/week) and FBG (mg/dL) in those with diabetes (Model 3), p for interaction = 0.32, ARIC, 1987-1998.

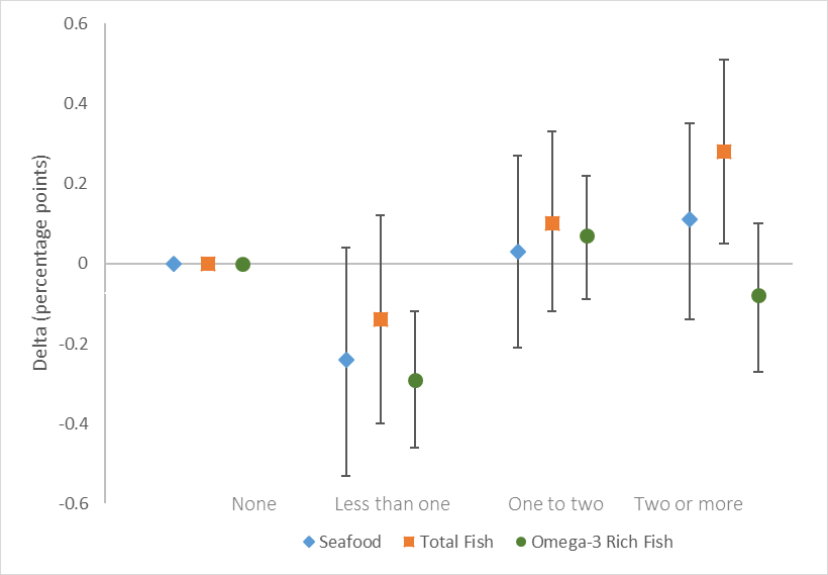

Figure 3-9. Associations among seafood types (servings/week) and HbA1c in those with diabetes (Model 3), ARIC, 1987-1993.

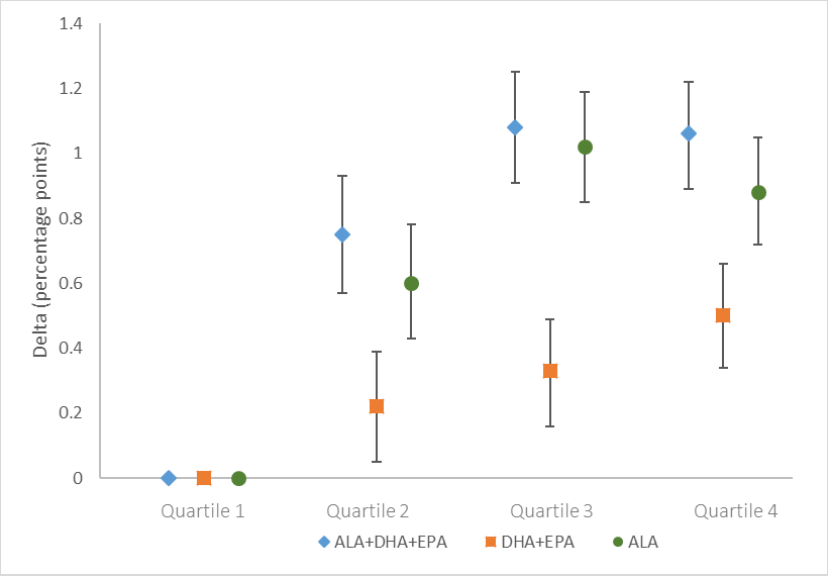

Figure 3-10. Associations among omega-3 PUFA (quartiles) and HbA1c in those with diabetes (Model 3), ARIC, 1987-1993.

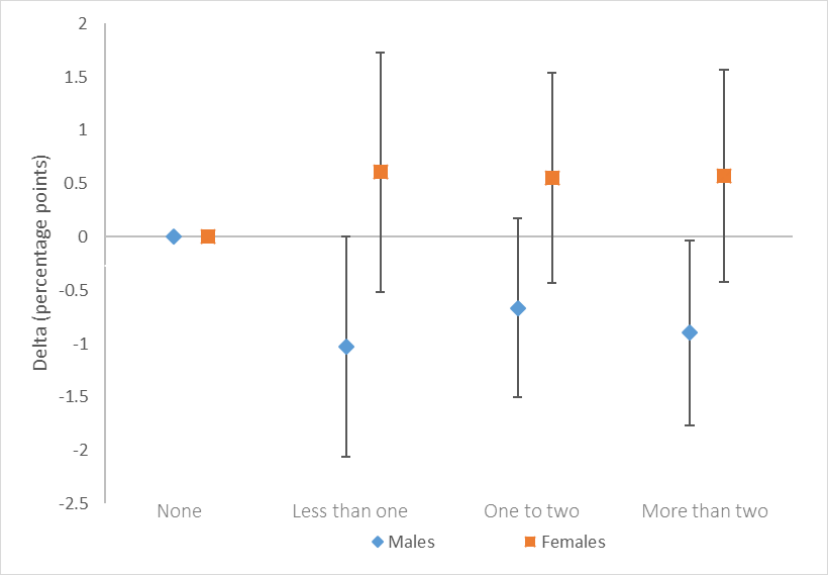

Figure 3-11. Sex modifies the associations between total seafood (servings/week) and HbA1c in those with diabetes (Model 3), p for interaction = 0.14, ARIC, 1987-1993.

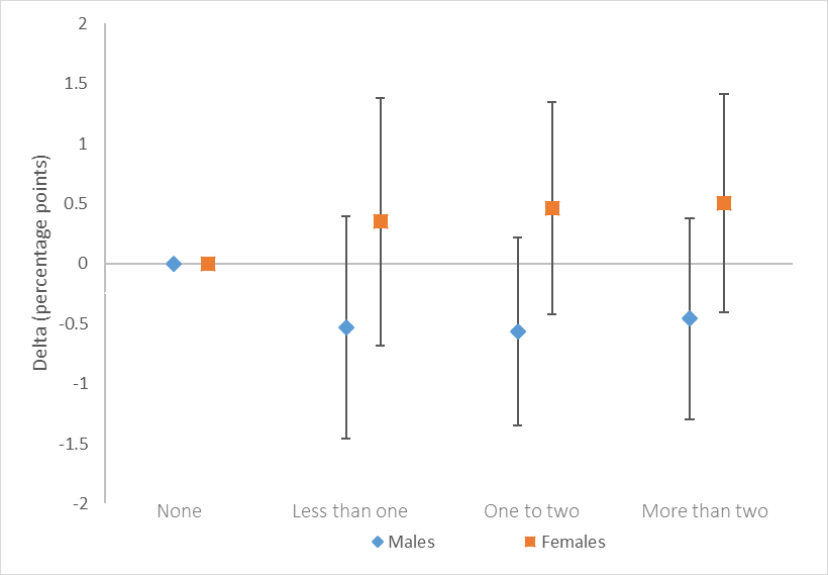

Figure 3-12. Sex modifies the associations between total fish (servings/week) and HbA1c in those with diabetes (Model 3), p for interaction = 0.40, ARIC, 1987-1993.

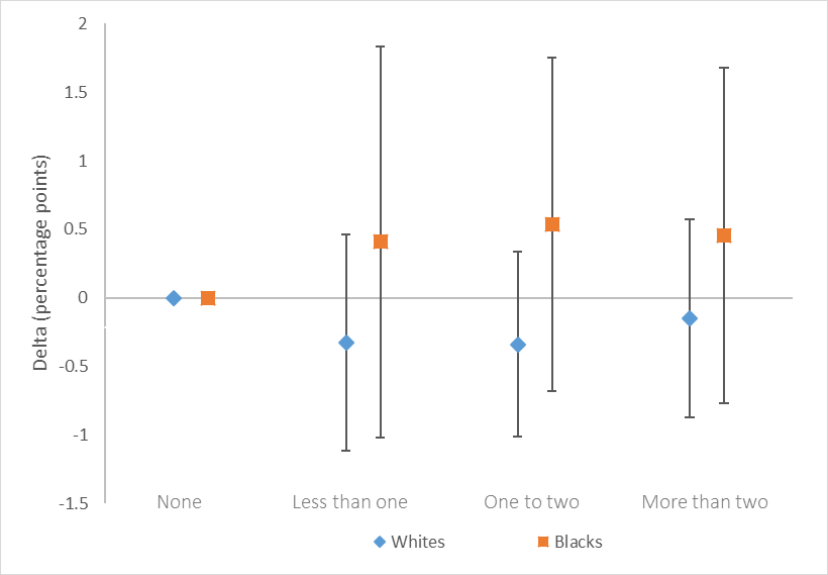

Figure 3-13. Race modifies the associations between total seafood (servings/week) and HbA1c in those with diabetes (Model 3), p for interaction = 0.75, ARIC, 1987-1993.

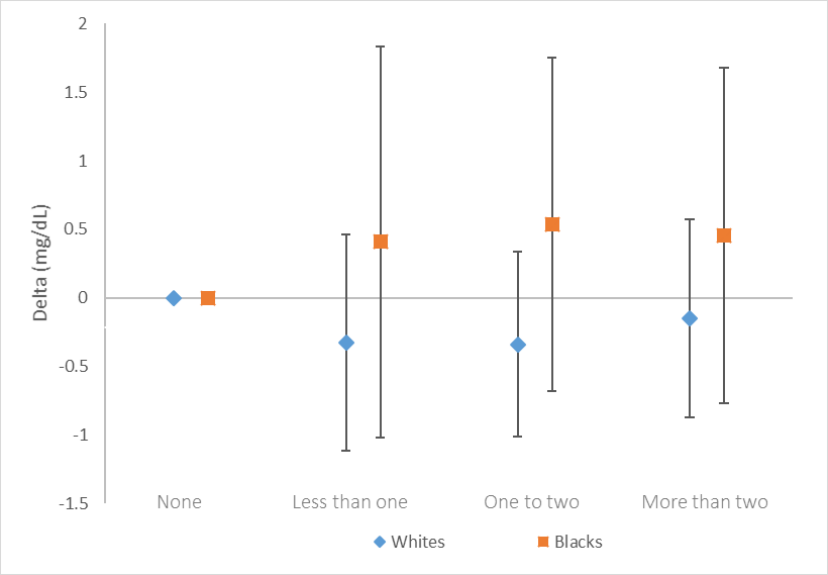

Figure 3-14. Race modifies the associations between total fish (servings/week) and HbA1c in those with diabetes (Model 3), p for interaction = 0.60, ARIC, 1987-1993.

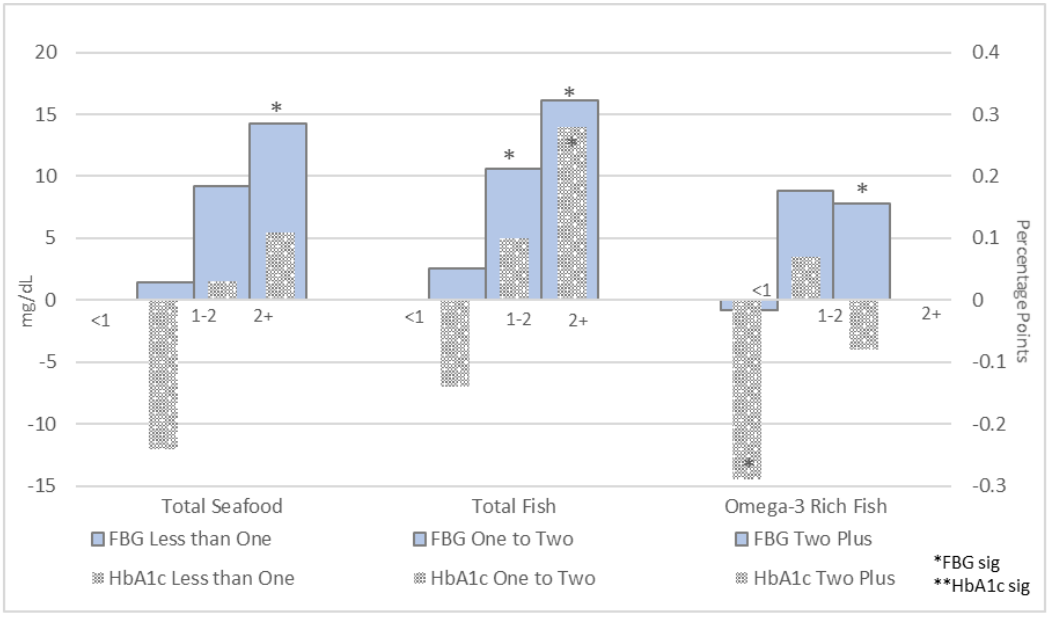

Figure 3-15. Deltas for FBG (mg/dL) and HbA1c (percentage points) across servings of total seafood, total fish, and total omega-3 rich fish in those with diabetes; fully adjusted model; ARIC, 1987-93 (HbA1c), 1987-1998 (FBG).

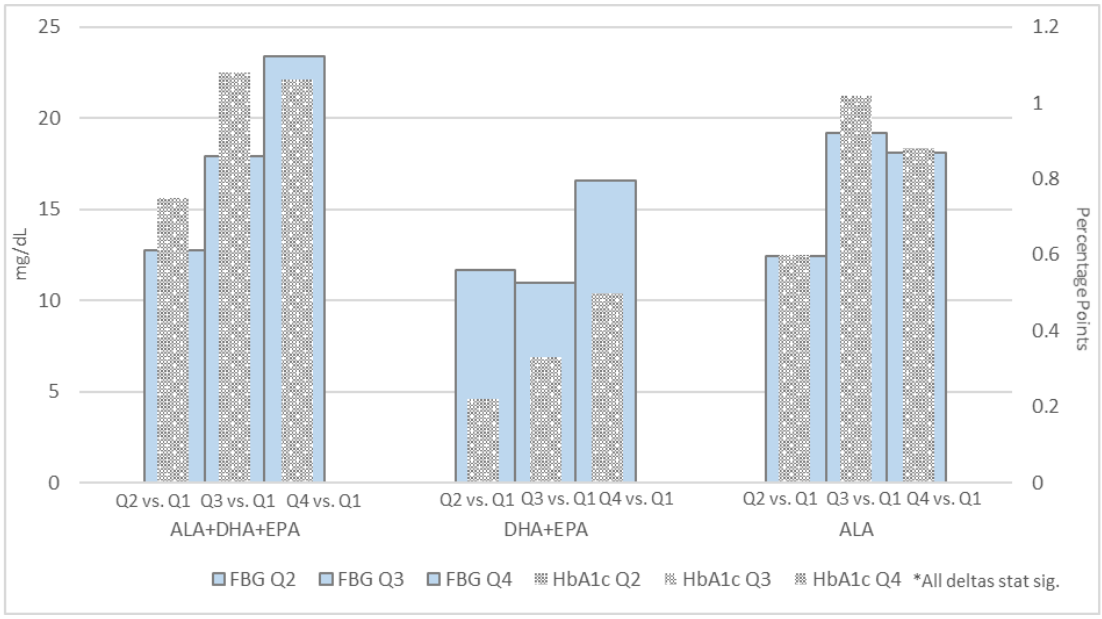

Figure 3-16. Deltas for FBG (mg/dL) and HbA1c (percentage points) across quartiles of ALA+DHA+EPA, DHA+EPA, and ALA in those with diabetes; fully adjusted model; ARIC, 1987-93 (HbA1c), 1987-1998 (FBG).

3.9 TABLES

Table 3-1. Baseline characteristics of ARIC participants (n=13,173) by weekly servings of seafood (total fish + shellfish), 1987–1989. Values correspond to mean (SD) or N (%)

	Zero Svgs/Week	Less Than 1 Svgs/Week	One to Two Svgs/Week	Two or More Svgs/Week
N	989 (7.5%)	1,624 (12.3%)	6,046 (45.9%)	4,514 (34.3%)
Sociodemographic Covariates				
Age (years)	54.7 (5.8)	54.0 (5.8)	53.9 (5.7)	53.8 (5.7)
Male	535 (54.1%)	762 (46.9%)	2,678 (44.3%)	1,785 (39.5%)
White	903 (91.3%)	1,419 (87.4%)	4,597 (76.0%)	2,951 (65.4%)
Education				
Basic	272 (27.5%)	313 (19.3%)	1,338 (22.1%)	956 (21.2%)
Intermediate	440 (44.5%)	779 (48.0%)	2,529 (41.8%)	1,706 (37.8%)
Advanced	277 (28.0%)	532 (32.8%)	2,179 (36.0%)	1,852 (41.0%)
Lifestyle Covariates				
BMI (kg/m^2)	26.7 (4.9)	26.8 (4.7)	27.3 (5.2)	28.0 (5.4)
Physical Activity^				
Low	359 (36.3%)	578 (35.6%)	2,112 (34.9%)	1,418 (31.4%)
Medium	317 (32.1%)	551 (33.9%)	2,035 (33.7%)	1,514 (33.5%)
High	313 (31.6%)	495 (30.5%)	1,899 (31.4%)	1,582 (35.0%)
Current Smokers	273 (27.6%)	439 (27.0%)	1,562 (25.8%)	1,096 (24.3%)
Current Alcohol Drinkers	517 (52.3%)	991 (61.0%)	3,551 (58.7%)	2,523 (55.9%)
Dietary Covariates				
Total Energy Intake (kcal/day)	1,519 (610)	1,522 (582)	1,583 (579)	1,752 (621)
Cereal Fiber (grams/day)	3.4 (2.5)	3.3 (2.2)	3.4 (2.2)	3.8 (2.5)
Dietary Fiber (grams/day)	15.0 (8.2)	14.8 (6.8)	16.2 (7.3)	19.7 (8.8)
Saturated Fats (grams/day)	21.5 (10.6)	22.0 (10.8)	21.9 (10.5)	22.6 (10.6)
Trans Fats (grams/day)	3.0 (1.9)	3.0 (1.8)	3.0 (1.8)	2.9 (1.8)
Clinical Covariates				
Hypertension	989 (25.9%)	1,624 (27.1%)	6,046 (30.5%)	4,514 (34.0%)
Systolic Blood Pressure, mmHg	120.0 (17.2)	119.6 (17.2)	120.6 (18.4)	121.3 (19.0)
Diastolic Blood Pressure, mmHg	72.4 (10.4)	72.7 (10.8)	73.5 (11.0)	74.0 (11.4)
High Density Lipoprotein Cholesterol, mg/dL	50.2 (15.7)	51.8 (16.7)	52.5 (16.9)	53.5 (17.3)
Low Density Lipoprotein Cholesterol, mg/dL	137.5 (15.7)	135.5 (38.1)	136.9 (38.7)	138.0 (40.4)
Triglycerides, mg/dL	126.8 (63.8)	124.2 (64.7)	122.2 (62.7)	122.4 (64.1)

*Svgs=Servings

^Work+Leisure+Sport Averaged and Divided into Low/Medium/High

Table 3-2. Associations of seafood consumption (servings/week) with fasting blood glucose in participants who are normoglycemic, ARIC, 1987-1998. Mg/dL deltas and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N (all Visits)	1,809	3,015	9,400	6,349			
	FBG Mean (Std)	92.5 (5.3)	92.3 (5.5)	92.5 (5.2)	92.6 (5.3)			
	Model 1	0 (ref)	-0.3 (-0.7, 0.1)	-0.6 (-1.0, -0.2)	-0.9 (-1.4, -0.5)	0.001	0.0004	0.003
	Model 2	0 (ref)	-0.3 (-0.7, 0.1)	-0.6 (-1.0, -0.2)	-1.1 (-1.7, -0.6)	0.0003	0.0001	0.003
	Model 3	0 (ref)	-0.3 (-0.7, 0.1)	-0.6 (-1.0, -0.2)	-1.1 (-1.6, -0.5)	0.001	0.0003	0.003
Total Fish	Total N (all Visits)	2,275	3,637	9,549	5,112			
	FBG Mean (Std)	92.5 (5.3)	92.4 (5.4)	92.4 (5.2)	92.6 (5.3)			
	Model 1	0 (ref)	-0.3 (-0.6, 0.1)	-0.7 (-1.1, -0.4)	-1.0 (-1.5, -0.5)	<.0001	<.0001	0.001
	Model 2	0 (ref)	-0.3 (-0.6, 0.1)	-0.8 (-1.2, -0.4)	-1.3 (-1.8, -0.7)	<.0001	<.0001	0.001
	Model 3	0 (ref)	-0.2 (-0.6, 0.1)	-0.7 (-1.1, -0.4)	-1.2 (-1.7, -0.7)	<.0001	<.0001	0.001
Omega-3 Rich Fish	Total N (all Visits)	4,562	5,525	7,506	2,980			
	FBG Mean (Std)	92.6 (5.3)	92.4 (5.3)	92.4 (5.3)	92.6 (5.3)			
	Model 1	0 (ref)	-0.1 (-0.4, 0.2)	-0.3 (-0.6, 0.0)	-0.3 (-0.8, 0.1)	0.19	0.08	0.02
	Model 2	0 (ref)	-0.1 (-0.4, 0.2)	-0.4 (-0.7, -0.1)	-0.7 (-1.2, -0.2)	0.03	0.01	0.02
	Model 3	0 (ref)	0.0 (-0.3, 0.3)	-0.4 (-0.7, 0.0)	-0.5 (-1.0, 0.0)	0.05	0.02	0.01

FBG: fasting blood glucose
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-3. Associations of fatty acid intake (in quartiles) with fasting blood glucose in participants who are normoglycemic, ARIC, 1987-1998. Mg/dL deltas and 95% confidence intervals from generalized estimating equations.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N (all Visits)	5,265	5,296	5,152	4,860			
	FBG Mean (Std)	92.5 (5.3)	92.5 (5.2)	92.5 (5.3)	92.4 (5.3)			
	Model 1	0 (ref)	0.0 (-0.3, 0.3)	-0.3 (-0.6, 0.0)	-0.6 (-0.9, -0.3)	0.0003	<.0001	<.0001
	Model 2	0 (ref)	-0.1 (-0.4, 0.2)	-0.5 (-0.9, -0.2)	-1.0 (-1.4, -0.5)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	0.0 (-0.4, 0.3)	-0.4 (-0.8, -0.1)	-1.0 (-1.4, -0.5)	<.0001	<.0001	<.0001
DHA + EPA	Total N (all Visits)	5,220	5,303	5,072	4,978			
	FBG Mean (Std)	92.7 (5.3)	92.4 (5.3)	92.4 (5.3)	92.5 (5.3)			
	Model 1	0 (ref)	-0.5 (-0.9, -0.2)	-0.9 (-1.2, -0.5)	-1.1 (-1.5, -0.7)	<.0001	<.0001	0.0002
	Model 2	0 (ref)	-0.6 (-0.9, -0.3)	-1.0 (-1.3, -0.6)	-1.4 (-1.8, -0.9)	<.0001	<.0001	0.0002
	Model 3	0 (ref)	-0.6 (-0.9, -0.2)	-1.0 (-1.3, -0.6)	-1.3 (-1.7, -0.8)	<.0001	<.0001	0.0001
ALA	Total N (all Visits)	5,311	5,330	5,032	4,900			
	FBG Mean (Std)	92.5 (5.4)	92.4 (5.3)	92.5 (5.2)	92.5 (5.3)			
	Model 1	0 (ref)	-0.1 (-0.3, 0.2)	0.0 (-0.3, 0.3)	0.1 (-0.2, 0.3)	0.84	0.28	<.0001
	Model 2	0 (ref)	0.0 (-0.4, 0.3)	0.0 (-0.4, 0.5)	0.1 (-0.5, 0.7)	0.87	0.56	<.0001
	Model 3	0 (ref)	-0.1 (-0.4, 0.3)	0.1 (-0.3, 0.5)	0.2 (-0.4, 0.8)	0.59	0.41	<.0001

FBG: fasting blood glucose
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-4. Associations of seafood consumption (servings/week) with fasting blood glucose in participants with pre-diabetes, ARIC, 1987-1998. Mg/dL deltas and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N (all Visits)	1,309	2,142	7,334	5,008			
	FBG Mean (Std)	107.6 (6.2)	107.9 (6.2)	108.0 (6.3)	107.9 (6.3)			
	Model 1	0 (ref)	0.2 (-0.2, 0.7)	-0.2 (-0.7, 0.2)	-0.8 (-1.3, -0.3)	0.0003	<.0001	0.003
	Model 2	0 (ref)	0.3 (-0.2, 0.7)	-0.3 (-0.7, 0.2)	-1.0 (-1.6, -0.4)	<.0001	<.0001	0.003
	Model 3	0 (ref)	0.3 (-0.2, 0.7)	-0.2 (-0.7, 0.3)	-1.0 (-1.6, -0.4)	0.0001	<.0001	0.003
Total Fish	Total N (all Visits)	1,680	2,610	7,447	4,056			
	FBG Mean (Std)	107.8 (6.2)	107.9 (6.2)	108.0 (6.3)	107.9 (6.3)			
	Model 1	0 (ref)	-0.1 (-0.5, 0.4)	-0.5 (-1.0, -0.1)	-1.1 (-1.6, -0.5)	<.0001	<.0001	0.001
	Model 2	0 (ref)	-0.1 (-0.5, 0.4)	-0.6 (-1.0, -0.2)	-1.3 (-1.9, -0.7)	<.0001	<.0001	0.001
	Model 3	0 (ref)	-0.1 (-0.5, 0.4)	-0.6 (-1.0, -0.1)	-1.3 (-1.9, -0.7)	<.0001	<.0001	0.001
Omega-3 Rich Fish	Total N (all Visits)	3,585	4,116	5,849	2,243			
	FBG Mean (Std)	108.0 (6.3)	107.9 (6.2)	108.0 (6.3)	107.7 (6.2)			
	Model 1	0 (ref)	0.1 (-0.3, 0.4)	-0.1 (-0.5, 0.2)	-0.5 (-0.9, 0.0)	0.20	0.04	0.02
	Model 2	0 (ref)	0.0 (-0.3, 0.4)	-0.3 (-0.6, 0.1)	-0.8 (-1.3, -0.3)	0.03	0.002	0.02
	Model 3	0 (ref)	0.1 (-0.3, 0.4)	-0.2 (-0.6, 0.1)	-0.8 (-1.3, -0.3)	0.02	0.003	0.01

FBG: fasting blood glucose
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-5. Associations of fatty acid intake (in quartiles) with fasting blood glucose in those with pre-diabetes, ARIC, 1987-1998. Mg/dL deltas and 95% confidence intervals from generalized estimating equations.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N (all Visits)	4,198	3,926	3,830	3,839			
	FBG Mean (Std)	107.9 (6.3)	107.8 (6.1)	108.0 (6.3)	108.0 (6.3)			
	Model 1	0 (ref)	-0.3 (-0.6, 0.1)	-0.2 (-0.6, 0.1)	-0.6 (-1.0, -0.2)	0.0004	<.0001	<.0001
	Model 2	0 (ref)	-0.5 (-0.8, -0.1)	-0.5 (-1.0, -0.1)	-1.1 (-1.6, -0.6)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	-0.4 (-0.8, 0.0)	-0.4 (-0.9, 0.0)	-1.0 (-1.5, -0.5)	<.0001	<.0001	<.0001
DHA + EPA	Total N (all Visits)	4,071	3,985	3,886	3,851			
	FBG Mean (Std)	107.9 (6.2)	107.9 (6.3)	107.9 (6.2)	107.9 (6.3)			
	Model 1	0 (ref)	-0.3 (-0.7, 0.1)	-0.8 (-1.2, -0.3)	-1.0 (-1.5, -0.6)	<.0001	<.0001	0.0002
	Model 2	0 (ref)	-0.4 (-0.7, 0.0)	-0.9 (-1.4, -0.5)	-1.3 (-1.8, -0.8)	<.0001	<.0001	0.0002
	Model 3	0 (ref)	-0.3 (-0.7, 0.1)	-0.9 (-1.3, -0.4)	-1.3 (-1.8, -0.8)	<.0001	<.0001	0.0001
ALA	Total N (all Visits)	4,128	3,993	3,855	3,817			
	FBG Mean (Std)	108.0 (6.3)	107.7 (6.2)	108.0 (6.3)	107.9 (6.2)			
	Model 1	0 (ref)	-0.4 (-0.7, 0.0)	-0.1 (-0.5, 0.2)	-0.1 (-0.5, 0.2)	0.45	0.56	<.0001
	Model 2	0 (ref)	-0.4 (-0.8, 0.0)	-0.2 (-0.7, 0.3)	-0.1 (-0.8, 0.5)	0.34	0.51	<.0001
	Model 3	0 (ref)	-0.4 (-0.8, 0.0)	-0.1 (-0.6, 0.4)	0.0 (-0.6, 0.6)	0.31	0.63	<.0001

FBG: fasting blood glucose
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-6. Associations of seafood consumption (servings/week) with fasting blood glucose in those with diabetes, ARIC, 1987-1998. Mg/dL deltas and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N (all Visits)	334	535	2,158	1,756			
	FBG Mean (Std)	174.8 (71.7)	176.5 (72.5)	185.0 (76.0)	190.7 (79.4)			
	Model 1	0 (ref)	1.4 (-10.1, 12.8)	9.4 (-0.5, 19.3)	14.6 (4.3, 24.8)	<.0001	<.0001	0.003
	Model 2	0 (ref)	1.3 (-10.1, 12.7)	9.3 (-0.6, 19.2)	14.3 (4.0, 24.5)	<.0001	<.0001	0.003
	Model 3	0 (ref)	1.5 (-9.9, 12.9)	9.2 (-0.7, 19.1)	14.2 (4.0, 24.5)	<.0001	<.0001	0.003
Total Fish	Total N (all Visits)	406	644	2,250	1,483			
	FBG Mean (Std)	173.9 (71.1)	176.9 (72.0)	185.8 (76.8)	191.8 (79.5)			
	Model 1	0 (ref)	2.5 (-7.5, 12.6)	10.8 (1.8, 19.8)	16.5 (6.9, 26.0)	<.0001	<.0001	0.001
	Model 2	0 (ref)	2.5 (-7.5, 12.5)	10.7 (1.7, 19.7)	16.1 (6.6, 25.6)	<.0001	<.0001	0.001
	Model 3	0 (ref)	2.5 (-7.4, 12.5)	10.6 (1.6, 19.6)	16.1 (6.5, 25.6)	<.0001	<.0001	0.001
Omega-3 Rich Fish	Total N (all Visits)	992	1,130	1,878	783			
	FBG Mean (Std)	180.7 (77.5)	179.9 (73.1)	189.8 (77.8)	189.1 (77.9)			
	Model 1	0 (ref)	-0.5 (-7.8, 6.8)	9.3 (2.2, 16.5)	8.6 (-0.1, 17.3)	0.01	0.03	0.02
	Model 2	0 (ref)	-0.7 (-7.9, 6.6)	9.0 (1.9, 16.2)	8.0 (-0.7, 16.7)	0.002	0.003	0.02
	Model 3	0 (ref)	-0.8 (-8.1, 6.4)	8.8 (1.7, 16.0)	7.8 (-0.9, 16.5)	0.003	0.01	0.01

FBG: fasting blood glucose
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-7. Associations of fatty acid intake (in quartiles) with fasting blood glucose in those with diabetes, ARIC, 1987-1998. Mg/dL deltas and 95% confidence intervals from generalized estimating equations.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N (all Visits)	856	1,086	1,351	1,490			
	FBG Mean (Std)	169.2 (68.4)	182.9 (77.8)	188.3 (76.3)	194.1 (79.4)			
	Model 1	0 (ref)	13.1 (5.7, 20.5)	18.3 (11.2, 25.4)	23.9 (16.9, 31.0)	<.0001	<.0001	<.0001
	Model 2	0 (ref)	12.9 (5.5, 20.3)	18.0 (10.9, 25.1)	23.4 (16.4, 30.5)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	12.7 (5.4, 20.1)	17.9 (10.8, 25.0)	23.4 (16.3, 30.4)	<.0001	<.0001	<.0001
DHA + EPA	Total N (all Visits)	971	1,056	1,320	1,436			
	FBG Mean (Std)	173.8 (69.7)	186.3 (77.8)	186.1 (78.3)	192.1 (78.4)			
	Model 1	0 (ref)	11.7 (4.4, 19.1)	11.1 (4.0, 18.3)	16.9 (9.7, 24.1)	<.0001	<.0001	0.0002
	Model 2	0 (ref)	11.7 (4.4, 19.0)	11.1 (3.9, 18.3)	16.5 (9.4, 23.7)	<.0001	<.0001	0.0002
	Model 3	0 (ref)	11.7 (4.3, 19.0)	11.0 (3.8, 18.1)	16.5 (9.4, 23.7)	<.0001	<.0001	0.0001
ALA	Total N (all Visits)	909	1,031	1,368	1,475			
	FBG Mean (Std)	171.5 (69.6)	184.0 (76.9)	191.2 (79.2)	189.8 (77.7)			
	Model 1	0 (ref)	12.3 (4.9, 19.7)	19.2 (12.2, 26.2)	17.9 (11.0, 24.8)	<.0001	<.0001	<.0001
	Model 2	0 (ref)	12.4 (5.0, 19.8)	19.3 (12.3, 26.3)	18.0 (11.0, 24.9)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	12.4 (5.0, 19.8)	19.2 (12.2, 26.2)	18.1 (11.2, 25.0)	<.0001	<.0001	<.0001

FBG: fasting blood glucose
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-8. Associations of seafood consumption (servings/week) with HbA1c in participants who are normoglycemic, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N	495	792	2,862	2,116			
	HbA1c Mean (Std)	5.4 (0.5)	5.3 (0.5)	5.4 (0.4)	5.4 (0.5)			
	Model 1	0 (ref)	0.0 (-0.1, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)	0.07	0.0244	0.002
	Model 2	0 (ref)	0.0 (-0.1, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)	0.07	0.02	0.005
	Model 3	0 (ref)	0.0 (-0.1, 0.1)	-0.1 (-0.2, 0.0)	-0.1 (-0.2, 0.0)	0.07	0.0155	0.005
Total Fish	Total N	614	999	2,927	1,725			
	HbA1c Mean (Std)	5.4 (0.5)	5.3 (0.5)	5.4 (0.5)	5.4 (0.5)			
	Model 1	0 (ref)	0.0 (-0.1, 0.1)	-0.1 (-0.1, 0.0)	-0.1 (-0.2, 0.0)	0.08	0.02	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.1)	-0.1 (-0.1, 0.0)	-0.1 (-0.2, 0.0)	0.06	0.01	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.1)	-0.1 (-0.1, 0.0)	-0.1 (-0.2, 0.0)	0.06	0.01	<.0001
Omega-3 Rich Fish	Total N	1,309	1,601	2,347	1,008			
	HbA1c Mean (Std)	5.4 (0.5)	5.4 (0.4)	5.4 (0.5)	5.4 (0.4)			
	Model 1	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.36	0.10	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.31	0.07	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.33	0.08	<.0001

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-9. Associations of fatty acid intake (in quartiles) with HbA1c in participants who are normoglycemic, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N	1,570	1,614	1,564	1,517			
	HbA1c Mean (Std)	5.3 (0.4)	5.4 (0.4)	5.4 (0.4)	5.4 (0.5)			
	Model 1	0 (ref)	0.0 (0.0, 0.1)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.84	0.60	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.31	0.06	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	0.31	0.06	<.0001
DHA + EPA	Total N	1,600	1,584	1,547	1,534			
	HbA1c Mean (Std)	5.4 (0.5)	5.4 (0.4)	5.4 (0.5)	5.4 (0.4)			
	Model 1	0 (ref)	-0.1 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	-0.1 (-0.2, 0.0)	0.04	0.02	<.0001
	Model 2	0 (ref)	-0.1 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	-0.1 (-0.2, 0.0)	0.03	0.01	<.0001
	Model 3	0 (ref)	-0.1 (-0.1, 0.0)	-0.1 (-0.1, 0.0)	-0.1 (-0.2, 0.0)	0.03	0.01	<.0001
ALA	Total N	1,562	1,633	1,547	1,523			
	HbA1c Mean (Std)	5.3 (0.4)	5.4 (0.4)	5.4 (0.4)	5.4 (0.6)			
	Model 1	0 (ref)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.1 (0.0, 0.1)	0.28	0.09	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.62	0.66	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.60	0.67	<.0001

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-10. Associations of seafood consumption (servings/week) with HbA1c in participants with pre-diabetes, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N	313	531	2,039	1,426			
	HbA1c Mean (Std)	5.6 (0.6)	5.5 (0.5)	5.7 (0.7)	5.7 (0.8)			
	Model 1	0 (ref)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.13	0.07	0.002
	Model 2	0 (ref)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.16	0.06	0.005
	Model 3	0 (ref)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.15	0.05	0.005
Total Fish	Total N	409	663	2,051	1,186			
	HbA1c Mean (Std)	5.6 (0.6)	5.6 (0.6)	5.7 (0.7)	5.7 (0.8)			
	Model 1	0 (ref)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.21	0.07	<.0001
	Model 2	0 (ref)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.17	0.05	<.0001
	Model 3	0 (ref)	-0.1 (-0.2, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.17	0.05	<.0001
Omega-3 Rich Fish	Total N	945	1,104	1,582	678			
	HbA1c Mean (Std)	5.6 (0.6)	5.6 (0.5)	5.7 (0.8)	5.7 (0.9)			
	Model 1	0 (ref)	-0.1 (-0.1, 0.0)	0.0 (0.0, 0.1)	0.0 (-0.1, 0.1)	0.10	0.09	<.0001
	Model 2	0 (ref)	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.14	0.10	<.0001
	Model 3	0 (ref)	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.15	0.10	<.0001

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-11. Associations of fatty acid intake (in quartiles) with HbA1c in those with pre-diabetes, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N	1,180	1,052	1,051	1,026			
	HbA1c Mean (Std)	5.6 (0.6)	5.6 (0.6)	5.7 (0.8)	5.7 (0.8)			
	Model 1	0 (ref)	0.0 (-0.1, 0.1)	0.1 (0.0, 0.1)	0.1 (0.0, 0.1)	0.42	0.15	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.48	0.15	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.51	0.14	<.0001
DHA + EPA	Total N	1,109	1,108	1,054	1,038			
	HbA1c Mean (Std)	5.6 (0.6)	5.6 (0.5)	5.7 (0.9)	5.7 (0.8)			
	Model 1	0 (ref)	-0.1 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.12	0.06	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.10	0.04	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.11	0.04	<.0001
ALA	Total N	1,168	1,087	1,032	1,022			
	HbA1c Mean (Std)	5.6 (0.7)	5.6 (0.6)	5.7 (0.8)	5.7 (0.8)			
	Model 1	0 (ref)	0.0 (-0.1, 0.1)	0.1 (0.0, 0.1)	0.1 (0.0, 0.2)	0.02	0.004	<.0001
	Model 2	0 (ref)	0.0 (-0.1, 0.0)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.32	0.42	<.0001
	Model 3	0 (ref)	0.0 (-0.1, 0.0)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.29	0.38	<.0001

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-12. Associations of seafood consumption (servings/week) with HbA1c in those with diabetes, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N	57	101	449	394			
	HbA1c Mean (Std)	8.4 (2.5)	8.2 (2.6)	8.5 (2.4)	8.7 (2.5)			
	Model 1	0 (ref)	-0.2 (-0.5, 0.1)	0.1 (-0.2, 0.3)	0.1 (-0.1, 0.4)	0.001	0.001	0.002
	Model 2	0 (ref)	-0.2 (-0.5, 0.0)	0.0 (-0.2, 0.3)	0.1 (-0.1, 0.4)	0.002	0.002	0.005
	Model 3	0 (ref)	-0.2 (-0.5, 0.0)	0.0 (-0.2, 0.3)	0.1 (-0.1, 0.4)	0.002	0.001	0.005
Total Fish	Total N	69	118	473	341			
	HbA1c Mean (Std)	8.3 (2.4)	8.1 (2.5)	8.5 (2.5)	8.7 (2.5)			
	Model 1	0 (ref)	-0.2 (-0.4, 0.1)	0.1 (-0.1, 0.3)	0.3 (0.1, 0.5)	<.0001	<.0001	<.0001
	Model 2	0 (ref)	-0.1 (-0.4, 0.1)	0.1 (-0.1, 0.3)	0.3 (0.1, 0.5)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	-0.1 (-0.4, 0.1)	0.1 (-0.1, 0.3)	0.3 (0.1, 0.5)	<.0001	<.0001	<.0001
Omega-3 Rich Fish	Total N	186	230	414	171			
	HbA1c Mean (Std)	8.6 (2.5)	8.3 (2.4)	8.7 (2.5)	8.5 (2.4)			
	Model 1	0 (ref)	-0.3 (-0.4, -0.1)	0.1 (0.0, 0.3)	0.0 (-0.2, 0.2)	<.0001	0.20	<.0001
	Model 2	0 (ref)	-0.3 (-0.5, -0.1)	0.1 (-0.1, 0.2)	-0.1 (-0.3, 0.1)	<.0001	0.20	<.0001
	Model 3	0 (ref)	-0.3 (-0.5, -0.1)	0.1 (-0.1, 0.2)	-0.1 (-0.3, 0.1)	<.0001	0.21	<.0001

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-13. Associations of fatty acid intake (in quartiles) with HbA1c in those with diabetes, ARIC, 1987-1993. Percentage Point deltas and 95% confidence intervals.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N	155	222	295	329			
	HbA1c Mean (Std)	7.6 (2.3)	8.4 (2.5)	8.8 (2.5)	8.8 (2.4)			
	Model 1	0 (ref)	0.8 (0.6, 1.0)	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)	<.0001	<.0001	<.0001
	Model 2	0 (ref)	0.7 (0.6, 0.9)	1.1 (0.9, 1.3)	1.1 (0.9, 1.2)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	0.7 (0.6, 0.9)	1.1 (0.9, 1.2)	1.1 (0.9, 1.2)	<.0001	<.0001	<.0001
DHA + EPA	Total N	176	232	284	309			
	HbA1c Mean (Std)	8.1 (2.4)	8.4 (2.5)	8.6 (2.5)	8.8 (2.4)			
	Model 1	0 (ref)	0.2 (0.0, 0.4)	0.3 (0.2, 0.5)	0.5 (0.4, 0.7)	<.0001	<.0001	<.0001
	Model 2	0 (ref)	0.2 (0.0, 0.4)	0.3 (0.2, 0.5)	0.5 (0.3, 0.7)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	0.2 (0.0, 0.4)	0.3 (0.2, 0.5)	0.5 (0.3, 0.7)	<.0001	<.0001	<.0001
ALA	Total N	168	212	302	319			
	HbA1c Mean (Std)	7.7 (2.3)	8.4 (2.6)	8.9 (2.5)	8.7 (2.4)			
	Model 1	0 (ref)	0.6 (0.4, 0.8)	1.0 (0.9, 1.2)	0.9 (0.7, 1.1)	<.0001	<.0001	<.0001
	Model 2	0 (ref)	0.6 (0.4, 0.8)	1.0 (0.9, 1.2)	0.9 (0.7, 1.0)	<.0001	<.0001	<.0001
	Model 3	0 (ref)	0.6 (0.4, 0.8)	1.0 (0.9, 1.2)	0.9 (0.7, 1.0)	<.0001	<.0001	<.0001

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-14. Associations of seafood consumption (servings/week) with incident T2D in non-diabetics, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend	P Interaction
Total Seafood	Total N	907	1,486	5,478	4,003			
	Incident T2D N (%)	69 (7.6%)	113 (7.6%)	468 (8.5%)	372 (9.3%)			
	Model 1	1 (ref)	0.9 (0.6, 1.5)	1.0 (0.7, 1.5)	0.9 (0.6, 1.4)	0.89	0.96	0.30
	Model 2	1 (ref)	0.9 (0.5, 1.3)	1.0 (0.6, 1.4)	0.9 (0.6, 1.4)	0.88	0.70	0.30
	Model 3	1 (ref)	0.9 (0.5, 1.3)	1.0 (0.7, 1.4)	0.9 (0.6, 1.4)	0.86	0.69	0.33
Total Fish	Total N	980	1,562	3,892	1,940			
	Incident T2D N (%)	90	126	400	244			
	Model 1	1 (ref)	1.0 (0.6, 1.4)	1.0 (0.7, 1.5)	1.0 (0.7, 1.6)	0.94	0.70	0.18
	Model 2	1 (ref)	0.9 (0.6, 1.3)	1.0 (0.7, 1.5)	1.1 (0.7, 1.6)	0.80	0.47	0.16
	Model 3	1 (ref)	0.9 (0.6, 1.3)	1.0 (0.7, 1.5)	1.0 (0.7, 1.5)	0.79	0.60	0.17
Omega-3 Rich Fish	Total N	1,933	2,304	3,047	1,090			
	Incident T2D N (%)	184	216	331	129			
	Model 1	1 (ref)	1.1 (0.8, 1.5)	1.0 (0.8, 1.3)	1.0 (0.7, 1.5)	0.85	1.00	0.63
	Model 2	1 (ref)	1.2 (0.9, 1.6)	1.0 (0.8, 1.4)	1.1 (0.7, 1.6)	0.74	0.88	0.54
	Model 3	1 (ref)	1.2 (0.9, 1.6)	1.0 (0.8, 1.4)	1.0 (0.7, 1.5)	0.79	0.97	0.57

T2D: type 2 diabetes

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-15. Associations of fatty acid intake (in quartiles) with incident T2D in non-diabetics, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
DHA + EPA	Total N	2,125	2,098	2,102	2,049			
	Incident T2D N (%)	214 (10.1%)	179 (8.5%)	229 (10.9%)	238 (11.6%)			
	Model 1	1 (ref)	0.9 (0.7, 1.2)	1.0 (0.7, 1.3)	0.9 (0.6, 1.2)	0.80	0.51	0.60
	Model 2	1 (ref)	0.9 (0.7, 1.2)	1.0 (0.7, 1.3)	0.9 (0.7, 1.2)	0.89	0.71	0.54
	Model 3	1 (ref)	0.9 (0.7, 1.3)	1.0 (0.7, 1.3)	0.9 (0.7, 1.3)	0.94	0.67	0.55

T2D: type 2 diabetes

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-16. Associations of fatty acid intake (in quartiles) with incident T2D among normoglycemic participants, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N	1,291	1,293	1,269	1,190			
	Incident T2D N (%)	37 (2.9%)	30 (2.3%)	46 (3.6%)	58 (4.9%)			
	Model 1	1 (ref)	1.0 (0.4, 2.6)	1.5 (0.7, 3.6)	2.1 (1.0, 4.8)	0.17	0.03	0.10
	Model 2	1 (ref)	1.0 (0.4, 2.6)	1.6 (0.7, 3.7)	2.3 (1.0, 5.2)	0.12	0.02	0.06
	Model 3	1 (ref)	1.1 (0.4, 2.8)	1.7 (0.7, 4.0)	2.4 (1.1, 5.4)	0.10	0.02	0.05
ALA	Total N	1,297	1,335	1,247	1,164			
	Incident T2D N (%)	35 (2.7%)	35 (2.6%)	50 (4.0%)	51 (4.4%)			
	Model 1	1 (ref)	1.8 (0.7, 4.7)	1.6 (0.6, 4.2)	3.4 (1.4, 7.9)	0.02	0.004	0.01
	Model 2	1 (ref)	2.0 (0.8, 5.1)	1.7 (0.6, 4.5)	3.8 (1.6, 9.1)	0.01	0.002	0.01
	Model 3	1 (ref)	2.0 (0.8, 5.2)	1.8 (0.6, 4.8)	4.0 (1.6, 9.6)	0.01	0.001	0.01

T2D: type 2 diabetes
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
P Interaction: Significance of the exposure*glycemia population term
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-17. Associations of fatty acid intake (in quartiles) with incident T2D among participants with pre-diabetes, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

		Q1	Q2	Q3	Q4	P Model	P Trend	P Interaction
ALA + DHA + EPA	Total N	868	807	847	809			
	Incident T2D N (%)	156 (18.0%)	152 (18.8%)	199 (23.5%)	182 (22.5%)			
	Model 1	1 (ref)	1.0 (0.7, 1.4)	1.2 (0.9, 1.7)	0.9 (0.6, 1.2)	0.17	0.08	0.10
	Model 2	1 (ref)	1.0 (0.7, 1.3)	1.3 (0.9, 1.8)	0.9 (0.6, 1.2)	0.12	0.05	0.06
	Model 3	1 (ref)	1.0 (0.7, 1.4)	1.3 (0.9, 1.8)	0.9 (0.6, 1.2)	0.10	0.04	0.05
ALA	Total N	844	831	817	839			
	Incident T2D N (%)	162 (19.2%)	152 (18.3%)	193 (23.6%)	182 (21.7%)			
	Model 1	1 (ref)	0.9 (0.6, 1.2)	1.4 (1.1, 1.9)	1.0 (0.7, 1.4)	0.02	0.01	0.01
	Model 2	1 (ref)	0.8 (0.6, 1.2)	1.4 (1.0, 2.0)	1.1 (0.7, 1.6)	0.01	0.01	0.01
	Model 3	1 (ref)	0.9 (0.6, 1.2)	1.5 (1.0, 2.0)	1.1 (0.7, 1.6)	0.01	0.004	0.01

T2D: type 2 diabetes

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

P Interaction: Significance of the exposure*glycemia population term

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 3-18. Summary of results for fish/shellfish consumption (servings/week), DHA+EPA, and ALA with glycemia outcomes in normoglycemic, pre-diabetic, and diabetic populations, ARIC, 1987-1998.

	Normoglycemic and Pre-Diabetic			Diabetic		Effect Modification in Diabetic Population	
	FBG	*HbA1c*	*Incident T2D*	*FBG*	*HbA1c*	*Black vs. White*	*Females vs. Males*
Fish/Shellfish	Favorable	Null	Null	Adverse	Adverse	n.s. Adverse (blacks) n.s. Favorable (whites)	Adverse (females) n.s. Favorable (males)
DHA+EPA	Favorable	Favorable (NGT) n.s. Favorable (Pre-T2D)	Null	Adverse	Adverse	None	None
ALA	Null	Null	Adverse (moreso in NGT)	Adverse	Adverse	None	None

NGT: normoglycemic
FBG: fasting blood glucose
T2D: type 2 diabetes

4 MANUSCRIPT 2: INTAKE OF OMEGA-3 POLYUNSATURATED FATTY ACIDS AND ELECTROCARDIOGRAPHIC PREDICTORS OF SUDDEN CARDIAC DEATH IN THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY

4.1 SYNOPSIS

Background: Intake of omega-3 polyunsaturated fatty acids (PUFAs) has been associated with lower incidence of sudden cardiac death (SCD). ECG predictors of SCD include prolonged QT interval and elevation of the QRS-ST junction or J-point elevation, and these may be markers of the mechanism underlying the association observed by intake of omega-3 PUFAs.

Methods: We studied the association of consumption of seafood, fish-derived omega-3 PUFAs (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), and vegetable-derived omega-3 PUFAs (alpha-linoleic acid (ALA)) with J-point height and heart rate-corrected QT (QTc) interval in individuals initially aged 45–64 from the Atherosclerosis Risk in Communities (ARIC) cohort (n = 12,611). Intake of seafood, DHA, EPA, and ALA were measured via food frequency questionnaire. QTc interval, and J-point height were measured using ECGs obtained during study visits. Generalized estimating equations were used to estimate odds ratios of prolonged QTc and J-point elevation and differences in continuous measures of QTc interval duration and J-point height by servings/week of seafood and quartiles of omega-3 PUFAs or by fish intake.

Results: The 12,611 participants contributed exposure/outcome data for a mean of 3.0 visits between 1987 and 1998. In multivariable analyses, higher intakes of ALA+DHA+EPA and ALA were associated with a shorter QTc interval (-0.8 ms, 95% CI -1.5, -0.2 and -1.0 ms, 95% CI -1.7, -0.3 comparing the top to the bottom quartile of ALA+DHA+EPA and ALA alone intake, respectively). The other exposures (DHA+EPA,

total fish + shellfish, total fish, and omega-3 rich fish) were not associated with QTc internal. None of the exposures were associated with the other outcomes examined (prolonged QTc, J-point elevation, J-point height).

Conclusions: Results from this population-based cohort provided limited evidence that omega-3 fatty acids are associated with prolonged QT interval, J-point height, or J-point elevation after controlling for potential confounders.

4.2 INTRODUCTION

Findings from observational and experimental trials indicate that individuals who regularly consume fish high in omega-3 polyunsaturated fatty acids (PUFAs) have lower incidence of sudden cardiac death (SCD) compared to those who consume no or little fish [46, 51, 194, 195], but the exact mechanisms underlying this association are unconfirmed. Alpha linoleic acid (ALA), a vegetable-derived omega-3 PUFA, has also been shown to be inversely associated with SCD [196].

Two ECG-measured variables associated with a higher risk of SCD are presence of prolonged QT interval and J-point elevation (JPE) [96-99, 142]. In an ECG, the QT interval represents electrical depolarization and repolarization of the left and right ventricles and the J-point – the junction of the QRS complex and the ST segment – marks the end of depolarization and the beginning of repolarization [100, 101]. A prolonged QT interval is indicative of abnormally prolonged repolarization and is associated with SCD [96, 97]. Additionally, early repolarization characterized by an elevation of the J-point has been associated with idiopathic ventricular fibrillation and SCD [98, 99].

There have been a limited number of studies evaluating whether omega-3 PUFAs are associated with repolarization abnormalities – prolonged repolarization (prolonged QT interval) [104-107] and early repolarization (JPE) [104]. With respect to prolonged QT interval, intake of the fish-derived omega-3 PUFAs docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA) have been shown to be associated with shorter QT intervals

in Greek adults [107] and predominately white Americans aged >65 years [106]. A study of white, middle-aged American adults found higher intakes of the vegetable-derived omega-3 PUFA ALA were associated with lower risk of prolonged QT [104]. With respect to JPE, a study of Japanese men found that higher intake of the fish-derived omega-3 PUFA DHA and EPA attenuated the association between JPE and cardiac death [105]. To our knowledge, no studies have investigated the association of ALA with JPE.

Although omega-3 PUFAs are inversely associated with SCD, and prolonged QT interval and JPE are positively associated with SCD, further details regarding the association between omega-3 PUFAs, QT interval, and JPE in a biracial cohort of middle-aged American populations may help elucidate the mechanisms relating omega-3 fatty acid consumptions and SCD. Thus, this study investigates whether consumption of seafood, the fish-derived omega-3 PUFAs DHA and EPA, and the vegetable-derived omega-3 PUFA ALA are associated with heart rate-corrected QT interval (QTc) or the height of the QRS-ST junction (J-point) in the Atherosclerosis Risk in Communities (ARIC) study. We hypothesize that higher dietary intake of fish, DHA, EPA, and ALA will be associated with shorter QTc intervals and lower J-points.

4.3 METHODS

4.3.1 STUDY POPULATION

The ARIC study has been described previously [155]. Briefly, ARIC is a prospective study of cardiovascular disease including 15,792 men and women 45–64 years of age at baseline (visit 1). Participants were recruited from four US communities using probability sampling techniques. The communities and racial composition were: predominately white subjects from suburbs of Minneapolis, Minnesota, and Washington County, Maryland; black subjects from Jackson, Mississippi; and white and black subjects from Forsyth County, North Carolina.

Visit 1 data were collected in 1987–89 and three additional exams were performed at approximately 3-year intervals (1990–92, 1993–95, 1996–98). A fifth exam was conducted in 2011-13 (visit 5), but those data were not utilized in this study as outcomes would have occurred more than 20 years after our exposure assessment.

Our exclusion criteria were as follows (Figure 4-1). We excluded participants with missing values for exposures, outcomes (missing ECG data), or covariates. Those whose race was neither black nor white (n = 48) were excluded, and we further excluded black participants at the Minneapolis and Washington County sites (n = 55) due to small n. We excluded those participants who had prevalent or incident coronary heart disease, heart failure, or stroke as (1) prevalent conditions influence how patients have their comorbidities managed, diagnosed and treated; and (2) diagnoses may result in changes to previously reported dietary behaviors. We excluded participants who self-reported use of antiarrhythmic medications and those whose duration of the QRS complex was ≥120 ms, as those individuals have major conduction defects that make the interpretation of primary repolarization abnormalities inappropriate [197]. Finally, participants who reported implausible caloric intakes were excluded for potentially unreliable exposure data. Implausible was defined as less than 500 kcal/day for women and 700 kcal/day for men or more than 3500 kcal/day for women and 4500 kcal/day for men. These ranges represent the sex-specific first and 99th percentiles for ARIC energy intake distributions – see Tell et al. for the initial description of the exclusion methodology and justification [156] and Steffen et al. for first use of current ranges [157].

4.3.2 Outcome Assessment

At each visit, a standard, resting, supine 12-lead ECG was obtained for each subject a minimum of 1 hour after any smoking or caffeine ingestion using MAC PC personal cardiography equipment (Marquette Electronics, Inc., Milwaukee, WI).

Subsequent processing of the ECGs took place at EPICARE (Epidemiological Cardiology Research Center at Wake Forest University, Winston-Salem, NC, USA). Outcomes of interest were assigned using these ECG data where the participant met the inclusion/exclusion criteria.

4.3.2.1 QTc Interval

We used a heart rate-corrected QT interval (QTc) – as recommended by the AHA, the American College of Cardiology, and the Heart Rhythm Society for the Standardization and Interpretation of the Electrocardiogram [138]. The most appropriate formula for correction is the one resulting in the least amount of correlation between heart rate and the calculated rate-corrected QT [139]. We tested Framingham [140] and Hodges [141] and found that the Framingham formula had the least correlation with heart rate in our study population (r= -0.23 and r=-0.38, respectively). In addition to the continuous measure of QT interval, we defined prolonged QT as QTc values of 460 ms or longer in women and 450 ms or longer in men [138].

4.3.2.2 J-Point Elevation

We calculated a continuous measure of the J-point as the maximum amplitude of the 12 STJ leads. As has been done in other ARIC studies, JPE was defined as a ST amplitude greater than 100 microvolts in at least two contiguous leads [142].

4.3.3 EXPOSURE ASSESSMENT

In this study, we focused on dietary consumption of fish and shellfish, the fish-derived omega-3 fatty acids DHA and EPA, and the vegetable-derived ALA.

Participants' usual dietary intake was assessed by an interviewer-administered, 66-item food frequency questionnaire (FFQ). The FFQ was based on the instrument developed by Willett et al. [167], with three principal modifications: (1) Data regarding

alcohol consumption were obtained using a separate, more detailed instrument; (2) Several food items were added (e.g., donuts, biscuits, and cornbread); and (3) Some items were split into detailed subcategories – notably a single item on fish consumption was separated into three specific fish items.

The 61-item Willett version has been validated against 28-day food record, but the validation took place in a population of educated, predominately white women [124, 167]. The ARIC questionnaire was also validated in a sample (n=419) of black and white ARIC participants who repeated the FFQ after three years [125]. The study found that, after adjusting for total caloric intake, the median reliability coefficient for blacks was 0.42 and the reliability for white ARIC participants was 0.49 – a value similar to that of other studies of white subjects. The study found no difference in the median reliability coefficients of men and women after adjusting for total calorie intake.

Another study investigated the validity of the ARIC FFQ by comparing Minnesota field center participants' dietary fat FFQ data against their plasma fatty acid concentrations [126]. Plasma measures reflect the types of fats proportionally consumed over the past several weeks to months [127] and the proportionate composition in plasma was moderately correlated with dietary intake, with highest correlations in the fish-derived omega-3 fatty acids DHA and EPA (r=0.42 and r=0.20 for plasma phospholipid measures of DHA and EPA, respectively) [126].

4.3.3.1 Fish/Shellfish Servings

Fish and other seafood intake was assessed through four FFQ questions with nine response categories. Participants were asked how often they consumed: 3–4 ounces of canned tuna fish; 3–5 ounces of dark meat fish such as salmon, mackerel, swordfish, sardines, and bluefish; 3–5 ounces of other fish such as cod, perch, catfish, etc.; and shrimp, lobster, scallops as a main dish. Interviewers used food models to help

participants with portion size estimation. Subjects could provide answers to each question ranging from ‘‘never or less than once per month’’ to ‘‘6 times per day.’’

Each of the participants’ seafood-related FFQ responses were grouped into three exposure categories: (1) omega-3 rich fish (tuna + dark); (2) total fish (tuna + dark + other); and (3) total seafood (tuna + dark + other + shellfish). Exposure categories were categorized into four weekly serving categories: none, less than one, one to two, and more than two.

4.3.3.2 Quartiles of Omega-3 PUFA

Daily intake of macro- and micronutrients was calculated via the FFQ by multiplying the nutrient content of each food by the frequency of daily consumption and then summing the results [124]. This process yielded daily intake of nutrients expressed as grams per day. Three different classifications of omega-3 fatty acids were investigated: (1) vegetable-derived ALA, (2) fish-derived DHA+EPA; and (3) total omega-3 PUFA ALA+DHA+EPA.

Intake of nutrients was adjusted using the residual method [168, 169]. In this method, nutrient residuals (observed intake – predicted intake) are obtained from the regression of total nutrient intake on total energy intake. The nutrient residuals are then rescaled by adding the overall mean nutrient intake to each participant’s residual. For this manuscript, we created rescaled residuals for the three nutrient classifications – ALA, DHA+EPA, and ALA+DHA+EPA – and categorized each into quartiles.

We selected the residual method rather than the standard multivariable method (quartiles of raw nutrient values as the exposure with total energy intake as a covariate) because (1) with the residual method, differences in exposure values amongst participants are due to differences in nutrient intake from the nutrient composition of the diet (versus overall variation in nutrient intake, which is due to diet composition and calorie amount) [169]; (2) when dietary exposure variables are categorized, the residual

and the standard multivariable models are no longer mathematically equivalent [168-170]; and (3) the residual model allows for greater precision [168]. All regression models where residual-adjusted nutrients were the exposure of interest included total energy intake (kcal/day) as a covariate.

4.3.4 POTENTIAL CONFOUNDERS

We selected potential confounders a priori based on their hypothesized relationship with exposure and outcome. While most variables were measured at multiple visits, potential confounders were measured contemporaneously with exposure values (visits 1 and 3) to avoid adjusting for confounders measured after our exposure of interest [143]. Potential confounders were grouped into four main categories: sociodemographic, lifestyle, dietary, and clinical variables.

4.3.4.1 Sociodemographic Variables

Sociodemographic variables included age, sex, race, field center, and education level. Age, sex, self-reported race, and center were obtained at visit 1 and confirmed at subsequent visits. Education level was measured at visit 1 via self-report and categorized based on years of education. We grouped education level as basic (no high school degree), intermediate (completed high school), and advanced (at least some college).

4.3.4.2 Lifestyle Variables

Lifestyle variables included body mass index (BMI), physical activity, smoking status, and drinking status and amount. Technicians measured height and weight, and BMI was calculated as weight (kilograms) divided by height squared (meters^2). Physical activity was measured at visits 1 and 3 using the Baecke questionnaire [144]. The questionnaire included 16 items about usual exertion, and three indexes ranging from 1

(low) to 5 (high) were derived for physical activity at work, during leisure time, and in sports. The reliability and validity of the Baecke questionnaire are good for both male and female subjects, and equal to many other physical activity instruments [145]. The three physical activity scores were summed and then translated into tertiles of physical activity (low, medium, and high). Smoking status was assessed via self-report and participants classified as current smokers, former smokers, and never smokers. Alcohol intake status (current, former, never) and amount (grams/day) were measured at visits 1 and 3.

4.3.4.3 Dietary Variables

Dietary variables included *trans* fatty acids, saturated fatty acids, and dietary fiber. Intake of *trans* fatty acids, saturated fatty acids, and dietary fiber were measured at visits 1 and 3 via FFQ and translated into nutrient values as described in the exposure section (Section 4.3.3).

4.3.4.4 Clinical Variables

Clinical variables included hypertension, LDL, HDL, and triglycerides. During each visit, three blood pressure measurements were taken with a random-zero sphygmomanometer and the mean of the last two measurements was used. Hypertension was defined as a systolic blood pressure above 140 mmHg, a diastolic above 90 mmHg, or self-reported use of antihypertensive medication. Participants with missing hypertension values (n=62 visit 1; n=56 visit 3) were imputed as not having hypertension (no disease). For metrics requiring phlebotomy, blood was drawn after a minimum 8-hour fasting period with minimal trauma from an antecubital vein [146]. Plasma total cholesterol and triglycerides were measured by enzymatic methods [132], and LDL cholesterol was calculated using the Friedewald formula [147]. HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins [132].

4.3.5 STUDY DESIGN

Figure 4-2 depicts the study design for all three outcomes of interest including the temporality of the exposure and outcomes. The blue arrow gives the timeline for each of the ARIC visits. The green ovals represent exposures and covariates obtained at visit 1 and visit 3. As mentioned in the potential confounder section (Section 4.3.4), visit 1 values were used in regressions involving outcomes obtained at visit 1 and visit 2, whereas visit 3 values were used for outcomes obtained at visits 3 and 4. The green oval shapes represent that participants' outcomes and inclusion/exclusion criteria updated at each of the four ARIC visits (where applicable). The pink box lists the inclusion/exclusion criteria, and demonstrates the consistency across all four visits.

As depicted in the Figure 4-2, we used a quasi-repeated cross-sectional design where each participant who met the inclusion/exclusion criteria at visit 1 could be included in the analysis up to four times (once at visit 1 and again for each subsequent visit where he/she met that visit's inclusion/exclusion criteria). Outcome data were obtained from each visit meeting the inclusion/exclusion criteria. As previously mentioned, exposure and confounder values from visit 1 were used in observations for visits 1 and 2, and observations for visits 3 and 4 used exposure and covariate values from visit 3.

4.3.6 STATISTICAL ANALYSES

To evaluate the associations of fish/shellfish servings and quartiles of omega-3 PUFA intake with continuous outcomes (QTc, J-point height), we used generalized estimating equations to account for repeated measures, using a normal distribution and an identity link. Associations with dichotomous outcomes (prolonged QTc, JPE) were estimated using generalized estimating equations, with a binomial distribution, a logit link. All analyses assumed an independent working correlation structure. A sensitivity analysis was conducted evaluating the impact of assuming an unstructured working

correlation structure and this modification did not appreciably alter our results. Finally, we tested for a linear trend across categories of intake by modeling the category medians as a continuous variable.

All statistical analyses were performed with SAS (version 9.4, Enterprise guide 7.1, SAS Institute Inc., Cary, NC, USA).

4.3.6.1 Covariate Adjustment Models

Three models were used to adjust for potential confounders measured contemporaneously with exposure values: Model 1 adjusted for sociodemographic variables (age, sex, race, center, education); Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount) and dietary variables (*trans* fatty acids, saturated fatty acids, and dietary fiber); Model 3 further adjusted for clinical variables (hypertension, HDL, LDL, triglycerides). All models included total energy intake (kcal/day) as a covariate.

4.4 RESULTS

During the first four ARIC visits (1987-1998), the 12,611 participants contributed exposure/outcome data for a mean of 3.0 visits (range 1-4, standard deviation 1.1) generating 38,345 observations. J-point elevation was identified in 2,071 ECGs among 1,205 (9.6%) participants, while prolonged QTc was measured in 558 ECGs among 452 (3.6%) participants. Baseline characteristics of the 12,611 eligible participants by categories of total seafood intake (total fish + shellfish) are shown in Table 4-1.

The greatest proportion of participants (46%) consumed one to two servings of seafood per week, while only 8% reported no seafood consumption at all. Those who consumed more seafood tended to be younger, female, and were more educated. Additionally, there was a greater proportion of black participants at higher levels of total seafood consumption compared to lower levels. Clinically, cardiovascular risk profiles

varied across seafood categories. Prevalence of hypertension was higher with greater seafood consumption, as was average BMI; smoking rates were lower with greater seafood consumption.

4.4.1 Associations with QTc Related Outcomes

4.4.1.1 Prolonged QTc

4.4.1.1.1 Fish/Shellfish Servings

Overall, fish intake was not associated with prolonged QTc (Table 4-2). The fully adjusted multivariable odds ratio of prolonged QTc in those consuming >2 servings seafood/week was 1.1 (95% CI: 0.74, 1.60) when compared to those who did not consume any seafood (p for trend = 0.71). Results were similar for total fish (OR=1.0, 95% CI: 0.73, 1.50) and omega-3 rich fish (OR=1.2, 95% CI: 0.86, 1.65), comparing >2 servings/week to no intake in the fully adjusted model.

4.4.1.1.2 Omega-3 PUFA Intake

Intake of ALA+DHA+EPA was not associated with prolonged QTc interval. The fully adjusted multivariable odds ratio of prolonged QTc in those in the highest quartile of ALA+DHA+EPA was 0.9 (95% CI: 0.67, 1.17). Results were similar when considering DHA+EPA (Model 3 OR=1.0; 95% CI: 0.74, 1.33) and ALA (Model 3 OR=0.7; 95% CI: 0.54, 1.02) separately. See Table 4-3 for detailed results.

4.4.1.2 QTc Interval

4.4.1.2.1 Fish/Shellfish Servings

Higher total seafood intake was associated with shorter mean QTc interval, though this association was not statistically significant (Model 3 highest vs. lowest

intake: -0.7 ms, 95% CI: -1.59, 0.16 comparing highest versus lowest category, p for trend = 0.07). Neither total fish nor omega-3 rich fish was associated with QTc interval (Table 4-4).

4.4.1.2.2 Omega-3 PUFAs Intake

QTc interval was shorter in individuals with higher intake of ALA+DHA+EPA, with 0.8 ms shorter QTc in the top versus bottom quartiles (Model 3: 95% CI: -1.51, -0.22, p for trend = 0.01) (Table 4-5). This association was mostly due to an association between ALA intake and QTc duration. Individuals in the highest quartile of ALA intake had a 1 ms shorter QTc compared to the lowest in the fully adjusted models (95% CI: -1.73, -0.30, p for trend = 0.01). No association was observed between DHA+EPA intake and QTc duration.

4.4.2 ASSOCIATIONS WITH J-POINT RELATED OUTCOMES

4.4.2.1 J-Point Elevation

4.4.2.1.1 Fish/Shellfish Servings

Overall, consumption of total seafood, total fish, or omega-3 rich fish was not associated with the odds of JPE. Total seafood and total fish each had their highest point estimates in the "less than one serving/week" category (Total Seafood Model 3 OR= 1.2, 95% CI: 0.89, 1.53; Total Fish Model 3 OR= 1.1, 95% CI: 0.89, 1.44), but overall the association was null (Table 4-6).

4.4.2.1.2 Omega-3 PUFA Intake

As with fish/shellfish intake, intake of ALA+DHA+EPA was not associated with JPE (Model 3, Q4 vs. Q1 OR= 1.0, 95% CI: 0.86, 1.24). Results were similar when considering DHA+EPA (fish-derived) and ALA (vegetable derived) separately (Table 4-7).

4.4.2.2 J-Point Height

Consistent with the analysis for presence of J-point elevation, neither fish/shellfish consumption, nor omega-3 fatty acid intake (all, fish-derived, or vegetable-derived) were associated with J-point height (tables 4-8 and 4-9).

4.5 DISCUSSION

In this population-based study of middle-aged adults, our investigation of the relationship among seafood, omega-3 PUFA, and ECG predictors of SCD suggested that higher total omega-3 fatty acid intake, particularly from the vegetable-derived ALA, was associated with shorter QTc interval and lower odds of prolonged QTc in multivariable adjusted models. All other analyses were null.

4.5.1 QTc Interval

Our null findings regarding the association of fish and fish-derived PUFA with QTc did not replicate the results of previous studies, but there were differences in population attributes. While intake of fish-derived omega-3 PUFAs was shown to be associated with shorter QT intervals in Greek adults [107] and predominately white Americans aged >65 years [106], the ARIC study included black participants whereas the other two did not. Also, while intake of seafood and fish-derived omega-3 PUFAs was similar in the two studies, the U.S. study participants were significantly older at baseline compared to ARIC participants (65 versus 45 years) [106].

Our finding that ALA is favorably associated with QTc is similar to a study of white, middle aged adults in the U.S. that found higher intakes of ALA were associated with lower risk of prolonged QT [104]. The authors posited many biological mechanisms through which ALA could affect arrhythmias including modification of the eicosanoid system and modulation of L-type calcium channels in the sarcolemma of cardiac myocytes [104].

4.5.2 J-POINT ELEVATION AND J-POINT HEIGHT

We did not find any significant association between seafood intake and measures of J-point height or JPE. Nor did we find any significant associations with omega-3 fatty acids as the exposure of interest. In a single study that investigated middle-aged Japanese men, the authors found a relationship between omega-3 PUFAs and J-point elevation [105]. It is possible that the Japanese study was better powered to detect an association as it had a much higher consumption of seafood compared to the ARIC population (half of the Japanese subjects had fish-derived omega-3 fatty acid intake ≥0.35% of total energy intake compared to ≥0.12% in ARIC participants).

In spite of the lack of strong and consistent epidemiological evidence, there is biological plausibility for an effect. Studies suggest that fish-derived PUFAs could have anti-arrhythmic effects, thus reducing SCD risk. Specifically, fish-derived omega-3 PUFAs may inhibit the fast, voltage-dependent sodium current and the L-type calcium currents [102, 103] that allow pre-SCD arrhythmias to be sustained [46].

4.6 STRENGTHS AND LIMITATIONS

This study has many strengths. It is a population-based, biracial cohort of participants who were followed over several years with multiple measures of exposures, outcomes, and covariates. However, our study is not without limitations. With dietary data, there is always the potential for misclassification bias. FFQs have been shown to work reasonably well at ranking subjects' food intake. While absolute values may not be reflective of actual intake, they are reflective of intake relative to other study participants [169, 198]. FFQs have also been shown to underestimate total caloric intake when compared to doubly labeled water [193]. Additionally, data were not available on fish preparation technique. Analysis in the Cardiovascular Health Study has shown that fish preparation method differentially affects the association between fish-derived omeag-3 PUFAs and CHD, with only intake of tuna and other baked or broiled

fish associated with cardiovascular benefits, but no or deleterious associations for fried fish or fish sandwiches [8]. Also, as previously mentioned, the range of seafood intake in the ARIC population was limited, and this inhibits our ability to find associations that exist at higher intake levels. Finally, as with all observational epidemiology studies, there is potential for unmeasured confounding. While we attempted to adjust for confounders in our analysis, there may be common causes of fish intake and ECG results that we have missed or mis-measured.

4.7 CONCLUSION

In summary, our results suggest that higher dietary intake of omega-3 fatty acids, particularly of vegetable-derived ALA, is associated with favorable differences in QT measures but not J-point height. However, given the number of statistical tests carried out, our finding regarding QTc may be due to type I error. Additional studies in different populations are needed to substantiate our results.

4.8 FIGURES

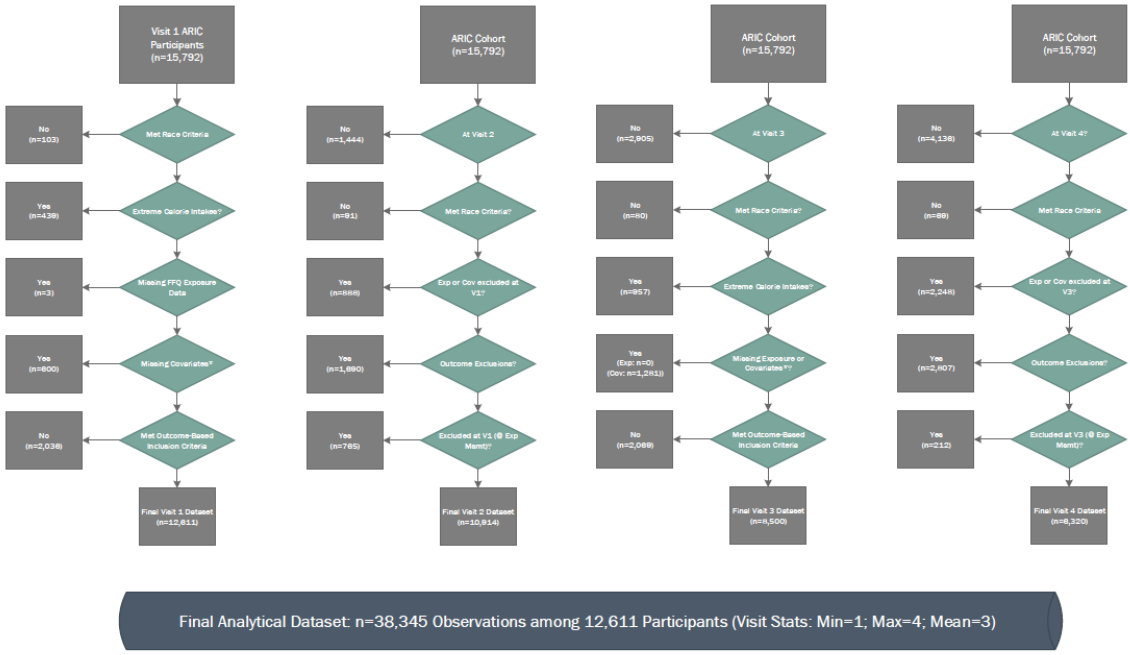

Figure 4-1. Flowchart of inclusion/exclusion criteria, ARIC, 1987-1998

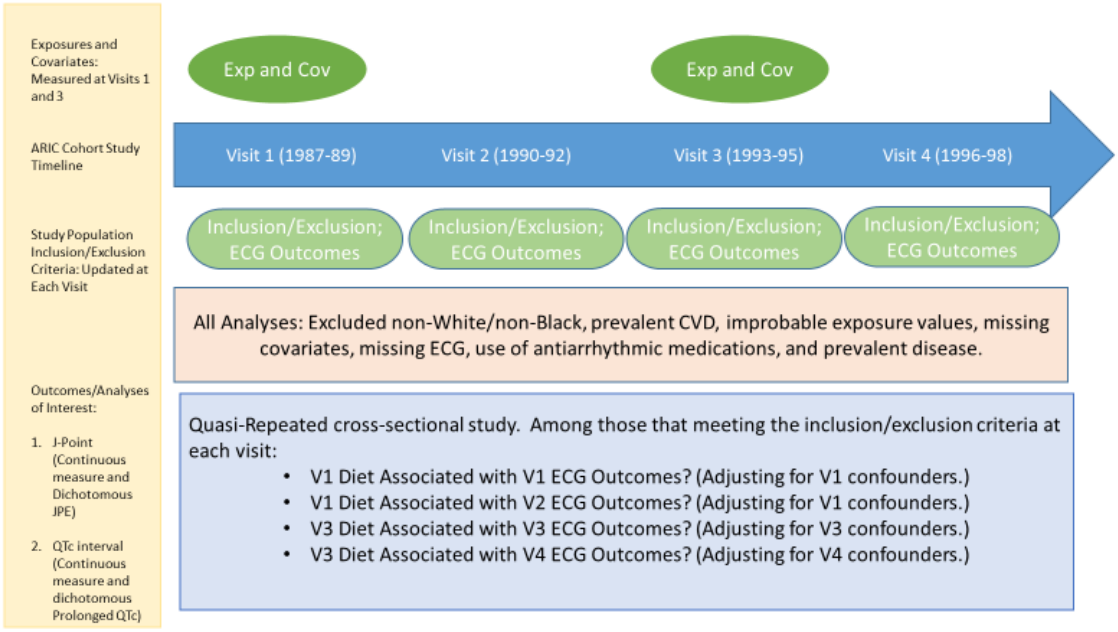

Figure 4-2. Study design depiction with variable temporality, ARIC, 1987-1998

4.9 TABLES

Table 4-1. Baseline characteristics of ARIC participants (n=12,611) by weekly servings of seafood consumption (tuna, dark meat fish, other fish, and shellfish), 1987–1989. Values correspond to mean (SD) or N (%)

	Zero Svgs/Week	Less Than 1 Svgs/Week	One to Two Svgs/Week	Two or More Svgs/Week
N	945 (7.5%)	1,560 (12.4%)	5,791 (45.9%)	4,315 (34.2%)
Sociodemographic Covariates				
Age (years)	54.6 (5.8)	53.9 (5.8)	53.9 (5.7)	53.8 (5.7)
Male	502 (53.1%)	718 (46.0%)	2,531 (43.7%)	1,706 (39.5%)
White	864 (91.4%)	1,362 (87.3%)	4,399 (76.0%)	2,825 (65.5%)
Education				
Basic	259 (27.4%)	300 (19.2%)	1,279 (22.1%)	903 (20.9%)
Intermediate	423 (44.8%)	749 (48.0%)	2,418 (41.8%)	1,646 (38.1%)
Advanced	263 (27.8%)	511 (32.8%)	2,094 (36.2%)	1,766 (40.9%)
Lifestyle Covariates				
BMI (kg/m^2)	26.8 (4.9)	26.8 (4.7)	27.3 (5.2)	27.9 (5.4)
Physical Activity^				
Low	342 (36.2%)	558 (35.8%)	2,022 (34.9%)	1,344 (31.1%)
Medium	302 (32.0%)	527 (33.8%)	1,963 (33.9%)	1,455 (33.7%)
High	301 (31.9%)	475 (30.4%)	1,806 (31.2%)	1,516 (35.1%)
Current Smokers	260 (27.5%)	429 (27.5%)	1,499 (25.9%)	1,056 (24.5%)
Current Alcohol Drinkers	498 (52.7%)	953 (61.1%)	3,405 (58.8%)	2,420 (56.1%)
Dietary Covariates				
Total Energy Intake (kcal/day)	1,512 (609)	1,521 (579)	1,580 (577)	1,749 (620)
Cereal Fiber (grams/day)	3.4 (2.5)	3.3 (2.2)	3.4 (2.2)	3.8 (2.5)
Dietary Fiber (grams/day)	15.0 (8.1)	14.7 (6.7)	16.2 (7.3)	19.7 (8.8)
Saturated Fats (grams/day)	21.4 (10.6)	21.9 (10.7)	21.8 (10.4)	22.5 (10.6)
Trans Fats (grams/day)	3.0 (1.9)	3.0 (1.8)	2.9 (1.8)	2.9 (1.8)
Clinical Covariates				
Hypertension	945 (25.6%)	1,560 (26.8%)	5,791 (30.3%)	4,315 (33.7%)
Systolic Blood Pressure, mmHg	120.1 (17.2)	119.4 (17.3)	120.5 (18.5)	121.2 (18.9)
Diastolic Blood Pressure, mmHg	72.4 (10.4)	72.7 (10.8)	73.5 (11.1)	73.9 (11.3)
High Density Lipoprotein Cholesterol, mg/dL	50.3 (15.8)	51.8 (16.7)	52.6 (17.0)	53.5 (17.3)
Low Density Lipoprotein Cholesterol, mg/dL	137.6 (15.8)	135.3 (38.4)	136.6 (38.7)	137.8 (40.3)
Triglycerides, mg/dL	126.8 (63.7)	124.0 (64.6)	121.7 (62.4)	122.2 (63.8)

*Svgs=Servings

^Work+Leisure+Sport Averaged and Divided into Low/Medium/High

Table 4-2. Associations of seafood consumption (in servings/week) with prolonged QTc in ARIC participants (n=12,611), 1987-1998. Odds ratios and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend
Total Seafood	N	3,213	5,355	17,574	12,203		
	Prol QTc n (%)	40 (1.2%)	85 (1.6%)	251 (1.4%)	182 (1.5%)		
	Model 1	1.0 (ref)	1.3 (0.9, 2.0)	1.1 (0.8, 1.6)	1.2 (0.8, 1.7)	0.62	0.95
	Model 2	1.0 (ref)	1.3 (0.9, 1.9)	1.1 (0.8, 1.6)	1.1 (0.7, 1.6)	0.63	0.70
	Model 3	1.0 (ref)	1.3 (0.9, 1.9)	1.1 (0.8, 1.6)	1.1 (0.7, 1.6)	0.64	0.71
Total Fish	N	4,070	6,436	17,926	9,913		
	Prol QTc n (%)	54 (1.3%)	106 (1.6%)	239 (1.3%)	159 (1.6%)		
	Model 1	1.0 (ref)	1.3 (0.9, 1.8)	1.0 (0.7, 1.3)	1.1 (0.8, 1.6)	0.21	0.80
	Model 2	1.0 (ref)	1.2 (0.9, 1.8)	0.9 (0.7, 1.3)	1.1 (0.7, 1.5)	0.21	0.98
	Model 3	1.0 (ref)	1.2 (0.9, 1.8)	0.9 (0.7, 1.3)	1.0 (0.7, 1.5)	0.21	0.96
Omega-3 Rich Fish	N	8,524	10,018	14,208	5,595		
	Prol QTc n (%)	116 (1.4%)	141 (1.4%)	206 (1.4%)	95 (1.7%)		
	Model 1	1.0 (ref)	1.1 (0.8, 1.4)	1.1 (0.8, 1.4)	1.2 (0.9, 1.7)	0.63	0.18
	Model 2	1.0 (ref)	1.0 (0.8, 1.4)	1.0 (0.8, 1.3)	1.2 (0.9, 1.7)	0.75	0.28
	Model 3	1.0 (ref)	1.0 (0.8, 1.4)	1.0 (0.8, 1.3)	1.2 (0.9, 1.7)	0.74	0.28

Prol QTc: Prolonged QTc

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 4-3. **Associations of fatty acid intake (in quartiles) with prevalence of prolonged QTc in ARIC participants (n=12,611), 1987-1998. Odds ratios and 95% confidence intervals from generalized estimating equations.**

		Q1	Q2	Q3	Q4	P Model	P Trend
ALA + DHA + EPA	N	9,701	9,795	9,459	9,390		
	Prol QTc n (%)	137 (1.4%)	141 (1.4%)	126 (1.3%)	154 (1.6%)		
	Model 1	1.0 (ref)	1.0 (0.7, 1.3)	0.8 (0.6, 1.0)	1.0 (0.7, 1.2)	0.17	0.57
	Model 2	1.0 (ref)	0.9 (0.7, 1.2)	0.7 (0.5, 1.0)	0.9 (0.7, 1.2)	0.12	0.35
	Model 3	1.0 (ref)	0.9 (0.7, 1.2)	0.7 (0.5, 1.0)	0.9 (0.7, 1.2)	0.13	0.32
DHA + EPA	N	10,372	9,007	9,514	9,452		
	Prol QTc n (%)	138 (1.3%)	133 (1.5%)	128 (1.3%)	159 (1.7%)		
	Model 1	1.0 (ref)	1.0 (0.8, 1.4)	0.9 (0.7, 1.2)	1.0 (0.8, 1.4)	0.71	0.79
	Model 2	1.0 (ref)	1.0 (0.8, 1.3)	0.9 (0.7, 1.2)	1.0 (0.7, 1.3)	0.66	0.97
	Model 3	1.0 (ref)	1.0 (0.8, 1.3)	0.9 (0.7, 1.1)	1.0 (0.7, 1.3)	0.64	0.97
ALA	N	9,872	9,566	9,615	9,292		
	Prol QTc n (%)	144 (1.5%)	136 (1.4%)	146 (1.5%)	132 (1.4%)		
	Model 1	1.0 (ref)	0.8 (0.6, 1.0)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.23	0.27
	Model 2	1.0 (ref)	0.7 (0.6, 1.0)	0.8 (0.6, 1.0)	0.8 (0.5, 1.0)	0.14	0.12
	Model 3	1.0 (ref)	0.7 (0.6, 1.0)	0.8 (0.6, 1.0)	0.7 (0.5, 1.0)	0.12	0.10

Prol QTc: Prolonged QTc

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, LDL, HDL, triglycerides)

Table 4-4. Associations of seafood consumption (in servings/week) with QTc interval (in milliseconds) in ARIC participants (n=12,611), 1987-1998. Millisecond deltas and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend
Total Seafood	Total N (all Visits)	3,213	5,355	17,574	12,203		
	QTc Mean (Std)	412.7 (17.0)	412.6 (16.3)	412.2 (17.1)	412.3 (16.9)		
	Model 1	0.0 (ref)	-0.2 (-1.1, 0.7)	-0.4 (-1.2, 0.4)	-0.3 (-1.1, 0.6)	0.81	0.47
	Model 2	0.0 (ref)	-0.3 (-1.2, 0.6)	-0.6 (-1.4, 0.2)	-0.7 (-1.6, 0.1)	0.34	0.05
	Model 3	0.0 (ref)	-0.4 (-1.2, 0.5)	-0.6 (-1.4, 0.2)	-0.7 (-1.6, 0.2)	0.39	0.07
Total Fish	Total N (all Visits)	4,070	6,436	17,926	159 (9,913)		
	QTc Mean (Std)	412.3 (16.8)	412.2 (16.4)	412.3 (17.0)	412.4 (17.1)		
	Model 1	0.0 (ref)	-0.3 (-1.1, 0.5)	-0.1 (-0.9, 0.6)	-0.1 (-0.9, 0.7)	0.86	0.86
	Model 2	0.0 (ref)	-0.4 (-1.2, 0.4)	-0.4 (-1.1, 0.4)	-0.6 (-1.4, 0.2)	0.56	0.25
	Model 3	0.0 (ref)	-0.5 (-1.3, 0.3)	-0.4 (-1.1, 0.3)	-0.6 (-1.4, 0.2)	0.53	0.28
Omega-3 Rich Fish	Total N (all Visits)	8,524	10,018	14,208	5,595		
	QTc Mean (Std)	411.8 (16.8)	412.1 (16.8)	412.5 (17.0)	412.9 (17.0)		
	Model 1	0.0 (ref)	-0.1 (-0.7, 0.5)	0.2 (-0.4, 0.8)	0.4 (-0.4, 1.1)	0.57	0.26
	Model 2	0.0 (ref)	-0.2 (-0.8, 0.4)	-0.1 (-0.6, 0.5)	-0.1 (-0.9, 0.6)	0.92	0.85
	Model 3	0.0 (ref)	-0.2 (-0.8, 0.4)	0.0 (-0.6, 0.5)	-0.1 (-0.8, 0.7)	0.90	0.99

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 4-5. Associations of fatty acid intake (in quartiles) with QTc interval (in milliseconds) in ARIC participants (n=12,611), 1987-1998. Millisecond deltas and 95% confidence intervals from generalized estimating equations.

		Q1	Q2	Q3	Q4	P Model	P Trend
ALA + DHA + EPA	Total N (all Visits)	9,701	9,795	9,459	9,390		
	QTc Mean (Std)	412.7 (16.4)	412.4 (17.3)	412.1 (16.8)	412.1 (17.1)		
	Model 1	0.0 (ref)	-0.4 (-1.0, 0.2)	-0.6 (-1.2, 0.0)	-0.4 (-1.0, 0.2)	0.30	0.17
	Model 2	0.0 (ref)	-0.5 (-1.1, 0.1)	-0.8 (-1.4, -0.2)	-0.9 (-1.5, -0.2)	0.03	0.01
	Model 3	0.0 (ref)	-0.5 (-1.1, 0.1)	-0.8 (-1.4, -0.2)	-0.8 (-1.5, -0.2)	0.04	0.01
DHA + EPA	Total N (all Visits)	10,372	9,007	9,514	9,452		
	QTc Mean (Std)	412.1 (16.5)	412.1 (16.9)	412.4 (16.9)	412.7 (17.4)		
	Model 1	0.0 (ref)	0.1 (-0.5, 0.6)	0.3 (-0.3, 0.9)	0.1 (-0.5, 0.8)	0.79	0.66
	Model 2	0.0 (ref)	-0.1 (-0.7, 0.5)	0.0 (-0.6, 0.6)	-0.3 (-1.0, 0.3)	0.67	0.32
	Model 3	0.0 (ref)	-0.1 (-0.7, 0.5)	0.0 (-0.6, 0.7)	-0.3 (-0.9, 0.3)	0.68	0.35
ALA	Total N (all Visits)	9,872	9,566	9,615	9,292		
	QTc Mean (Std)	412.7 (16.5)	412.6 (17.0)	412.5 (17.6)	411.4 (16.5)		
	Model 1	0.0 (ref)	-0.4 (-0.9, 0.2)	-0.3 (-0.9, 0.3)	-0.7 (-1.3, -0.1)	0.20	0.03
	Model 2	0.0 (ref)	-0.5 (-1.1, 0.1)	-0.6 (-1.2, 0.1)	-1.0 (-1.7, -0.3)	0.05	0.005
	Model 3	0.0 (ref)	-0.5 (-1.1, 0.1)	-0.5 (-1.2, 0.1)	-1.0 (-1.7, -0.3)	0.05	0.01

Std: standard deviation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 4-6. Associations of seafood consumption (in servings/week) with prevalence of J-point elevation in ARIC participants (n=12,611), 1987-1998. Odds ratios and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend
Total Seafood	N	3,213	5,355	17,574	12,203		
	JPE n (%)	144 (4.5%)	262 (4.9%)	910 (5.2%)	755 (6.2%)		
	Model 1	1.0 (ref)	1.1 (0.9, 1.5)	1.0 (0.8, 1.2)	0.9 (0.7, 1.2)	0.31	0.40
	Model 2	1.0 (ref)	1.2 (0.9, 1.5)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	0.36	0.77
	Model 3	1.0 (ref)	1.2 (0.9, 1.5)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	0.36	0.76
Total Fish	N	4,070	6,436	17,926	9,913		
	JPE n (%)	188 (4.6%)	328 (5.1%)	932 (5.2%)	623 (6.3%)		
	Model 1	1.0 (ref)	1.1 (0.9, 1.4)	0.9 (0.7, 1.1)	1.0 (0.8, 1.2)	0.07	0.61
	Model 2	1.0 (ref)	1.1 (0.9, 1.5)	0.9 (0.7, 1.1)	1.0 (0.8, 1.3)	0.07	0.95
	Model 3	1.0 (ref)	1.1 (0.9, 1.4)	0.9 (0.7, 1.1)	1.0 (0.8, 1.3)	0.07	1.00
Omega-3 Rich Fish	JPE n (Total N)	8,524	10,018	14,208	5,595		
	ST Seg Ht Mean (Std)	490 (5.7%)	518 (5.2%)	775 (5.5%)	288 (5.1%)		
	Model 1	1.0 (ref)	1.0 (0.9, 1.2)	1.0 (0.8, 1.2)	1.0 (0.8, 1.2)	0.99	0.82
	Model 2	1.0 (ref)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.8, 1.3)	0.97	0.81
	Model 3	1.0 (ref)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.8, 1.3)	0.98	0.87

JPE: J-point elevation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 4-7. Associations of fatty acid intake (in quartiles) with prevalence of J-point elevation in ARIC participants (n=12,611), 1987-1998. Odds ratios and 95% confidence intervals from generalized estimating equations.

		Q1	Q2	Q3	Q4	P Model	P Trend
ALA + DHA + EPA	N	9,701	9,795	9,459	9,390		
	JPE n (%)	449 (4.6%)	499 (5.1%)	536 (5.7%)	587 (6.3%)		
	Model 1	1.0 (ref)	0.9 (0.8, 1.1)	0.9 (0.7, 1.0)	0.9 (0.8, 1.1)	0.55	0.50
	Model 2	1.0 (ref)	1.0 (0.8, 1.2)	1.0 (0.8, 1.2)	1.0 (0.9, 1.3)	0.81	0.60
	Model 3	1.0 (ref)	1.0 (0.8, 1.2)	1.0 (0.8, 1.1)	1.0 (0.9, 1.2)	0.84	0.68
DHA + EPA	N	10,372	9,007	9,514	9,452		
	JPE n (%)	506 (4.9%)	446 (5.0%)	546 (5.7%)	573 (6.1%)		
	Model 1	1.0 (ref)	0.9 (0.8, 1.1)	0.9 (0.7, 1.1)	0.9 (0.8, 1.1)	0.64	0.49
	Model 2	1.0 (ref)	1.0 (0.8, 1.1)	0.9 (0.8, 1.1)	1.0 (0.8, 1.2)	0.80	0.94
	Model 3	1.0 (ref)	1.0 (0.8, 1.1)	0.9 (0.8, 1.1)	1.0 (0.8, 1.2)	0.80	0.87
ALA	N	9,872	9,566	9,615	9,292		
	JPE n (%)	484 (4.9%)	500 (5.2%)	508 (5.3%)	579 (6.2%)		
	Model 1	1.0 (ref)	1.0 (0.8, 1.1)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.79	0.53
	Model 2	1.0 (ref)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.1 (0.9, 1.3)	0.86	0.40
	Model 3	1.0 (ref)	1.0 (0.8, 1.2)	1.0 (0.8, 1.2)	1.1 (0.9, 1.3)	0.92	0.50

JPE: J-point elevation

P Model: p value for the exposure term

P Trend: p value for exposure modeled linearly using the median values in each category

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, HDL, triglycerides)

Table 4-8. Associations of seafood consumption (in servings/week) with J-point height (in millivolts) in ARIC participants (n=12,611), 1987-1998. Millivolt deltas and 95% confidence intervals from generalized estimating equations.

		Zero Servings/Week	Less Than 1 Servings/Week	One to Two Servings/Week	Two or More Servings/Week	P Model	P Trend
Total Seafood	Total N (all Visits)	3,213	5,355	17,574	12,203		
	J-Point Ht Mean (Std)	53.2 (33.6)	52.6 (34.0)	55.0 (35.8)	56.3 (36.8)		
	Model 2b	0.0 (ref)	-0.1 (-2.0, 1.8)	0.0 (-1.7, 1.7)	-0.8 (-2.6, 1.0)	0.47	0.20
	Model 4b	0.0 (ref)	0.0 (-1.8, 1.9)	0.2 (-1.4, 1.9)	-0.7 (-2.4, 1.1)	0.44	0.21
	Model 5b	0.0 (ref)	0.0 (-1.8, 1.9)	0.2 (-1.5, 1.8)	-0.7 (-2.5, 1.1)	0.42	0.18
Total Fish	Total N (all Visits)	4,070	6,436	17,926	159 (9,913)		
	J-Point Ht Mean (Std)	53.4 (33.7)	53.1 (34.2)	55.3 (36.0)	56.2 (37.0)		
	Model 2b	0.0 (ref)	0.2 (-1.5, 1.9)	-0.1 (-1.6, 1.5)	-0.6 (-2.4, 1.2)	0.74	0.29
	Model 4b	0.0 (ref)	0.3 (-1.4, 2.0)	0.1 (-1.4, 1.7)	-0.3 (-2.1, 1.4)	0.82	0.42
	Model 5b	0.0 (ref)	0.3 (-1.4, 1.9)	0.1 (-1.5, 1.6)	-0.4 (-2.2, 1.3)	0.79	0.37
Omega-3 Rich Fish	Total N (all Visits)	8,524	10,018	14,208	5,595		
	J-Point Ht Mean (Std)	55.8 (36.3)	54.3 (35.7)	55.4 (35.7)	53.6 (35.1)		
	Model 1	0.0 (ref)	0.4 (-0.9, 1.7)	0.6 (-0.7, 1.8)	-0.6 (-2.2, 1.0)	0.39	0.30
	Model 2	0.0 (ref)	0.6 (-0.7, 1.9)	0.8 (-0.5, 2.1)	-0.2 (-1.8, 1.4)	0.38	0.52
	Model 3	0.0 (ref)	0.5 (-0.8, 1.8)	0.7 (-0.5, 2.0)	-0.3 (-1.9, 1.3)	0.38	0.45

Ht: height
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 4-9. Associations of fatty acid intake (in quartiles) with J-point height (in millivolts) in ARIC participants (n=12,611), 1987-1998. Millivolt deltas and 95% confidence intervals from generalized estimating equations.

		Q1	Q2	Q3	Q4	P Model	P Trend
ALA + DHA + EPA	Total N (all Visits)	9,701	9,795	9,459	9,390		
	J-Point Ht Mean (Std)	52.8 (34.5)	54.4 (35.2)	55.4 (36.1)	57.3 (37.0)		
	Model 1	0.0 (ref)	-0.5 (-1.8, 0.7)	-0.9 (-2.2, 0.4)	-1.0 (-2.3, 0.4)	0.47	0.15
	Model 2	0.0 (ref)	-0.1 (-1.3, 1.2)	-0.2 (-1.4, 1.1)	-0.2 (-1.6, 1.1)	0.99	0.69
	Model 3	0.0 (ref)	-0.2 (-1.4, 1.1)	-0.3 (-1.6, 1.0)	-0.4 (-1.7, 1.0)	0.96	0.58
DHA + EPA	Total N (all Visits)	10,372	9,007	9,514	9,452		
	J-Point Ht Mean (Std)	53.2 (34.9)	54.8 (35.1)	55.9 (36.3)	56.0 (36.6)		
	Model 1	0.0 (ref)	-0.2 (-1.5, 1.0)	0.3 (-1.1, 1.6)	-0.9 (-2.3, 0.5)	0.30	0.20
	Model 2	0.0 (ref)	0.3 (-1.0, 1.5)	0.2 (-1.1, 1.5)	-1.0 (-2.4, 0.4)	0.21	0.10
	Model 3	0.0 (ref)	0.3 (-1.0, 1.5)	0.2 (-1.1, 1.5)	-0.9 (-2.3, 0.4)	0.24	0.12
ALA	Total N (all Visits)	9,872	9,566	9,615	9,292		
	J-Point Ht Mean (Std)	53.4 (34.9)	54.3 (35.1)	54.8 (36.3)	57.5 (36.6)		
	Model 1	0.0 (ref)	-0.3 (-1.6, 0.9)	-0.7 (-2.0, 0.5)	-0.6 (-1.9, 0.6)	0.67	0.27
	Model 2	0.0 (ref)	0.2 (-1.0, 1.5)	0.3 (-1.0, 1.6)	0.6 (-0.8, 2.0)	0.88	0.49
	Model 3	0.0 (ref)	0.2 (-1.1, 1.4)	0.2 (-1.1, 1.5)	0.5 (-0.9, 1.9)	0.93	0.62

Ht: height
Std: standard deviation
P Model: p value for the exposure term
P Trend: p value for exposure modeled linearly using the median values in each category
Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.
Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)
Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

5 MANUSCRIPT 3: BIOSTATISTICAL METHODS TO ADDRESS DIETARY EXPOSURE MEASUREMENT ERROR (IN FULFILLMENT OF THE BIOSTATISTICS MINOR)

5.1 SYNOPSIS

Background: A common issue in studies with a dietary exposure is measurement error in self-reported intake. The true (unknown) exposure value can be treated as a missing value problem, and it can be imputed using multiple imputation for measurement error (MIME). We expand on this idea using Multiple Imputation by Chained Equations (MICE).

Methods: We utilized data from the Atherosclerosis in Communities (ARIC) study, a prospective cohort of adults recruited from four field centers in the United States. One field center collected plasma fatty acid biomarker values from participants, and these data were used to augment self-report dietary data obtained via food frequency questionnaire. We imputed biomarker values using MICE to investigate the associations of biomarker measures of the omega-3 polyunsaturated fatty acids alpha linoleic acid (ALA), docosahexaenoic acid (DHA) + eicosapentaenoic acid (EPA), and ALA+DHA+EPA with prolonged QT, HbA1c, and incident type 2 diabetes (T2D). We also qualitatively compared associations in the full cohort – imputed and observed plasma values – to associations obtained using observed plasma values only.

Results: In both the full cohort and the observed plasma populations, none of the exposures were significantly associated with our outcomes of interest. Point estimates in both populations were similar across three different covariate-adjustment models, and confidence intervals were narrower for associations from the full cohort population than those from the observed plasma population.

Conclusions: Using MICE to augment self-report dietary data with biomarker data did not fundamentally change the associations between exposure and outcome, but it did increase precision.

5.2 INTRODUCTION

Measurement error in dietary exposures is a significant challenge for nutritional epidemiological studies [199] and understanding the direction and magnitude of the error is necessary for accurate interpretation of nutritional epidemiology study results [200]. Different dietary assessment methods can have varying levels of measurement error [169]; this manuscript will focus on food frequency questionnaire (FFQ)-derived measures and biomarker measures of dietary intake of omega-3 polyunsaturated fatty acids (PUFAs).

FFQs are relatively inexpensive ways to obtain dietary data from a large group of subjects without a high participant burden [169]. Some epidemiologists view FFQs as a way to qualitatively rank subjects' dietary intake from low to high and/or to quantitatively estimate subjects' absolute nutrient intakes [169]. However, many commentaries have been written regarding the fallibility of the data obtained from FFQs [201-203]. Key in those commentaries is evidence suggesting that data from FFQs are imprecise and subject to measurement error.

Biomarker values are another measure of dietary intake. These are objective measures that indicate how much of the nutrient was absorbed (bioavailability), and may be a good measure of usual intake provided between-season variability in an individual's intake is not large [169]. Given the potential biases that can result from measurement error in the exposure variable, several techniques have been developed to augment FFQ data using biomarker data [108, 114, 121, 122, 204-220]. For this manuscript, we used biomarker data collected from participants in the Minnesota (MN)

field center of the Atherosclerosis Risk in Communities (ARIC) Study to address potential measurement error in omega-3 PUFA intake as measured via FFQ.

Specifically, we expanded upon the premise embodied in Multiple Imputation for Measurement Error (MIME) and imputed biomarker data for non-MN ARIC participants using Multiple Imputation by Chained Equations (MICE) [221-223]. We then investigated the associations of plasma ALA, DHA+EPA, and ALA+DHA+EPA with three outcomes previously analyzed with respect to their associations with FFQ exposures: (1) prolonged QTc, (2) HbA1c, and (3) incident type 2 diabetes (T2D). Finally, we compared the measures of association in the full cohort (imputed + observed biomarker data) with those in the MN field center (observed biomarker data).

The remainder of this manuscript is divided into four parts. Part 1 introduces the ARIC study, our research questions of interest, and we describe the methods that are independent of multiple imputation. In Part 2, we define MIME and MICE and describe the methods specific to the multiple imputation process. Part 3 describes how we analyzed the post-imputation data and compared the results from our two populations of interest: results derived using observed plasma values from MN participants versus results derived from the full cohort (observed and imputed plasma exposure values). We conclude in Part 4 by describing our results and offering discussion points and overall conclusions.

5.3 PART 1 – THE ASSOCIATION OF OMEGA-3 PUFA WITH PROLONGED QTC, HBA1C, AND INCIDENT TYPE 2 DIABETES

5.3.1 BACKGROUND

5.3.1.1 Omega-3 PUFA and ECG Predictors of SCD

Intake of omega-3 PUFAs has been associated with lower incidence of sudden cardiac death (SCD) [46, 51, 194, 195]. ECG predictors of SCD include prolonged QT interval [96-99, 142], and prolonged QT interval may be the mechanism underlying the

association observed between intake of omega-3 PUFAs and SCD. We previously investigated whether FFQ-measured consumption of seafood, the fish-derived omega-3 PUFAs DHA and EPA, and the vegetable-derived omega-3 PUFA ALA were associated with heart rate-corrected QT interval (QTc) in the ARIC study. In this manuscript, we addressed potential FFQ measurement error by utilizing a biomarker sub-study to augment our ALA, DHA+EPA, and ALA+DHA+EPA exposure estimates. These updated exposures were used to reevaluate the association of omega-3 PUFA with prolonged QTc.

5.3.1.2 Omega-3 PUFA and Markers of Glucose Homeostasis

Studies investigating seafood intake and intake of fish-derived omega-3 PUFAs with markers of glucose homeostasis have been mixed with some reporting favorable [86], adverse [85-88], and null [85, 86] associations, depending on study population and exposure definition. Studies investigating intake of the vegetable-derived ALA have been similarly mixed with both favorable [85] and null [86] results. Given the inconsistent nature of the previous literature, we previously tested the associations among FFQ-obtained intakes of seafood, DHA + EPA, and ALA with glycemia outcomes in the ARIC study. In this manuscript, we addressed potential FFQ measurement error by utilizing a biomarker sub-study to augment our ALA, DHA+EPA, and ALA+DHA+EPA exposure estimates. These updated exposures were used to reevaluate the association of omega-3 PUFA with HbA1c and Incident T2D.

5.3.2 METHODS

5.3.2.1 Study Population

The ARIC study has been described previously [155]. Briefly, it is a prospective study of cardiovascular disease including 15,792 men and women 45–64 years of age at baseline (visit 1). Participants were recruited from four US communities using

probability sampling techniques. The communities and racial composition were: predominately white subjects from suburbs of Minneapolis, Minnesota, and Washington County, Maryland; black subjects from Jackson, Mississippi; and white and black subjects from Forsyth County, North Carolina.

For the analyses described in this manuscript, there were two sets of inclusion/exclusion criteria. The first set included criteria that were specific to the outcome of interest and the study design used in investigating that outcome. These are described later in this section (Part 1). The second set of inclusion/exclusion criteria are specific to the imputation methodology. These are described in Part 2.

5.3.2.2 Exposures: Measures of Dietary Omega-3 PUFA intake

Although our exposure of interest was circulating concentrations of omega-3 PUFA biomarkers, we will briefly revisit the FFQ measures of omega-3 PUFA before describing the biomarker exposures.

5.3.2.2.1 Food Frequency Questionnaire

Participants' usual dietary intake was assessed at visit 1 using an interviewer-administered, 66-item FFQ. The FFQ was a based on the instrument developed by Willett et al. [167], with three principal modifications: (1) Data regarding alcohol consumption were obtained using a separate, more detailed instrument; (2) Several food items were added (e.g., donuts, biscuits, and cornbread); and (3) Some items were split into detailed subcategories – notably a single item on fish consumption was separated into three specific fish items.

Daily intake of macro- and micronutrients was calculated via the FFQ by multiplying the nutrient content of each food by the frequency of daily consumption and then summing the results [124]. This process yielded daily intake of nutrients expressed as grams per day. Three different classifications of omega-3 fatty acids were

investigated in prior analyses: (1) vegetable-derived ALA, (2) fish-derived DHA+EPA; and (3) total omega-3 PUFA ALA+DHA+EPA.

5.3.2.2.2 Biomarker Measures of Plasma Fatty Acids

Blood samples were obtained from MN field participants at visit 1 (n= 3,757) and plasma fatty acids were measured in cholesterol esters and phospholipids using gas chromatography [126]. The fatty acid profile of cholesterol esters reflects medium-term (weeks) dietary intake of fatty acids while phospholipids reflect intake over a slightly longer duration (weeks to months) [127]. Phospholipid measurements were used for the present analysis. Previous analyses in ARIC have shown that correlation coefficients for FFQ values compared to plasma phospholipid values are r=0.15 (ALA), r=0.20 (EPA), and r=0.42 (DHA) [126].

Biomarker values of omega-3 PUFA were expressed as percentage of total fatty acids. We considered three exposure categories: ALA, DHA+EPA, and ALA+DHA+EPA. Each of these three exposures was categorized into quartiles.

5.3.2.3 Outcomes: ECG Predictors of Sudden Cardiac Death and Markers of Glucose Homeostasis

5.3.2.3.1 Prolonged QTc

At visit 1, a standard, resting, supine 12-lead ECG was obtained for each subject using MAC PC personal cardiography equipment (Marquette Electronics, Inc., Milwaukee, WI). We defined prolonged QT as a heart rate-corrected (QTc) value of 460 ms or longer in women and 450 ms or longer in men [138].

5.3.2.3.2 HbA1c

HbA1c was measured from whole blood samples using high-performance liquid chromatography. The blood was collected during visit 2 (1990-92) and stored at -70 °C

for 14-18 years until HbA1c measurements could be obtained. Selvin et al. give a detailed description of the HbA1c measurement process [158].

5.3.2.3.3 Incident Type 2 Diabetes

Diabetes was defined as (1) self-report of physician-diagnosed diabetes; (2) self-reported use of diabetes medication in the past two weeks; (3) fasting glucose level ≥ 7.0 mmol/liter (126 mg/dl); or (4) non-fasting glucose level > 11.1 mmol/liter (200 mg/dl).

5.3.2.4 Covariates: Potential Confounders

All potential confounders were measured at visit 1. Variables of interest included sociodemographic variables (age, sex, race, and education), lifestyle variables (physical activity, smoking status, drinking status, and drinking amount), dietary variables (*trans* fatty acids, saturated fatty acids, dietary fiber), and clinical variables (body mass index (BMI), hypertension, LDL, HDL, triglycerides).

Several variables were obtained via self-report. Age, sex, and race were obtained at visit 1 and confirmed at subsequent visits. Participants' smoking status and education level were obtained via self-report and categorized. Education level was categorized based on years of education: basic (less than high school graduate), intermediate (high school graduate, no college), and advanced (at least some college). Smoking status had three categories: current smokers, former smokers (more than 100 cigarettes in the past), and never smokers.

Physical activity, alcohol intake, and dietary intake were measured using validated instruments. Physical activity was measured using the Baecke questionnaire [144]. The questionnaire translated into three indexes ranging from 1 (low) to 5 (high) for physical activity at work, during leisure time, and in sports. The three physical activity scores were summed and then translated into tertiles of physical activity (low,

medium, and high). Alcohol intake and amount (grams/day) was measured via an interviewer-administered questionnaire. Dietary intake of *trans* fatty acids, saturated fatty acids, and dietary fiber were obtained with the same methodology used to translate FFQ responses into grams of omega-3 PUFA per day.

Finally, the remaining variables were measured by trained technicians. Weight and height were measured with the participant wearing light clothes. BMI was calculated as weight (kilograms) divided by height squared (meters^2). Technicians also obtained three blood pressure measurements with a random-zero sphygmomanometer and the mean of the last two measurements was used. Hypertension was defined as a systolic blood pressure above 140 mmHg, a diastolic above 90 mmHg, or self-reported use of antihypertensive medication. Participants with missing hypertension values were imputed as not having hypertension (no disease). For metrics requiring phlebotomy, blood was drawn after a minimum 8-hour fasting period with minimal trauma from an antecubital vein [146]. Plasma total cholesterol and triglycerides were measured by enzymatic methods [132], and LDL cholesterol was calculated using the Friedewald formula [147]. HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins [132].

5.3.2.5 Statistical Analysis

The statistical methods used were dependent on the study design used to investigate the relationship between the exposure and the outcome of interest. Outcome-specific study populations, study designs, and statistical methods are described below. Figure 5-1 represents this pictorially. All analyses were limited to white participants – additional details regarding rationale are in Part 2.

5.3.2.5.1 Prolonged QTc

Analyses investigating this outcome utilized data from a cross-sectional study design using data from visit 1. Participants were excluded if they were non-white and if

they had prevalent cardiovascular disease (CVD) – defined as coronary heart disease (CHD), heart failure (HF), or stroke – as prevalent conditions influence how patients have their comorbidities managed, diagnosed and treated. If a participant was missing data on CVD prevalence, it was assumed that the participant did not have prevalent CVD. We excluded participants who self-reported use of antiarrhythmic medications and those whose duration of the QRS complex was ≥120 ms, as those individuals have major conduction defects that make the interpretation of primary repolarization abnormalities inappropriate [197].

Data were analyzed using logistic regression – prolonged QTc was the outcome of interest and quartiles of plasma ALA, DHA+EPA, and ALA+DHA+EPA were the exposures, with the lowest quartile as the reference category. Three covariate-adjustment models were tested. Model 1 included age, sex, and education level. Model 2 was Model 1 plus BMI, physical activity, smoking status, drinking status and amount, and the dietary variables *trans* fatty acids, saturated fatty acids, and dietary fiber. Model 3 was Model 2 plus the clinical covariates: hypertension, LDL, HDL, and triglycerides.

5.3.2.5.2 Hemoglobin A1c

Analyses investigating this outcome utilized data from a modified cross-sectional study design, with exposures and potential confounders measured at visit 1 and the outcome measured at visit 2. Our exclusion criteria were non-white race and CVD. We excluded those participants who reported diagnoses of CHD, HF, or stroke at either visit 1 or 2 – not only because their clinical treatment may differ from those who do not have those conditions, but because incident disease between visits 1 and 2 may have led to changes in the exposure and covariate behaviors reported at visit 1. Finally, we restricted our analysis to diabetic participants as previous analyses suggested this population was most likely to show differences in HbA1c levels across different strata of omega-3 PUFA intake.

For those participants who reported taking anti-hyperglycemic medications, we applied a correction factor using the approach described in Tobin et al. [159] and used in other studies with glycemia outcomes [160, 161]. Specifically, for medicated participants, we added a constant of 1 percentage point to HbA1c values. These constants were based on pharmaceutical studies, systematic reviews, and meta-analyses of the effect of medication on glycemia lab values [162-166].

Data were analyzed using linear regression – HbA1c was the outcome of interest and quartiles of plasma ALA, DHA+EPA, and ALA+DHA+EPA were the exposures, with the lowest quartile as the reference category. The same three covariate-adjustment models were tested: Model 1 (sociodemographic variables), Model 2 (Model 1 + lifestyle and dietary variables), and Model 3 (Model 2 + clinical variables).

5.3.2.5.3 Incident Type 2 Diabetes

This was a prospective study design, with exposures and potential confounders measured at visit 1 and the outcome of interest being time to incident T2D or censoring. Our inclusion/exclusion criteria were similar to the other two outcomes with respect to race and CVD. Assignment of variables followed the algorithm depicted in Figure 5-2, with participants censored if lost to follow-up, experienced incident CVD, or reached visit 4 without developing T2D.

Data were analyzed using Cox proportional hazards regression. Exposures were quartiles of plasma ALA, DHA+EPA, and ALA+DHA+EPA, with the lowest quartile as the reference category. The same three covariate-adjustment models were tested: Model 1 (sociodemographic variables), Model 2 (Model 1 + lifestyle and dietary variables), and Model 3 (Model 2 + clinical variables). We tested for violations of the proportional hazards assumption using a *log(time)*exposure* interaction term in our model and an α of 0.10 (Ho=no violation).

5.4 PART 2 – MULTIPLE IMPUTATION METHODS

As previously mentioned, this section will describe our imputation process. Here we present an overview of multiple imputation followed by our imputation-specific methods.

5.4.1 OVERVIEW

When investigating the associations between dietary exposures and an outcome of interest, the investigators attempt to quantify subjects' true intake. Researchers must consider participant burden and expense along with measurement error and accuracy. FFQs are inexpensive and have low participant burden [169], but their output can have measurement error [198].

Our paradigm can be described as follows: consumption of omega-3 PUFA and other foods, behaviors (smoking, exercise), and biological factors (race, sex) influence how dietary omega-3 PUFA are digested and absorbed into circulating concentrations of biomarker omega-3 PUFA. Data exist for diet, behaviors, and biological factors in the full ARIC cohort. Data exist for biomarkers in the MN sub-study. We can use the MN data to establish the relationship of diet and other variables with plasma values of omega-3 PUFA.

This is not without precedent. Several approaches exist to leverage biomarker data to address measurement error [108, 114, 121, 122, 209]. One such approach is multiple imputation for measurement error or MIME – a technique first proposed by Rubin in 1987 [216, 224] that treats measurement error as a missing data problem where the "true" value is missing. We expand on this premise, but instead of imputing corrected FFQ values using data from a validation study, we impute plasma biomarker values utilizing the validation sub-study and FFQ data.

The multiple imputation process can be summarized as three steps. The first is that missing values are replaced with a simulated value to create a complete dataset. This process is repeated until there are *m* datasets. After the imputation process is

finished, step two is to analyze each of the *m* complete datasets separately using standard methods – these are the methods that were described in Part 1. As an example, for our HbA1c outcome, each dataset was analyzed using linear regression with the imputed exposure values and relevant covariates. Each of the *m*-sets of analyses result in *m*-sets of regression results. Finally, step three combines the *m*-sets of regression data into a single result using "Rubin's Rules" – simplistically they are "averaged" to get an overall beta coefficient and standard error [220, 225, 226].

5.4.2 METHODS

Our overall manuscript methods can be simplistically described in three steps: impute missing values, analyze the data for our two populations of interest (full cohort and MN only), compare the population-specific results. This section describes the imputation process – specifically the inclusion/exclusion criteria, imputation method used, variables used in the imputation, and model specifications. All statistical analyses were performed with SAS (version 9.4, Enterprise guide 7.1, SAS Institute Inc., Cary, NC, USA).

5.4.2.1 Inclusion and Exclusion Criteria

As previously mentioned, there were two sets of inclusion/exclusion criteria: those specific to imputation, and those specific to our outcomes (e.g., diabetics only for HbA1c analyses). Here we will describe our imputation inclusion/exclusion criteria.

We could not eliminate the possibility that race could be a confounder or an effect modifier in the relationship between dietary intake of omega-3 fatty acids and biomarker measures of omega-3 fatty acids. There is evidence that the translation of FFQ responses into nutrients can vary by race [227]. Additionally, race can be associated with FFQ exposure through dietary patterns and cooking methods and affect biomarker concentrations through potential genetic differences in converting dietary PUFA to plasma PUFA [228]. Because MN field center participants were overwhelmingly

white, we could not include race in our imputation model. Consequently, we limited our analyses to white participants at the MN and other field centers.

Additionally, we excluded those participants who did not fill out an FFQ (n=12), whose FFQ responses were not translatable into nutrient intakes (n=207), and those who had implausible responses on their FFQ (n=47; 43 males with values below 700 kcal/day and 4 females with values above 3500 kcal/day). The improbable calorie cut-points (males: >4500, <700; females: >3500, <500) represent the 1st and 99th sex-specific percentiles of all ARIC FFQ responses [156]. Because we hypothesized biomarker imputation would depend on dietary intakes, we did not want implausible data to skew our results.

5.4.2.2 Multiple Imputation by Chained Equations

Step 1 in the imputation process is to replace missing values with a simulated value. We calculated our simulated values via MICE [221-223] , also known as fully conditional specification [212, 223]. Azur et al.[221] describes the process in five steps:

1. Impute a placeholder value for all the missing values in your dataset. For example, a random sample of observed values from your validation study.
2. Select a single variable, and set all placeholder values for that variable back to missing.
3. Regress the variable of interest on the variables of the imputation model (e.g., biomarker values of ALA regressed on all other variables in the dataset)
4. Replace the missing values from step 2 with predictions from step 3. This is like step 1 above except, instead of placeholders, we are imputing predicted values based on regression.
5. Complete this process (steps 2-4) for all variables with missing values

A cycle is defined completing these steps for all variables with missing values. For subsequent cycles, steps 2 through 5 are repeated. Typically at least 10 cycles are performed [229] and, once a predetermined number of cycles are complete, the final iteration is output. This output is an imputed dataset. This process is repeated until *m* imputed datasets are output.

5.4.2.3 Selecting Imputation Model Parameters

Here we describe how we selected our variables, regression techniques, cycles to run before outputting an imputed dataset, and total number of imputed datasets.

5.4.2.3.1 Variable Selection

We had FFQ data on all ARIC participants and biomarker measures of plasma fatty acids for MN field center participants. We wanted to address FFQ measurement error by utilizing the biomarker values – specifically, using the MN biomarker sub-study to elucidate the relationship between FFQ-derived intake of omega-3 PUFA and plasma measures of omega-3 PUFA – and then use these findings to impute plasma measures for the rest of the ARIC cohort.

As previously stated, variables with missing values are imputed by regressing their observed values (dependent variable) against a set of related variables (independent variables) [221-223]. Given our theoretical paradigm (biomarkers are a function of dietary intake), our imputation model for our exposure variables (plasma biomarkers) must at a minimum include their FFQ-derived analogs.

For the full imputation model, we selected our variables based on two criteria adapted from Azur et al. [221]: (1) the imputation model should be more general than the models used in post-imputation analyses; and (2) impute variables at lowest level possible.

For #1, a “more general” imputation model means that the imputation model should include all covariates used in the post-imputation analyses [221] along with post-imputation outcomes [217, 230]. If interaction terms will be used in post-imputation regressions, then those terms should be in the imputation model as well [221]. Variables that predict missingness should also be included in the model [217]. In general, increasing the number of variables in the imputation model does not deleteriously influence precision or bias [221, 231], but failing to include important variables can [217, 230].

For #2, to impute variables at the lowest level possible means that if a continuous variable was categorized, we included its continuous form in the imputation model and re-categorized it post-imputation [221, 232]. Similarly, if a variable was a function of other variables (e.g., x = f(a, b, c)) then we included a, b, and c in the imputation model and generated x post-imputation.

In addition to our exposures (and dietary analogs), outcomes, potential confounders, and variables used for inclusion/exclusion, we included other variables in the imputation model that could have predicted omega-3 PUFA intake. These included fish consumption in servings/week as measured via FFQ (tuna, dark fish, other fish, and shellfish) and dietary intake of omega-6 PUFA in grams/day as these fatty acids influence the bioavailability of dietary omega-3 PUFAs [233].

The “more general” approach for selecting covariates relies on including interaction terms if necessary [221]. We did not include interaction terms in our imputation model for two reasons. The first is because previous analyses did not show evidence of interaction by sex for the association of omega-3 PUFAs and HbA1c, prolonged QTc, or time to incident T2D. The second is that the MN data did not suggest there were differences by sex for dietary intake of omega-3 PUFAs and plasma levels after adjusting for total caloric intake (data not shown).

Figure 5-3 lists the variables that were part of our imputation model along with the regression method used for imputation.

5.4.2.3.2 Regression Method

To justify our selection of regression method (linear, logistic, or discriminant analysis), we will first provide some theoretical details. There are two components to multiple imputation: the model (variables) and the distribution assumptions. That is, imputation is a function of our data Y and distributions theta: $f(Y|\Theta)$ [212, 234]. Imputing a missing value is a random draw of the set of all imputed values from the posterior predictive distribution of the missing data: $f(Y_{miss}|Y_{obs}|\Theta)$ where Θ is the vector of parameters or functions of these that uniquely define the predictive distribution [212]. When software performs the imputation using MICE, part of the process is deriving the posterior predictive distribution $f(Y_{miss}|Y_{obs}|\Theta)$ [212, 226, 234].

Continuous variables with missing data use linear regression derive the posterior predictive distribution, and categorical variables can use logistic regression or discriminant analysis [212, 234]. For categorical variables, we tried logistic regression first as it makes the fewest assumptions about model parameters [234]. However, sometimes maximum likelihood estimation failed to estimate a posterior predictive distribution without augmenting the data [235]. An alternative to augmenting data is to use discriminant analysis instead of logistic regression – discriminant analysis also estimates categorical outcomes, but assumes covariates are approximately multivariate normal and the within-group covariance matrices are approximately equal [234]. We did not test these assumptions, but relied on our ample sample size [171].

Finally, regardless of regression method, all regressions were set to have a minimum value of zero by using the minimum option in SAS PROC MI. Although setting a minimum value can introduce bias if the data are skewed [236], post-imputation rounding of values also introduces bias [236]. Since all plasma values were biologically constrained from 0 to 100 (as a percentage of total fatty acids), we set the minimum

value as zero. In a sensitivity analysis, we considered how many negative values were imputed if we did not include the minimum option. Six (out of 49) variables had negative values (across all imputations) and the percentages of negative values were all less than 0.1% except for the physical activity via work variable, which had 2.3% negative values.

5.4.2.3.3 Cycles and Datasets

As previously mentioned, typically at least 10 MICE cycles (burn-ins) are performed before outputting an imputed dataset [229]. We used 40 burn-in iterations.

Theoretically, five imputed datasets (*m*=5) are sufficient for the imputation technique [210, 218, 237]. However, using a large number of imputed datasets (*m*>20) provides a greater ability to reduce sampling variability from the imputation process and better confidence interval coverage [214, 217, 218]. Table 5-1 shows the relative efficiency given percentage of missingness as derived using the equation $RE = (1 + \frac{\lambda}{m})^{-1}$ where *RE* is relative efficiency, λ is the percentage of missing data, and *m* is the number of datasets [211].

Because we imputed biomarker values for all white ARIC participants who were not part of the biomarker subsample, we had a high proportion of missingness (approximately 2,500 MN participants among approximately 9,500 white participants is roughly 75% missingness). We used m=40 imputations for our analysis.

5.5 PART 3 – POST IMPUTATION

Here we describe how we utilized the imputed data to investigate the associations between omega-3 biomarker exposures and our outcomes of interest, and how we compared results from our two populations of interest (full cohort and MN-only).

5.5.1 POST-IMPUTATION ANALYSES

Our imputation process generated *m*=40 datasets where all missing values were imputed. Using these data, we considered our two populations. For simplicity, we will use the term "imputed" for the full cohort even though those data contain both imputed and observed plasma values. Similarly, we will use "observed" for the MN participants with observed plasma values, even though some covariates had missing values imputed.

Each of the two populations were analyzed as described in Part 1. That is, each of the *m* datasets had outcome-specific inclusion/exclusion criteria applied and were analyzed using the outcome-specific statistical methods. The *m* sets of results were combined into a single set of regression results using Rubin's rules [220, 225, 226] via SAS PROC MIANALYZE.

5.5.2 COMPARING RESULTS

All exposures of interest were categorized into quartiles with the lowest quartile as the reference category. Thus, for any given exposure, we had six beta estimates and six standard errors – three for the full cohort (imputed) results (Q2 vs. Q1, Q3 vs. Q1, and Q4 vs. Q1) and three for the MN population (observed) results.

We considered if our full cohort (imputed) results were qualitatively different from the observed (MN field center) results. That is, if imputing missing plasma values fundamentally changed the measures of association compared to results obtained using observed plasma values. We did this by comparing the beta coefficients and their confidence intervals for the imputed and observed populations.

5.6 RESULTS

In this section, we will describe the results from the imputation process first, followed by the results from regressions involving the three outcomes of interest. For

each outcome, we report and compare the imputed (full cohort) results and the observed (MN-specific) results.

5.6.1 IMPUTATION RESULTS

Our goal was to impute plasma values for the white, non-MN participants and impute any missing covariates in the full cohort. There were 11,478 white participants at visit 1. Of those, n=266 were excluded for missing or implausible FFQ results. Data from the remaining n=11,212 participants were available for the multiple imputation procedure. Table 5-2 lists variables that had missing values prior to the imputation process and the relative efficiency after 40 imputations. Table 5-3 shows the baseline characteristics post-imputation for the MN field center participants, non-MN field center participants, and the full cohort. Counts were calculated by averaging across the *m*=40 imputations. In general, Minnesotans were more highly educated and exercised more. A greater proportion of Minnesotans were current alcohol drinkers, but fewer were current smokers.

5.6.2 POST IMPUTATION REGRESSION RESULTS: PROLONGED QTC.

For the prolonged QTc analysis we excluded those with prevalent CVD, those who used antiarrhythmic medications, and those whose QRS duration was greater than 120 ms. Figure 5-4 shows the inclusion/exclusion results for the prolonged QTc analysis. Average counts across the 40 imputations were n=8,791 full cohort; n=3,061 MN only.

5.6.2.1 ALA, DHA+EPA, ALA+DHA+EPA and Prolonged QTc

In both the full cohort and the MN-only analyses, quartiles of plasma ALA, DHA+EPA, or ALA+DHA+EPA were not associated with prolonged QTc. See tables 5-4, 5-5, and 5-6 and figures 5-5, 5-6, and 5-7.

5.6.2.2 Full Cohort vs. Minnesota-Only: Prolonged QTc

Results for the imputed exposures (full cohort) analyses were similar to those for the observed exposures (MN-only). There were no instances where the point estimate in one population suggested lower odds but the other population suggested higher odds. Furthermore, the point estimate for one population was always within the 95% confidence interval for the point estimate of the other population. Finally, the confidence intervals for the MN analyses were always wider than those for the full cohort analyses. Figure 5-8 shows all nine analyses.

5.6.3 Post Imputation Regression Results: HbA1c.

Analyses investigating the HbA1c outcome were limited to those with T2D at visit 2. Additionally, the exclusion criteria for the HbA1c outcome was prevalent CVD at visit 1 or visit 2. Figure 5-4 shows the inclusion/exclusion criteria. Final counts were n=1,019 for the full cohort; and n=299 for the MN population.

5.6.3.1 ALA, DHA+EPA, ALA+DHA+EPA and HbA1c

In both the full cohort and the MN-only analyses, there was no association of quartiles of plasma ALA with HbA1c. See Table 5-7 and Figure 5-9.

In both the full cohort and the MN-only analyses, there was a suggestion that higher concentrations of DHA+EPA and of ALA+DHA+EPA are associated with lower HbA1c in those with T2D, although the association was not statistically significant (ALA+DHA+EPA Q4 vs. Q1 percentage point difference (95% CI); Model 3; full cohort: -0.4 (-1.0, 0.2); MN-only: -0.6 (-1.3, 0.2)). See tables 5-8 and 5-9 and figures 5-10 and 5-11.

5.6.3.2 Full Cohort vs. Minnesota-Only: HbA1c

Results for the imputed exposures (full cohort) analyses were similar to those for the observed exposures (MN-only). There were only two instances where the point estimate in one population suggested lower HbA1c values with higher concentrations of omega-3 PUFAs and the other population suggested higher HbA1c with higher concentrations (Q4 vs. Q1, ALA, Model 3; Q2 vs. Q1, ALA+DHA+EPA, Model 1). Regardless, the point estimate for one population was always within the 95% confidence interval for the point estimate of the other population. Finally, the confidence intervals for the MN analyses were always wider than those for the full cohort analyses. Figure 5-12 shows all nine analyses.

5.6.4 POST IMPUTATION REGRESSION RESULTS: INCIDENT T2D.

Our exclusion criteria for incident T2D analysis were prevalent CVD or T2D at visit 1. Figure 5-4 shows the inclusion/exclusion criteria for the incident T2D analysis. Final counts were n=8,638 full cohort; n=3,131 MN only. In all analyses, the proportional hazards assumption was not violated (data not shown).

5.6.4.1 ALA, DHA+EPA, ALA+DHA+EPA and Incident T2D

In both the full cohort and the MN-only analyses, there was an association of quartiles of plasma ALA with incident T2D, with higher circulating concentrations associated with lower risk (Q4 vs. Q1 HR (95% CI), Model 1; full cohort: 0.6 (0.5, 0.9); MN-only: 0.6 (0.4, 0.9)), although this association disappeared after adjustment (Q4 vs. Q1 HR (95% CI), Model 3; full cohort: 0.8 (0.6, 1.1); MN-only: 0.9 (0.6, 1.3)). See Table 5-10 and Figure 5-13.

In both the full cohort and the MN-only analyses, there was no association of DHA+EPA or ALA+DHA+EPA with incident T2D. See tables 5-11 and 5-12 and figures 5-14 and 5-15.

5.6.4.2 Full Cohort vs. Minnesota-Only: Incident T2D

Results for the imputed exposures (full cohort) analyses were similar to those for the observed exposures (MN-only). There were no instances where the point estimate in one population suggested lower incidence of T2D in those with higher concentrations of omega-3 PUFAs and the other population suggested higher incidence, although there were multiple instances where one hazard ratio rounded to 1.0 and the other did not. Furthermore, the point estimate for one population was always within the 95% confidence interval for the point estimate of the other population. Finally, the confidence intervals for the MN analyses were always wider than those for the full cohort analyses. Figure 5-16 shows all nine analyses.

5.7 DISCUSSION

In this population-based study of middle aged adults, our investigation of the relationship among plasma omega-3 PUFA observed in MN participants suggested that higher circulating concentrations of ALA may be associated with lower incidence of T2D. The data also suggested that circulating concentrations of DHA+EPA in those with T2D may be associated with lower HbA1c. Using the MN data to impute plasma values for other white participants at other field centers yielded similar results, and the standard errors were lower for the full cohort analyses compared to the MN-only analyses as can be seen by the narrower confidence intervals.

Our plasma-exposure results were different from the FFQ results for some exposures and outcomes. Specifically, higher intake of ALA (grams/day) was associated with higher incidence of T2D in Manuscript 1 results (HR 4.0, 95% CI: 1.7, 9.6, comparing

extreme quartiles) whereas there was a nonsignificant trend in the plasma analysis towards lower incidence of T2D with higher levels of circulating ALA. Similarly, the HbA1c results from Manuscript 1 indicated that higher versus lower intakes of omega-3 PUFAs, especially ALA, were associated with approximately 1 percentage point higher HbA1c values in those with T2D. In contrast, plasma analyses suggested no relationship between circulating concentrations of ALA and HbA1c, and further suggested a non-significant association of DHA+EPA and ALA+DHA+EPA with HbA1c, with higher concentrations non-significantly associated with lower HbA1c values.

These differences could be due to measurement error in FFQ data that was corrected using biomarker data. However, other plausible explanations exist. Disease status (T2D) could limit the absorption of omega-3 PUFAs, so higher plasma levels could be an indicator for less disease (and thus result in a favorable association). There could also be unmeasured confounding – dietary variables that influence the bioavailability of omega-3 PUFAs that that were not included in our imputation model, or geographically differences in the omega-3 content of foods in MN compared to other areas of the United States. Finally, there was evidence of effect modification by race in the relationship of dietary intake of fish and seafood with HbA1c values, with whites showing null-to-favorable associations with seafood exposures. Thus, differences could be due to limiting analyses to whites only.

The approach used in this study was motivated by MIME, where we sought to address dietary measurement error by utilizing a plasma biomarker sub-study. Other researchers have combined biomarker data with FFQ data where participants had both measures [121, 128] but, to our knowledge, ours was the first to utilize multiple imputation in harnessing the relationship between dietary intake and circulating biomarker values and expanding the analysis beyond those participants in the sub-study.

In our own analysis, our comparison of the observed and imputed results is akin to comparing a complete case analysis (observed MN results) to imputed results (full cohort). A minor difference is that our observed MN results had observed plasma values, but missing covariate values were imputed using MICE.

Our results are similar to other studies comparing multiple imputation results to complete case analysis where point estimates were similar and confidence intervals/standard errors were more narrow/smaller [238, 239]. In a case-control study of diet and breast cancer, multiple imputation had similar point estimates and smaller standard errors compared to complete case analysis, but imputation also revealed associations between other covariates and breast cancer that the complete case analysis did not [240].

One study did not perform a complete case analysis, but their design does demonstrate the viability of imputing a large number of missing values and obtaining an unbiased measure of association [241]. This study utilized an existing case-cohort design with waist circumference data available for the entire cohort. The authors simulated a multiple imputation analysis by deleting waist circumference values for all participants except those the comparison sample. This resulted in approximately 90% missing values for the exposure of interest. In their analysis, the association of waist circumference with incident T2D using imputed data was similar to the association calculated using full cohort data, demonstrating that multiple imputation can provide unbiased estimates of association when compared to those obtained with observed values [241].

In general, multiple imputation is more efficient than complete case analysis when data are missing completely at random and is less biased when data are MAR [14]. Other missing data mechanisms can lead to increased bias with multiple imputation, and merely comparing standard errors is insufficient because that does not account for bias – standard errors may be smaller because the estimate was biased towards the null

[14]. In general, methodologists suggest that multiple imputation and complete case analysis offer a tradeoff between bias and precision [114, 213] and that the increase in precision is reduced as the size of the validation sample increases [242].

5.8 CONCLUSION

While our results were predominately null, the similarity of our results between the observed and the imputed populations and the narrower confidence intervals in the imputed full cohort results suggest we gained efficiency/precision through imputation. Utilizing biomarker data in conjunction with self-report dietary data could be an important tool in investigating diet/disease relationships.

5.9 FIGURES

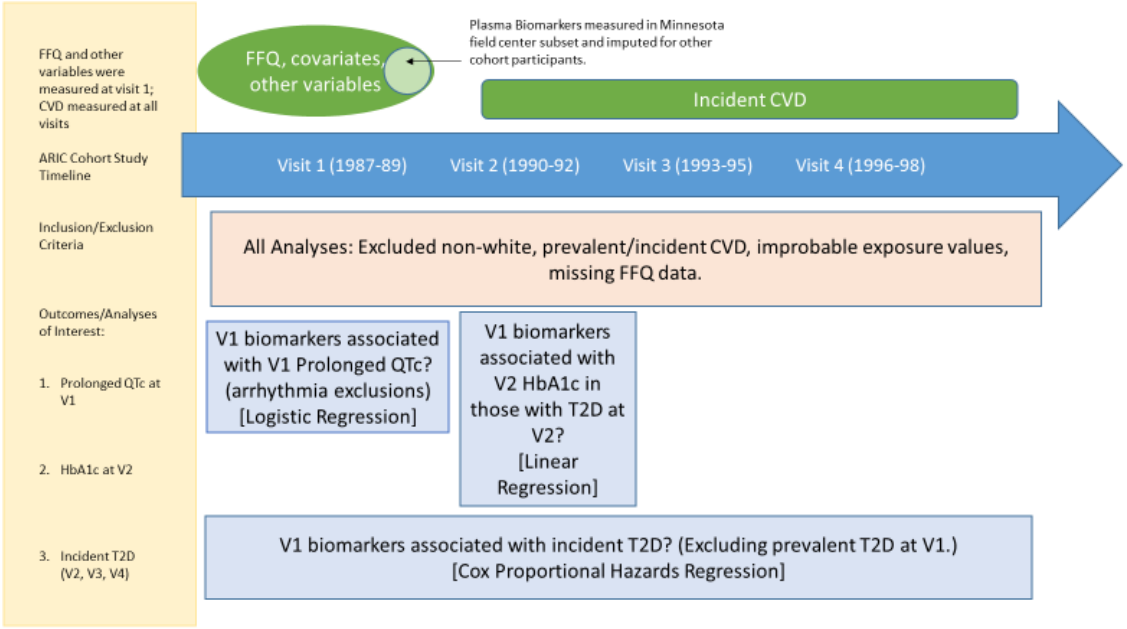

Figure 5-1. Study design depiction with variable temporality, ARIC, 1987-1998

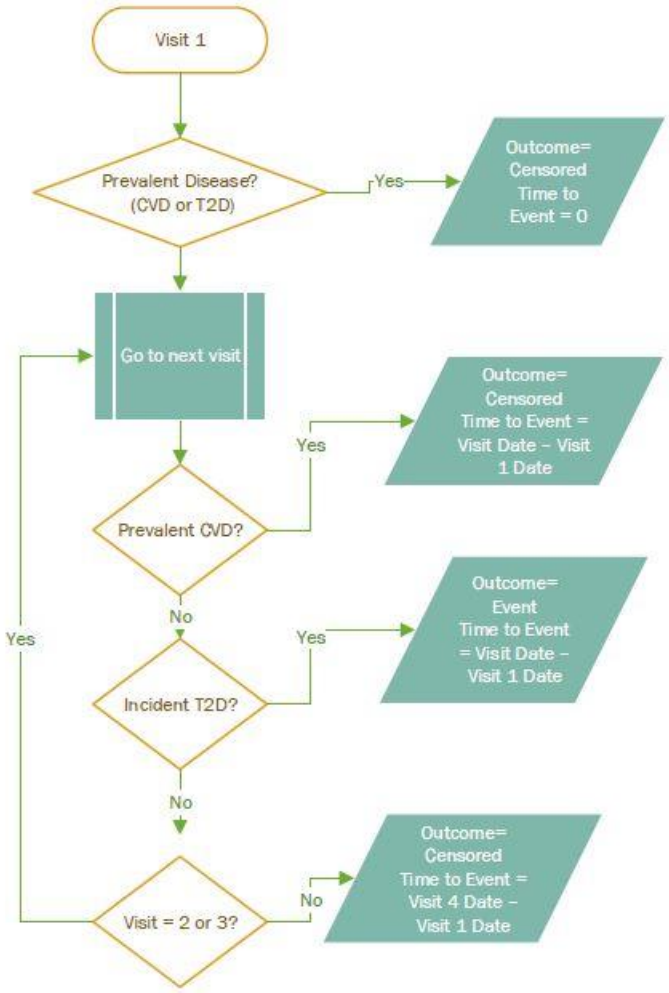

Figure 5-2. Assignment of outcome and time to event data for incident T2D analysis, ARIC, 1987-1998.

Post-Imputation Exposures	Post-Imputation Model Covariates		Outcome or Used in Derivation of Outcome
Plasma ALA	Age	Saturated Fatty Acids g/day	Heart Rate
Plasma DHA	Sex[D]	Diastolic Blood Pressure	QT duration
Plasma EPA	Education Level[LR]	Systolic Blood Pressure	HbA1c
Dietary Predictors of Plasma Omega-3 PUFA	BMI	Hypertension[LR]	Antihyperglycemic medications at Visit 2[D]
ALA grams/day	Indices from Baecke (Work, Sport, Leisure)	HDL	Time to event
EPA grams/day	Smoking status (current, former, never)[LR]	LDL	Censored/T2D[D]
DHA grams/day	Drinking status (current, former, never)[D]	Triglycerides	**Inclusion Exclusion Criteria**
Fish servings/week (tuna, dark, other, shell)*	Drinking amount g/day		QRS duration
Omega-6 fatty acids grams/day	TCAL per day		Type 2 Diabetes at Visit 2[D]
(octadecadienoic acid, eicosatetraenoic acid,	Trans fatty acids g/day		Anti-arrhythmic medications[LR]
and docosapentaenoic acid)*	Dietary Fiber g/day		**Other Relevant Variables**
[D] Modeled using Discriminant Function	*Each parenthetical is a separate continuous variable		Fasting Blood Glucose, T2D[D]
[LR] Modeled using Logistic Regression	Unlabeled variables used linear regression. Variables Visit 1 unless noted.		Antihyperglycemic medications[D]

Figure 5-3. Variables and regression techniques used in multiple imputation model, ARIC, 1987-1998.

Visit 1 Participants (MN, NC, MD)	11,212	3,861	Visit 1 Participants (MN Only)
With T2D @ Visit 2	1,342	377	With T2D @ Visit 2

	Prolonged QTc		
QRS>120	420	150	QRS>120
anti-arrhythmic Rx	1,593	533	anti-arrhythmic Rx
V1 Prevalent CVD	1,086	323	V1 Prevalent CVD
Prol QTc (all exclusions)	2,421	800	Prol QTc (all exclusions)
Total N	**8,791**	**3,061**	**Total N**

	HbA1c		
V1 Prevalent CVD	273	67	V1 Prevalent CVD
V2 Prevalent CVD	196	46	V2 Prevalent CVD
HbA1c - T2D only (all exclusions)	323	78	HbA1c - T2D only (all exclusions)
Total N	**1,019**	**299**	**Total N**

	Incident T2D		
Exclusions for Prevalant Disease	2,574	730	Exclusions for Prevalent Disease
Total N	**8,638**	**3,131**	**Total N**

Figure 5-4. Inclusion/Exclusion criteria, ARIC, 1987-1998.

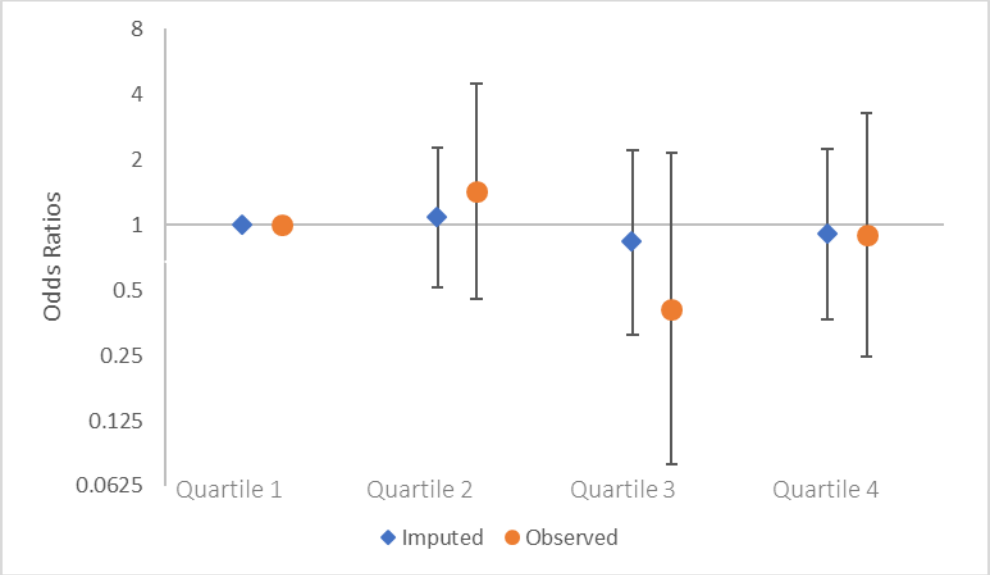

Figure 5-5. Odds ratios for prolonged QTc by quartiles of ALA; fully adjusted model; multiple imputation vs. observed.

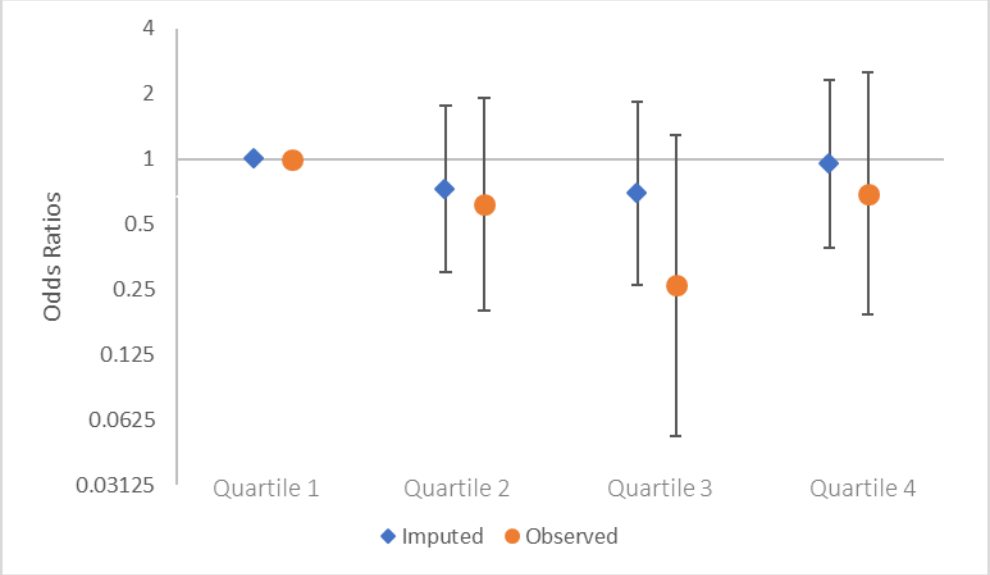

Figure 5-6. Odds ratios for prolonged QTc by quartiles of DHA+EPA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1989.

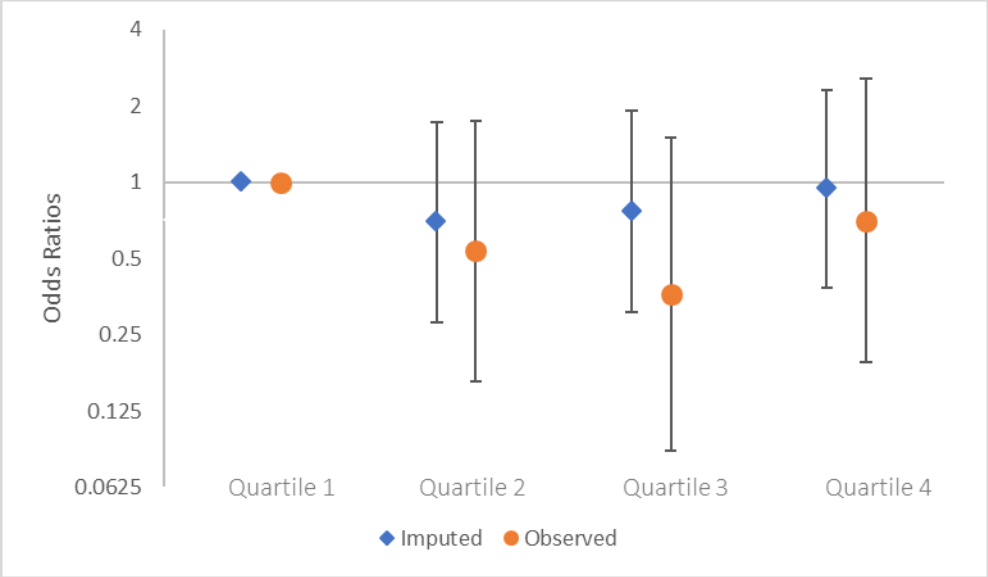

Figure 5-7. Odds ratios for prolonged QTc by quartiles of ALA+DHA+EPA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1989.

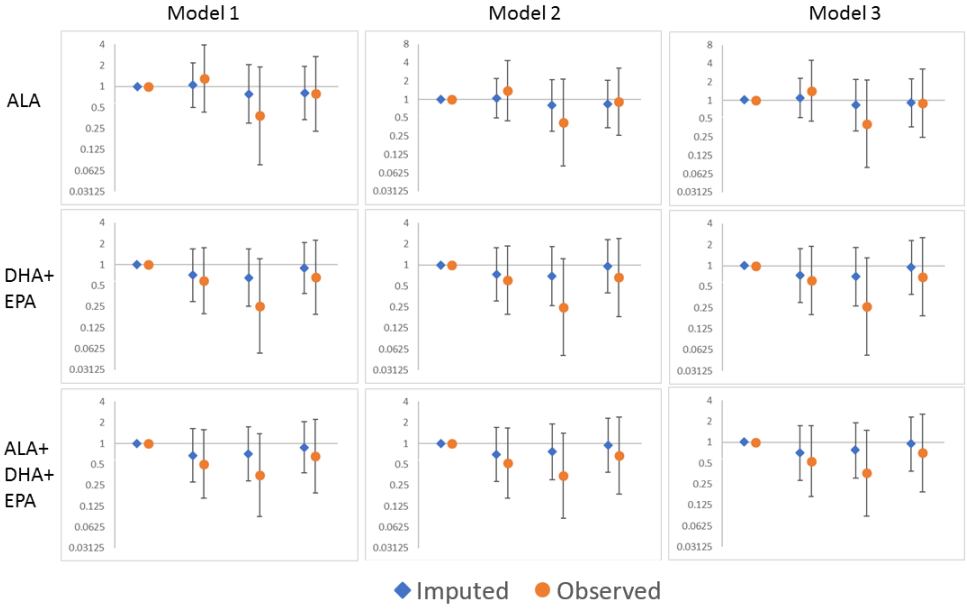

Figure 5-8. Odds ratios for prolonged QTc by quartiles of ALA, DHA+EPA, and ALA+DHA+EPA; all covariate adjustment models; multiple imputation vs. observed plasma values, ARIC, 1987-1989.

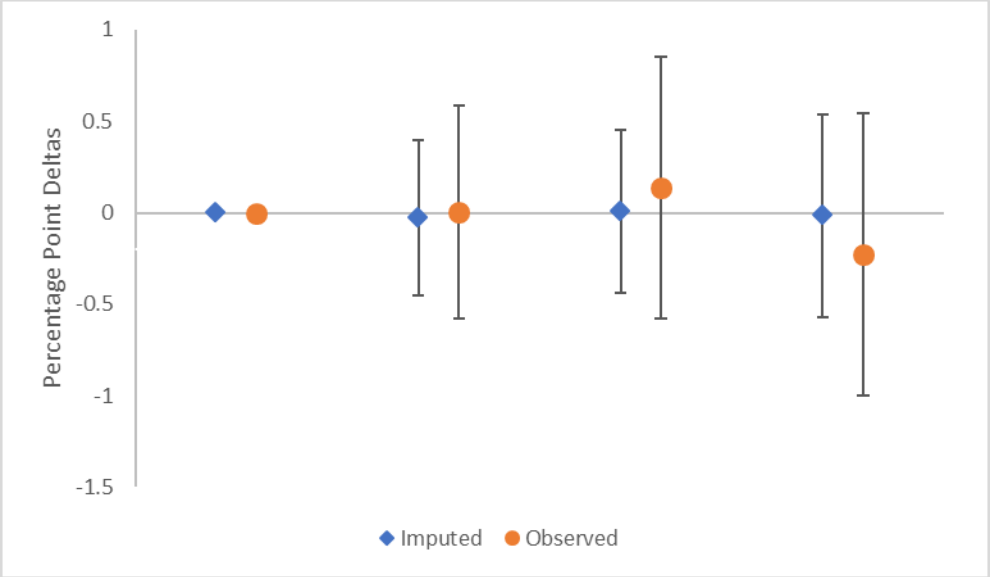

Figure 5-9. Deltas in HbA1c by quartiles of ALA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1993.

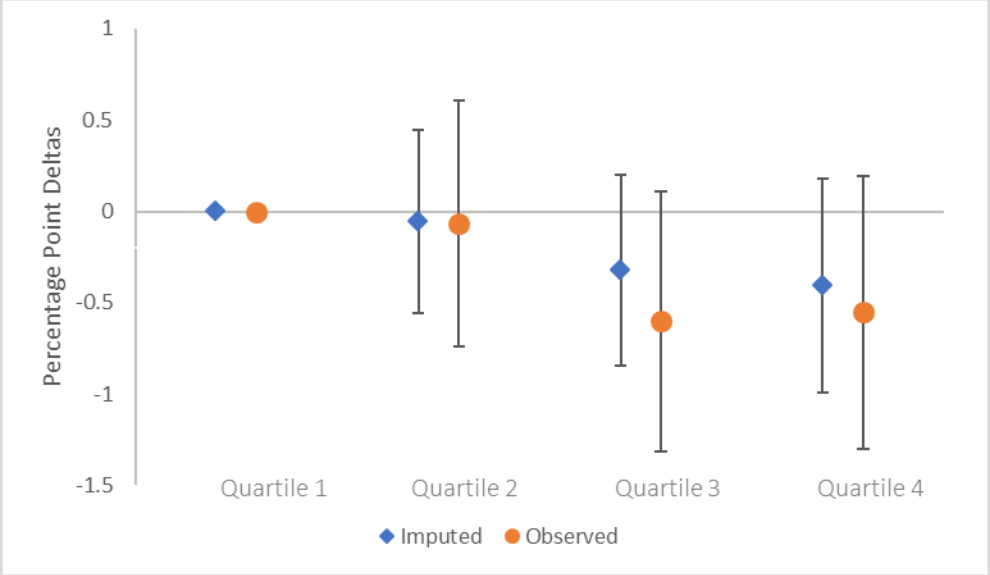

Figure 5-10. Deltas in HbA1c by quartiles of DHA+EPA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1993.

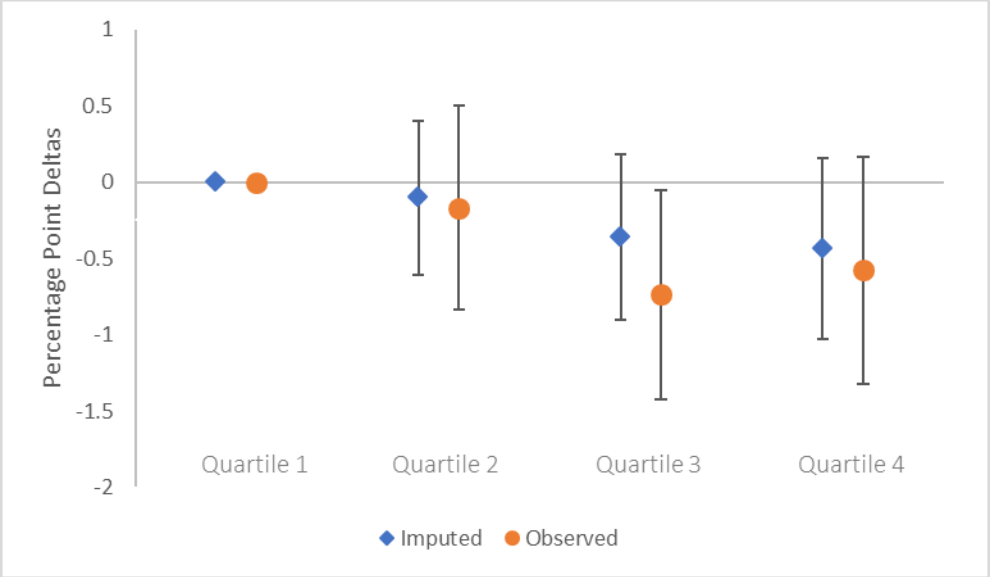

Figure 5-11. Deltas in HbA1c by quartiles of ALA+DHA+EPA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1993.

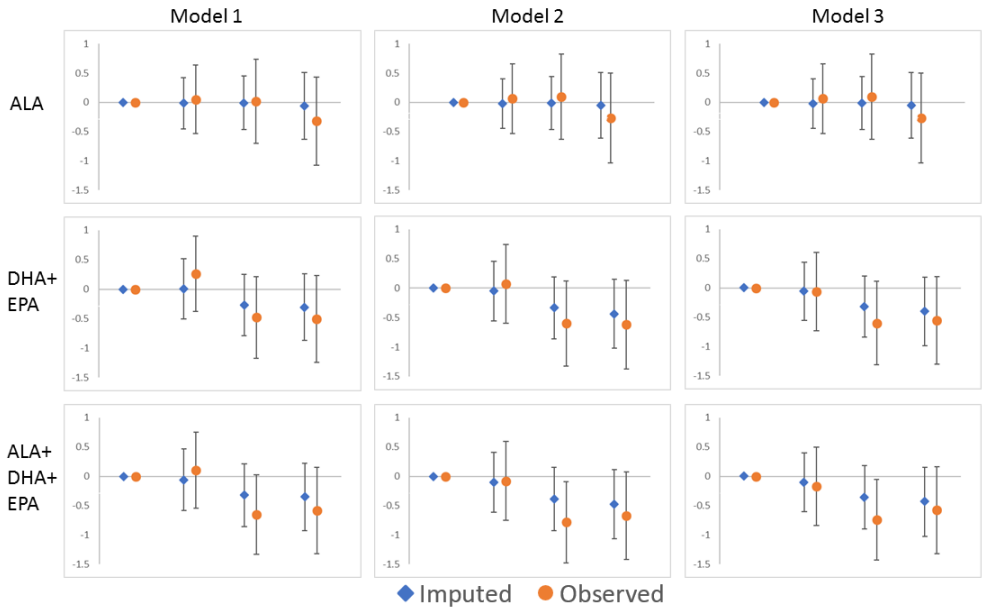

Figure 5-12. Percentage point deltas in HbA1c QTc by quartiles of ALA, DHA+EPA, and ALA+DHA+EPA; all covariate adjustment models; multiple imputation vs. observed plasma values, ARIC, 1987-1993.

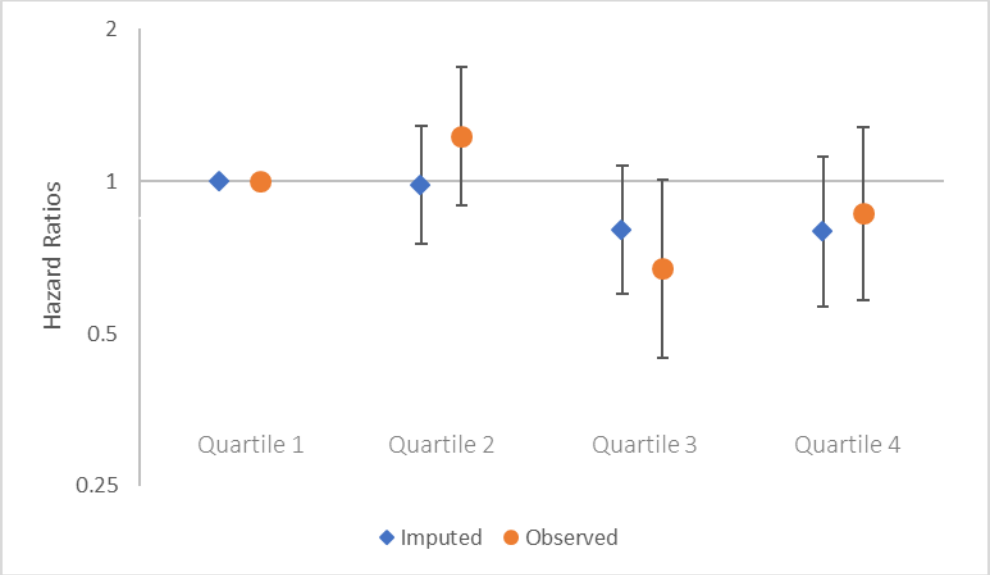

Figure 5-13. Hazard ratios for incident T2D by quartiles of ALA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1998.

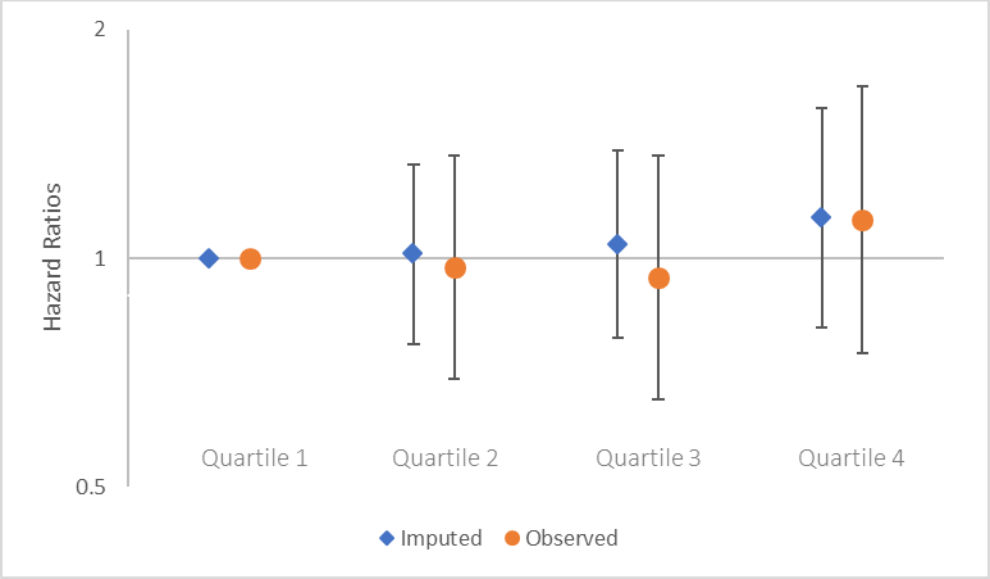

Figure 5-14. Hazard ratios for incident T2D by quartiles of DHA+EPA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1998.

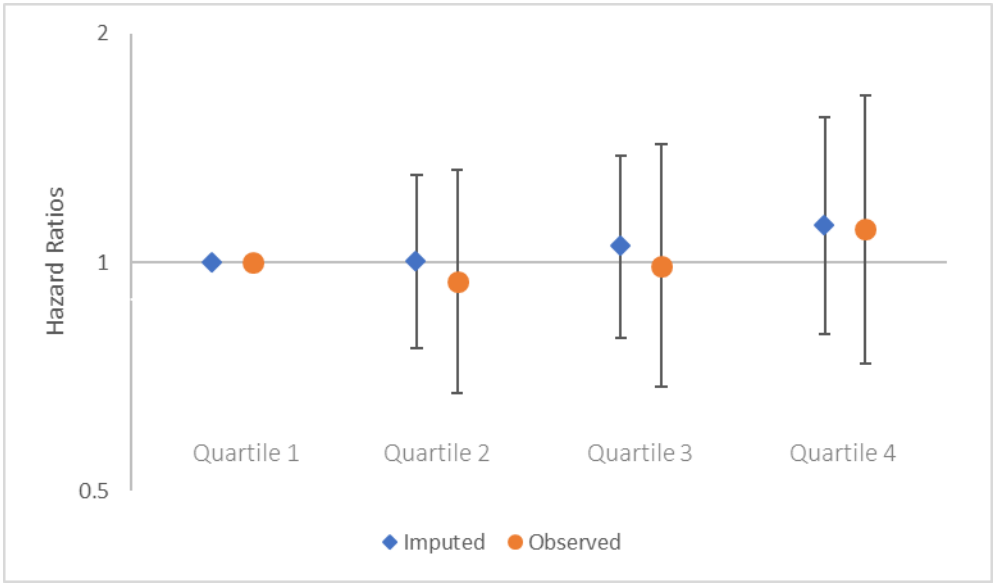

Figure 5-15. Hazard ratios for incident T2D by quartiles of ALA+DHA+EPA; fully adjusted model; multiple imputation vs. observed plasma values, ARIC, 1987-1998.

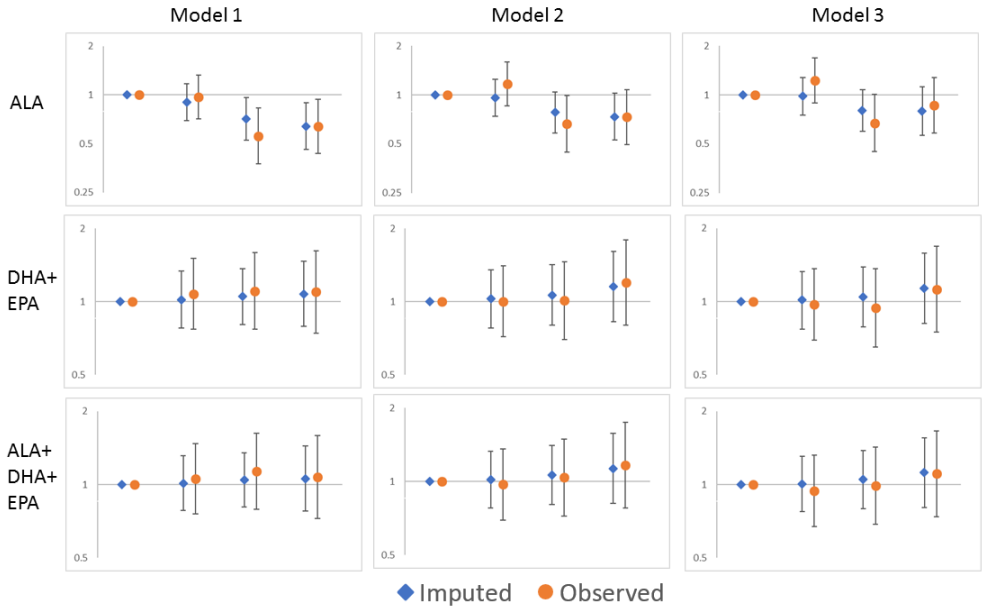

Figure 5-16. Hazard ratios for incident T2D by quartiles of ALA, DHA+EPA, and ALA+DHA+EPA; all covariate adjustment models; multiple imputation vs. observed plasma values, ARIC, 1987-1993.

5.10 TABLES

Table 5-1. Relative Efficiency of Multiple Imputation Datasets (m) Given Percentage of Missingness (λ). [18]

	λ			
M	25%	50%	75%	90%
3	0.92	0.86	0.80	0.77
5	0.95	0.91	0.87	0.85
10	0.98	0.95	0.93	0.92
20	0.99	0.98	0.96	0.96

Table 5-2. Pre- and post-imputation characteristics for variables with missing values in white ARIC participants (n=11,212), 1987-1998.

Variable	Number Observed Values	Number Missing Values	% Missing	Variable Category	Relative Efficiency
Plasma ALA	3,861	7,351	65.6%	Exposure	97.9%
Plasma DHA	3,861	7,351	65.6%	Exposure	98.1%
Plasma EPA	3,861	7,351	65.6%	Exposure	98.2%
HbA1c	10,338	874	7.8%	Outcome	99.9%
Antihyperglycemic Rx at Visit 2	10,497	715	6.4%	Outcome	99.8%*
QT Duration	11,094	118	1.1%	Outcome	100.0%
Heart Rate	11,094	118	1.1%	Outcome	100.0%
LDL	11,001	211	1.9%	Confounder	99.9%
Sport Physical Activity	11,179	33	0.3%	Confounder	100.0%
HDL	11,188	24	0.2%	Confounder	100.0%
Triglycerides	11,188	24	0.2%	Confounder	100.0%
Alcohol Intake	11,196	16	0.1%	Confounder	100.0%
Education Level	11,199	13	0.1%	Confounder	100.0%*
Drinking Status	11,202	10	0.1%	Confounder	100.0%*
Leisure Physical Activity	11,204	8	0.1%	Confounder	100.0%
BMI	11,204	8	0.1%	Confounder	100.0%
Work Physical Activity	11,205	7	0.1%	Confounder	100.0%
Smoking Status	11,205	7	0.1%	Confounder	100.0%*
Diastolic Blood Pressure	11,208	4	0.0%	Confounder	100.0%
Systolic Blood Pressure	11,208	4	0.0%	Confounder	100.0%
T2D (Visit 2)	10,476	736	6.6%	Inclusion/Exclusion	99.8%*
Fasting Blood Glucose (Visit 2)	10,486	726	6.5%	Related Variable	99.9%
QRS Duration	11,091	121	1.1%	Inclusion/Exclusion	100.0%
T2D (Visit 1)	11,187	25	0.2%	Related Variable	100.0%*
Fasting Blood Glucose (Visit 1)	11,200	12	0.1%	Related Variable	100.0%

*Calculated using $(1+(\%\ \text{missing}/40))^{-1}$

Table 5-3. Baseline characteristics of ARIC participants (n=11,212) by field center, 1987–1989. Values correspond to mean N (%) or Mean (min, max), m=40 imputations.

	Non-MN	MN	Full Cohort
N	7,351	3,861	11,212
Sociodemographic Variable			
Age (years)	54.6 (44, 66)	54.0 (44, 65)	54.4 (44, 66)
Male	3,428 (49.9%)	1,855 (50.0%)	5,283 (49.9%)
Education			
Basic	1,673 (22.8%)	239 (6.2%)	1,913 (17.1%)
Intermediate	3,267 (44.4%)	1,812 (46.9%)	5,079 (45.3%)
Advanced	2,410 (32.8%)	1,810 (46.9%)	4,221 (37.6%)
Lifestyle Variables			
BMI	27.0 (14, 56)	27.1 (14, 51)	27.0 (14, 56)
Physical Activity^			
Low	2,817 (38.3%)	1,156 (29.9%)	3,973 (35.4%)
Medium	2,345 (31.9%)	1,314 (34.0%)	3,659 (32.6%)
High	2,189 (29.8%)	1,391 (36.0%)	3,580 (31.9%)
Current Smokers	1,907 (25.9%)	862 (22.3%)	2,769 (24.7%)
Current Alcohol Drinkers	4,061 (55.2%)	3,213 (83.2%)	7,274 (64.9%)
Dietary Covariates			
Total Energy Intake (kcal/day)	1,644 (501, 4,179)	1,640 (508, 4,176)	1,642 (501, 4,179)
Dietary Fiber (g/day)	18.4 (1, 82)	16.3 (2, 73)	17.7 (1, 82)
Saturated Fats (g/day)	22.4 (1, 82)	22.9 (3, 90)	22.6 (1, 90)
Trans Fats (g/day)	2.9 (0, 17)	3.2 (0, 17)	3.0 (0, 17)
Clinical Covariates			
Hypertension	2,061 (44.9%)	976 (43.5%)	3,037 (44.4%)
Systolic Blood Pressure	118.3 (61, 208)	118.8 (61, 198)	118.5 (61, 208)
Diastolic Blood Pressure	70.4 (12, 130)	73.7 (42, 116)	71.5 (12, 130)
High Density Lipoprotein	49.6 (4, 135)	52.1 (12, 143)	50.5 (4, 143)
Low Density Lipoprotein	138.7 (1, 505)	137.2 (4, 452)	138.2 (1, 505)
Triglycerides	142.3 (1, 1876)	129.6 (4, 1599)	137.9 (1, 1876)
Nutrient Intake (g/day)			
Alpha-linolenic acid (ALA)	0.7 (0, 3)	0.7 (0, 3)	0.7 (0, 3)
Docosahexaenoic acid (DHA)	0.2 (0, 4)	0.2 (0, 2)	0.2 (0, 4)
Eicosapentaenoic acid (EPA)	0.1 (0, 2)	0.1 (0, 1)	0.1 (0, 2)

^Work+Leisure+Sport Averaged and Divided into Low/Medium/High

Table 5-4. Association of plasma quartiles of ALA with prolonged QTc, ARIC, 1987-1989. Odds ratios and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (Imputed)	**ALA**	FA% mean (min, max)	0.1 (0.0, 0.1)	0.1 (0.1, 0.1)	0.2 (0.1, 0.2)	0.2 (0.2, 0.4)
		Total N	2,277	2,123	2,208	2,184
		Prolonged QTc (%)	19 (0.8%)	19 (0.9%)	16 (0.7%)	17 (0.8%)
		Model 1	1.0 (ref)	1.0 (0.5, 2.2)	0.8 (0.3, 2.1)	0.8 (0.3, 1.9)
		Model 2	1.0 (ref)	1.1 (0.5, 2.2)	0.8 (0.3, 2.1)	0.8 (0.3, 2.0)
		Model 3	1.0 (ref)	1.1 (0.5, 2.3)	0.8 (0.3, 2.2)	0.9 (0.4, 2.2)
Minnesota Only (Observed)	**ALA**	FA% mean (min, max)	0.1 (0.0, 0.1)	0.1 (0.1, 0.1)	0.2 (0.2, 0.2)	0.2 (0.2, 0.4)
		Total N	900	779	658	725
		Prolonged QTc (%)	6 (0.7%)	7 (0.9%)	2 (0.3%)	5 (0.7%)
		Model 1	1.0 (ref)	1.3 (0.4, 3.9)	0.4 (0.1, 1.9)	0.8 (0.2, 2.7)
		Model 2	1.0 (ref)	1.4 (0.4, 4.3)	0.4 (0.1, 2.1)	0.9 (0.3, 3.2)
		Model 3	1.0 (ref)	1.4 (0.5, 4.5)	0.4 (0.1, 2.1)	0.9 (0.2, 3.3)

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-5. Association of plasma quartiles of DHA+EPA with prolonged QTc, ARIC, 1987-1989. Odds ratios and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**DHA + EPA**	FA% mean (min, max)	2.3 (0.3, 2.8)	3.1 (2.8, 3.4)	3.7 (3.4, 4.1)	4.9 (4.1, 14.0)
		Total N	2,199	2,197	2,197	2,198
		Prolonged QTc (%)	20 (0.9%)	15 (0.7%)	15 (0.7%)	21 (0.9%)
		Model 1	1.0 (ref)	0.7 (0.3, 1.7)	0.7 (0.3, 1.7)	0.9 (0.4, 2.1)
		Model 2	1.0 (ref)	0.7 (0.3, 1.8)	0.7 (0.3, 1.8)	1.0 (0.4, 2.3)
		Model 3	1.0 (ref)	0.7 (0.3, 1.8)	0.7 (0.3, 1.8)	0.9 (0.4, 2.3)
Minnesota Only (obs)	**DHA + EPA**	FA% mean (min, max)	2.4 (0.7, 2.8)	3.1 (2.8, 3.4)	3.7 (3.4, 4.1)	5.1 (4.1, 12.2)
		Total N	869	943	704	545
		Prolonged QTc (%)	8 (0.9%)	6 (0.6%)	2 (0.3%)	4 (0.7%)
		Model 1	1.0 (ref)	0.6 (0.2, 1.8)	0.3 (0.1, 1.2)	0.7 (0.2, 2.3)
		Model 2	1.0 (ref)	0.6 (0.2, 1.9)	0.2 (0.1, 1.2)	0.7 (0.2, 2.4)
		Model 3	1.0 (ref)	0.6 (0.2, 1.9)	0.3 (0.1, 1.3)	0.7 (0.2, 2.5)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-6. Association of plasma quartiles of ALA+DHA+EPA with prolonged QTc, ARIC, 1987-1989. Odds ratios and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**ALA + DHA**	FA% mean (min, max)	2.4 (0.4, 2.9)	3.2 (2.9, 3.5)	3.9 (3.5, 4.2)	5.0 (4.2, 14.1)
	+ EPA	Total N	2,199	2,198	2,197	2,197
		Prolonged QTc (%)	20 (0.9%)	15 (0.7%)	16 (0.7%)	21 (0.9%)
		Model 1	1.0 (ref)	0.7 (0.3, 1.6)	0.7 (0.3, 1.7)	0.9 (0.4, 2.0)
		Model 2	1.0 (ref)	0.7 (0.3, 1.7)	0.8 (0.3, 1.9)	0.9 (0.4, 2.3)
		Model 3	1.0 (ref)	0.7 (0.3, 1.7)	0.8 (0.3, 1.9)	0.9 (0.4, 2.3)
Minnesota Only (obs)	**ALA + DHA**	FA% mean (min, max)	2.5 (0.9, 2.9)	3.2 (2.9, 3.5)	3.8 (3.5, 4.2)	5.2 (4.3, 12.3)
	+ EPA	Total N	869	939	710	544
		Prolonged QTc (%)	8 (0.9%)	5 (0.5%)	3 (0.4%)	4 (0.7%)
		Model 1	1.0 (ref)	0.5 (0.2, 1.6)	0.3 (0.1, 1.4)	0.7 (0.2, 2.2)
		Model 2	1.0 (ref)	0.5 (0.2, 1.7)	0.3 (0.1, 1.4)	0.7 (0.2, 2.4)
		Model 3	1.0 (ref)	0.5 (0.2, 1.7)	0.4 (0.1, 1.5)	0.7 (0.2, 2.6)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-7. Association of plasma quartiles of ALA with HbA1c, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**ALA**	FA% mean (min, max)	0.1 (0.0, 0.1)	0.1 (0.1, 0.1)	0.2 (0.1, 0.2)	0.2 (0.2, 0.3)
		Total N	331	266	230	192
		HbA1c Mean (Std)	7.6 (2.0)	7.6 (2.1)	7.7 (2.1)	7.7 (2.2)
		Model 1	1.0 (ref)	0.0 (-0.5, 0.4)	0.0 (-0.5, 0.5)	-0.1 (-0.6, 0.5)
		Model 2	1.0 (ref)	0.0 (-0.4, 0.4)	0.0 (-0.5, 0.4)	-0.1 (-0.6, 0.5)
		Model 3	1.0 (ref)	0.0 (-0.5, 0.4)	0.0 (-0.4, 0.5)	0.0 (-0.6, 0.5)
Minnesota Only (obs)	**ALA**	FA% mean (min, max)	0.1 (0.0, 0.1)	0.1 (0.1, 0.1)	0.2 (0.2, 0.2)	0.2 (0.2, 0.3)
		Total N	116	87	49	47
		HbA1c Mean (Std)	7.4 (2.0)	7.5 (2.2)	7.6 (2.2)	7.3 (2.2)
		Model 1	1.0 (ref)	0.1 (-0.5, 0.6)	0.0 (-0.7, 0.7)	-0.3 (-1.1, 0.4)
		Model 2	1.0 (ref)	0.1 (-0.5, 0.7)	0.1 (-0.6, 0.8)	-0.3 (-1.0, 0.5)
		Model 3	1.0 (ref)	0.0 (-0.6, 0.6)	0.1 (-0.6, 0.9)	-0.2 (-1.0, 0.5)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-8. Association of plasma quartiles of DHA+EPA with HbA1c, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**DHA + EPA**	FA% mean (min, max)	2.3 (0.6, 2.8)	3.1 (2.8, 3.4)	3.7 (3.4, 4.1)	4.9 (4.1, 10.0)
		Total N	237	247	259	276
		HbA1c Mean (Std)	7.8 (2.3)	7.8 (2.1)	7.5 (1.9)	7.5 (1.9)
		Model 1	1.0 (ref)	0.0 (-0.5, 0.5)	-0.3 (-0.8, 0.3)	-0.3 (-0.9, 0.3)
		Model 2	1.0 (ref)	0.0 (-0.6, 0.5)	-0.3 (-0.9, 0.2)	-0.4 (-1.0, 0.2)
		Model 3	1.0 (ref)	-0.1 (-0.6, 0.4)	-0.3 (-0.8, 0.2)	-0.4 (-1.0, 0.2)
Minnesota Only (obs)	**DHA + EPA**	FA% mean (min, max)	2.4 (1.7, 2.8)	3.1 (2.8, 3.4)	3.7 (3.4, 4.1)	5.1 (4.1, 10.0)
		Total N	80	94	72	52
		HbA1c Mean (Std)	7.6 (2.5)	7.9 (2.2)	7.1 (1.6)	7.0 (1.9)
		Model 1	1.0 (ref)	0.3 (-0.4, 0.9)	-0.5 (-1.2, 0.2)	-0.5 (-1.2, 0.2)
		Model 2	1.0 (ref)	0.1 (-0.6, 0.7)	-0.6 (-1.3, 0.1)	-0.6 (-1.4, 0.1)
		Model 3	1.0 (ref)	-0.1 (-0.7, 0.6)	-0.6 (-1.3, 0.1)	-0.6 (-1.3, 0.2)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-9. Association of plasma quartiles of ALA+DHA+EPA with HbA1c, ARIC, 1987-1993. Percentage point deltas and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**ALA**	FA% mean (min, max)	2.4 (0.7, 2.9)	3.2 (2.9, 3.5)	3.9 (3.5, 4.2)	5.0 (4.3, 10.2)
	+ DHA	Total N	242	246	259	273
	+ EPA	HbA1c Mean (Std)	7.8 (2.3)	7.8 (2.1)	7.5 (1.9)	7.5 (1.9)
		Model 1	1.0 (ref)	-0.1 (-0.6, 0.5)	-0.3 (-0.9, 0.2)	-0.4 (-0.9, 0.2)
		Model 2	1.0 (ref)	-0.1 (-0.6, 0.4)	-0.4 (-0.9, 0.2)	-0.5 (-1.1, 0.1)
		Model 3	1.0 (ref)	-0.1 (-0.6, 0.4)	-0.4 (-0.9, 0.2)	-0.4 (-1.0, 0.2)
Minnesota Only (obs)	**ALA**	FA% mean (min, max)	2.5 (1.8, 2.9)	3.2 (2.9, 3.5)	3.9 (3.5, 4.2)	5.3 (4.3, 10.2)
	+ DHA	Total N	84	91	73	51
	+ EPA	HbA1c Mean (Std)	7.7 (2.5)	7.8 (2.1)	7.0 (1.6)	7.1 (1.9)
		Model 1	1.0 (ref)	0.1 (-0.5, 0.8)	-0.7 (-1.3, 0.0)	-0.6 (-1.3, 0.2)
		Model 2	1.0 (ref)	-0.1 (-0.8, 0.6)	-0.8 (-1.5, -0.1)	-0.7 (-1.4, 0.1)
		Model 3	1.0 (ref)	-0.2 (-0.8, 0.5)	-0.7 (-1.4, 0.0)	-0.6 (-1.3, 0.2)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-10. Association of plasma quartiles of ALA with incident T2D, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**ALA**	FA% mean (min, max)	0.1 (0.0, 0.1)	0.1 (0.1, 0.1)	0.2 (0.1, 0.2)	0.2 (0.2, 0.4)
		Total N	2,199	2,120	2,189	2,130
		Incident T2D (%)	243 (11.0%)	208 (9.8%)	165 (7.5%)	140 (6.6%)
		Model 1	1.0 (ref)	0.9 (0.7, 1.2)	0.7 (0.5, 1.0)	0.6 (0.5, 0.9)
		Model 2	1.0 (ref)	1.0 (0.7, 1.2)	0.8 (0.6, 1.0)	0.7 (0.5, 1.0)
		Model 3	1.0 (ref)	1.0 (0.8, 1.3)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)
Minnesota Only (obs)	**ALA**	FA% mean (min, max)	0.1 (0.0, 0.1)	0.1 (0.1, 0.1)	0.2 (0.2, 0.2)	0.2 (0.2, 0.4)
		Total N	902	787	709	733
		Incident T2D (%)	89 (9.9%)	74 (9.4%)	36 (5.0%)	41 (5.6%)
		Model 1	1.0 (ref)	1.0 (0.7, 1.3)	0.6 (0.4, 0.8)	0.6 (0.4, 0.9)
		Model 2	1.0 (ref)	1.2 (0.9, 1.6)	0.7 (0.4, 1.0)	0.7 (0.5, 1.1)
		Model 3	1.0 (ref)	1.2 (0.9, 1.7)	0.7 (0.4, 1.0)	0.9 (0.6, 1.3)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-11. Association of plasma quartiles of DHA+EPA with incident T2D, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**DHA**	FA% mean (min, max)	2.3 (0.3, 2.8)	3.1 (2.8, 3.4)	3.7 (3.4, 4.1)	4.9 (4.1, 14.0)
	+ EPA	Total N	2,161	2,159	2,159	2,158
		Incident T2D (%)	189 (8.7%)	187 (8.7%)	188 (8.7%)	191 (8.9%)
		Model 1	1.0 (ref)	1.0 (0.8, 1.3)	1.0 (0.8, 1.4)	1.1 (0.8, 1.5)
		Model 2	1.0 (ref)	1.0 (0.8, 1.4)	1.1 (0.8, 1.4)	1.2 (0.8, 1.6)
		Model 3	1.0 (ref)	1.0 (0.8, 1.3)	1.0 (0.8, 1.4)	1.1 (0.8, 1.6)
Minnesota Only (obs)	**DHA**	FA% mean (min, max)	2.4 (0.7, 2.8)	3.1 (2.8, 3.4)	3.7 (3.4, 4.1)	5.0 (4.1, 12.2)
	+ EPA	Total N	890	950	718	573
		Incident T2D (%)	67 (7.5%)	74 (7.8%)	55 (7.7%)	44 (7.6%)
		Model 1	1.0 (ref)	1.1 (0.8, 1.5)	1.1 (0.8, 1.6)	1.1 (0.7, 1.6)
		Model 2	1.0 (ref)	1.0 (0.7, 1.4)	1.0 (0.7, 1.5)	1.2 (0.8, 1.8)
		Model 3	1.0 (ref)	1.0 (0.7, 1.4)	0.9 (0.7, 1.4)	1.1 (0.7, 1.7)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

Table 5-12. Association of plasma quartiles of ALA+DHA+EPA with incident T2D, ARIC, 1987-1998. Hazard Ratios and 95% confidence intervals.

			Q1	Q2	Q3	Q4
Full Cohort (imp)	**ALA**	FA% mean (min, max)	2.4 (0.4, 2.9)	3.2 (2.9, 3.5)	3.9 (3.5, 4.2)	5.0 (4.2, 14.1)
	+ DHA	Total N	2,161	2,160	2,159	2,158
	+ EPA	Incident T2D (%)	186 (8.8%)	188 (8.7%)	188 (8.7%)	189 (8.7%)
		Model 1	1.0 (ref)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	1.1 (0.8, 1.4)
		Model 2	1.0 (ref)	1.0 (0.8, 1.3)	1.1 (0.8, 1.4)	1.1 (0.8, 1.6)
		Model 3	1.0 (ref)	1.0 (0.8, 1.3)	1.0 (0.8, 1.4)	1.1 (0.8, 1.6)
Minnesota Only (obs)	**ALA**	FA% mean (min, max)	2.5 (0.9, 2.9)	3.2 (2.9, 3.5)	3.8 (3.5, 4.2)	5.2 (4.3, 12.3)
	+ DHA	Total N	892	945	725	569
	+ EPA	Incident T2D (%)	67 (7.6%)	73 (7.7%)	56 (7.8%)	43 (7.3%)
		Model 1	1.0 (ref)	1.1 (0.8, 1.5)	1.1 (0.8, 1.6)	1.1 (0.7, 1.6)
		Model 2	1.0 (ref)	1.0 (0.7, 1.4)	1.0 (0.7, 1.5)	1.2 (0.8, 1.7)
		Model 3	1.0 (ref)	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)	1.1 (0.7, 1.7)

obs: observed; imp: imputed

Model 1 adjusted for age, sex, sociodemographic variables (race, center, education), and total energy intake.

Model 2 further adjusted for lifestyle variables (BMI, physical activity, smoking status, drinking status and amount)

Model 3 further adjusted for dietary variables (*trans* fats, saturated fat, dietary fiber) and clinical variables (hypertension, HDL, LDL, triglycerides)

6 CONCLUSION

Currently, both the American Heart Association (AHA) and the American Diabetes Association (ADA) recommend at least two servings of oily fish a week to promote cardiovascular health [3, 4, 243]. However, results from observational studies have been mixed with respect to the associations of fish and omega-3 PUFAs with certain cardiovascular and glycemia outcomes. Specifically, the mechanism through which fish intake affects risk of SCD has yet to be elucidated, and associations of omega-3 PUFAs and fish with markers of glucose homeostasis have been mixed [73, 85-88, 174, 244-247].

The original research described in this dissertation examined the relationship among dietary intake of fish, fish-derived omega-3 PUFAs DHA and EPA, and the vegetable-derived omega-3 PUFA ALA with ECG predictors of SCD and measures of glycemia in a population-based cohort of middle aged adults.

Data from the first manuscript suggest that dietary intake of fish and omega-3 PUFAs may adversely affect glycemic control among those with diabetes. Furthermore, results suggested the deleterious associations were differential across race and sex.

Results from the second manuscript did not provide compelling evidence of a relationship between intake of fish or fish-derived omega-3 PUFAs (DHA+EPA) with J-point height or QT interval duration. We did find higher intakes of ALA were associated with shorter QT interval duration, but this finding should be interpreted with caution given the large number of models investigated in that study.

The results from the first two manuscripts were derived using exposure data collected via FFQ. Because data collected using FFQs are subject to measurement error [201-203], whereas biomarker values offer an objective measure of nutrient absorption (bioavailability) that can be a proxy for usual intake [169], we reanalyzed our data for select outcomes using plasma phospholipid values of DHA, EPA, and ALA obtained at visit 1 from MN field center participants. Associations among plasma omega-3 PUFAs

and prolonged QTc, HbA1c, and incident T2D in MN participants were null. Imputing biomarker values for white participants at other field centers using MICE increased our precision, but did not change the magnitude of association for these outcomes.

Taken together, the results presented in this dissertation suggest that neither fish nor the fish-derived omega-3 PUFAs DHA+EPA reduces SCD risk through changes in J-point height or QT interval duration; higher intake of ALA may be associated with more favorable (shorter) QT interval durations. Data do suggest that intake of fish and of the omega-3 PUFAs DHA, EPA, and ALA may adversely affect glycemic control in those with diabetes, especially in black participants and female participants.

To place these results into a context, a recent position statement by the AHA considered the evidence for the role of fish-derived omega-3 fatty acids supplements in primary/secondary prevention of CVD in various populations [74]. Supplementation was recommended for secondary prevention of CHD and SCD among those with prevalent CHD [74]. With respect to CVD mortality prevention in those with diabetes or pre-diabetes, the evidence indicated no benefit and the position was that treatment was not indicated [74]. While this suggests a tentative consensus that high doses of omega-3 PUFAs (as supplements) are beneficial for prevention of SCD, the benefits of supplementation in diabetes is attenuated by detrimental effects on glucose control.

It should be noted that these recommendations are for supplements, and that dietary intake of foods rich in omega-3 PUFAs may confer benefits beyond their nutrients, like displacing foods higher in saturated and *trans* fatty acids [3]. Regardless, the AHA and ADA continue to recommend at least two servings of oily fish per week.

The data and overall study design utilized in this dissertation have many strengths. The ARIC study is a population-based prospective study with multiple visits over several years. Participants were varied in their medical history and outcomes, and retention was high during follow-up. Data were available for several clinical covariates, and these data were refreshed over time. Dietary data were collected more than once,

and the FFQ was modified from its original version to add more questions about seafood intake. Additionally, biomarker values for circulating concentrations of omega-3 fatty acids were available for a subpopulation.

However, the analyses presented in this dissertation are not without limitations. Data were not available on food preparation technique, which can influence the nutritional composition of the foods consumed. Additionally, the FFQ was worded such that baked fish sticks, deep fried fish sticks, and poached cod are all equivalent. As such, there may be significant misclassification bias of dietary intake, even if FFQ questions were answered without error. We attempted to address this limitation by utilizing plasma fatty acid measures in Manuscript 3, but bioavailability and absorption of nutrients into circulating concentrations of biomarkers is not a perfect proxy for dietary intake of whole foods, and it is influenced by many factors – not all of them measurable.

A commentary published in 2015 by Subar et al. sought to address the criticism of nutritional epidemiology studies that rely on self-reported dietary data [248]. There were seven recommendations, and the first was to continue to collect self-reported dietary data as they are an invaluable resource. The authors also implored researchers to acknowledge the limitations of self-reported dietary data when presenting and interpreting results derived from them, and offered techniques for addressing measurement error. It is this last point that resonates the most. Dietary data are difficult to measure in a free-living population. Methods that do not place a high burden on participants are key in recruiting and maintain a diverse set of individuals willing to participate in long-term cohort studies. FFQs augmented with 24-hour recalls, 3-day food records, and other gold standard self-report instruments can help mitigate measurement error, especially when combined with sophisticated statistical methods like multiple imputation. Equal weight should be given to studies utilizing biomarkers and other objective measures of dietary intake, so that we can triangulate exposure status and address the criticism levied against nutritional epidemiological studies.

Additional research incorporating the suggestions made by Subar et al. may help elucidate the relationship of dietary intake of fish with markers of glucose homeostasis, and determine if revisions should be made in dietary guidelines recommending regular consumption of oily fish by those with diabetes.

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