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Modeling the relationships between urinary F2-isoprostanes, BMI, and
risk of type 2 diabetes

by

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BSc., GEORGIA STATE UNIVERSITY

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of the

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APPROVAL PAGE

Modeling the relationships between urinary F2-isoprostanes, BMI, and risk of type 2 diabetes

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ABSTRACT

Type 2 diabetes (T2DM) is a chronic condition affecting 1 in every 10 adults in the United States. There is evidence that individuals with greater levels of F2-isoprostanol at similar levels of adiposity have reduced risk of T2DM. F2-isoprostanol has been validated as markers of oxidative status in animal and human studies. Many cross-sectional studies found correlations between F2-isoprostanol and adiposity measured as body mass index (BMI). The connection of F2-isoprostanol to the lower risk of diabetes and BMI suggests that these markers can be interpreted as a part of some compensatory mechanisms involved in metabolic adaptation to body fat accumulation. The purpose of this study was to compare the additive relationship between BMI, as a measure of adiposity, and 2,3-dinor-iPF₂α-III (F2-isoP), as a measure of adaptation to increased BMI, to a model that proposes multiplicative relationships between F2-isoP to BMI expressed by the ratio of F2-isoP to BMI. The present analysis utilizes data from the Insulin Resistance Atherosclerosis Study (IRAS), a multicenter prospective cohort designed to study the relationships between insulin resistance, type 2 diabetes, cardiovascular disease risk factors and behaviors in a diverse population including non-Hispanic whites, African Americans, and Hispanics. Between October 1992 and April 1994 approximately 1625 participants, between 40-69 years of age at baseline, were recruited from four U.S. clinical centers located in San Antonio, TX; San Luis Valley, CO; Oakland, CA; Los Angeles, CA. Wilcoxon-rank sum/ Kruskal-Wallis tests and Wald-chi-square test were used to describe the study population. Logistic regression models were used to assess the relationships between the exposures of interest as well as age, gender, race/ethnicity, glucose tolerance status. The additive model estimated the association between F2-isoP and the risk of T2DM with BMI being a covariate. The multiplicative model estimated the association between F2-isoP/BMI ratio, F2-isoP and the risk of T2DM. Percent differences of odds ratios were calculated between the two models, with >10% difference indicating meaningful change. The results from the analysis show that the new variable F2-isoP/BMI ratio does not clearly indicate whether the multiplicative model represents a better way to evaluate the relationships between F2-isoP, BMI and risk for T2DM. Evaluation of the additive and multiplicative models for outcomes—such as weight change, decrease in insulin resistance, blood pressure and others—might clarify whether the additive or multiplicative relationships, between F2-isoP and BMI, better predict these outcomes.

Author's Statement Page

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Padmini Sangaraju

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Chapter I: Introduction

1.1 Purpose of study

Type 2 diabetes (T2DM) is a chronic disease which affects about 1 in every 10 adults in the U.S.¹. Diabetes is a major public health issue because of the many comorbidities it promotes including hypoglycemia, hyperglycemic crisis, kidney disease, cardiovascular disease and stroke, to name a few¹¹. Optimized body weight and physical activity can help in reducing T2DM risk thereby reducing risk of cardiovascular events³. Metabolically healthy obese adults are half as likely to develop diabetes when compared to the obese counterparts⁸. Cross-sectional studies suggest that oxidative stress plays an etiologic role in the pathology of various chronic diseases, including T2DM⁴. These associations can be explained by a compensatory mechanism known as metabolic adaptation, when fatty acid oxidation increases with increased adiposity. This was shown to be the case when it was found that individuals with greater levels of F2-isoprostanes at similar levels of adiposity would have a reduced risk of T2DM²⁰. This additive relationship between body mass index (BMI) as a measure of adiposity and 2,3-dinor-iPF₂α-III (F2-isoP) as a measure of oxidative status has been studied previously. We compared the additive model to the model that proposes multiplicative relationships between F2-isoP to BMI expressed by the ratio of F2-isoP to BMI.

Chapter II: Literature review

2.1 Diabetes Mellitus

Diabetes Mellitus (DM) is one of the most predominant chronic conditions in the U.S. and is one of the leading causes of death⁹. According to the Centers for Disease Control and Prevention (CDC) about 23 million adults in the U.S. have been diagnosed with diabetes in 2016, out of which 90% to 95% have type 2 diabetes (T2DM). The highest prevalence of T2DM was found to be among those who aged >65 years (25.2%) and lowest among those below 18 years (4%). There was a significantly higher prevalence among those who classified as non-Hispanic blacks (11.52%) when compared to non-Hispanic whites (7.99%), Hispanics (9.07%), and Asians (6.89%). However, those who had higher education attainment, more than high school education, had a lower prevalence of T2DM (6.89%) when compared to those with less than high school education (14.20%).

There are many comorbidities and complications associated with diabetes. Diabetes is a known risk factor for cardiovascular diseases (CVD). Approximately 65% of mortality among diabetic patients is due to heart disease or stroke³³. Other conditions associated with T2DM include retinopathy, chronic renal impairment, cardiovascular events, and amputation of lower body limbs. High comorbidity makes T2DM a high priority public health problem. Approximately 7.2 million hospital discharges were reported with diabetes as a diagnosis in 2014 in the United States. Hospitalizations were mainly due to CVD (70.4 per 1,000 individuals with diabetes), lower extremity amputation (5 per 1000 individuals with diabetes), ketoacidosis (7.7 per 1,000 individuals with diabetes)¹¹. In a study conducted by *Pantalone et al.* (2015), the most prevalent comorbidities included: hypertension (82.5%) and CVD (26.8%). Among all complications and comorbidities, retinopathy increased slightly from 3.2% to 3.4% in 2008 to 2013, respectively³⁹. Nephropathy, neuropathy, and peripheral vascular disease were among other complications in those with T2DM. In a study done in the United Arab Emirates, 83.74% of individuals with T2DM had more than one clinically diagnosed complication, including retinopathy—the primary complication, coronary artery disease, neuropathy and nephropathy¹⁴. The cost of diabetes has increased from \$245 billion in 2012 to \$327 in 2017—this is almost a 30% increase in five years

²⁸. People with diabetes spend approximately \$9,600 to manage their disease. Indirect costs include increased absenteeism (costing about \$3.3 billion) and reduced productivity in the workplace (\$27 billion, approximately), disease-related disability (\$37.5 billion), and many more costs leading to almost \$90 billion in indirect costs ²⁸.

T2DM has a long natural history and is more a deterioration of one's metabolic pathways than just an occurrence. Impaired glucose tolerance and impaired fasting glucose are intermediates of abnormal glucose regulation which exists between normal glucose homeostasis and diabetes ³¹. Impaired glucose tolerance is defined as an elevated 2-hour plasma glucose concentration, ≥ 140 and < 200 mg/dl, after a 75-grams glucose load from an oral glucose tolerance test (OGTT) in addition to a fasting plasma glucose concentration of < 126 mg/dl as described by Genuth et al. (2003) ³¹. IGT progression to diabetes is a strong risk predictor of cardiovascular disease (CVD). CVD risk increases from two-fold to four-fold in those who progress to diabetes ³¹. High levels of circulating fatty acids and fat deposits in skeletal muscle also disrupt insulin signaling pathways, which promotes the development of T2DM ¹⁸. Circulating free fatty acids in plasma were found to be elevated among obese individuals with diabetes when compared to metabolically healthy obese and normal weight individuals ³⁶.

2.2 T2DM risk factors and prevention

Two major risk factors for T2DM are obesity/overweight and physical inactivity ³⁴. Prospective studies show that both a low physical activity and obesity are associated with the risk of T2DM. Hu et al. (2004) found that there was an inverse association between physical activity and T2DM risk based on a subgroup analysis of BMI (< 30 kg/m² and ≤ 30 kg/m²) and glucose levels—either normal glucose level or impaired glucose level. Adjusting for age, BMI, blood pressure, education, obesity, current percent of individuals smoking, and physical activity, subjects with impaired glucose regulation showed approximately a five-fold increase of developing T2DM when compared to those with normal glucose levels.

Lifestyle changes related to obesity, eating habits, and physical activity are all factors that need to be suggested to individuals at high risk for diabetes along with medication adherence ¹⁵. Dietary modifications targeting high fiber, low calorie, low-saturated fat and moderate physical

activity of at least 150 minutes per week contributed to approximately 5% loss of initial body weight. There was a 58% risk reduction of diabetes in these studies ⁴¹. Heavy smokers—those who smoked more than 20 cigarettes per day—had a relative risk of 1.66 (95% CI: 1.43, 1.80) when compared with light smokers (1.29 (95% CI: 1.13, 1.48)) and former smokers (1.23 (95% CI: 1.14, 1.33)) ⁴². Low trans-fats and glycemic index, regular exercise ³⁵, abstinence from smoking and reduction of alcohol consumption ¹⁵ are also important considerations for managing T2DM.

2.3 Reactive oxygen species, oxidative stress, and F2-isoprostanes

Reactive oxygen species (ROS) are a group of highly reactive oxygen-containing molecules, which are produced in normal metabolic processes in all aerobic organisms ²³. ROS are generated through reduction-oxidation reactions, most of which happen in the mitochondria. Mitochondria within cells are major sources of endogenous ROS. ROS react with lipids, proteins, and nucleic acids which alter structural and functional properties of the target molecules ²². Antioxidant defense mechanisms protect cells from ROS-induced oxidative damage ²³. Redox homeostasis is achieved when ROS levels and antioxidant defenses are in balance. Oxidative stress is a concept considering an imbalance in redox homeostasis towards the oxidative process due to insufficient antioxidant defense system.

Polyunsaturated fatty acids easily react with ROS producing various oxidation products including F2-isoprostanes. F2-isoprostanes are prostaglandin-like stable compounds that are commonly used for assessing oxidative status ²⁷. Systemic levels of F2-isoprostanes reflect the overall levels of ROS and have been validated indicators of oxidative status in animal and human models ²⁴. Elevated systemic F2-isoprostane levels are commonly interpreted as indicators of harmful oxidative stress but can also indicate the intensity of mitochondrial metabolism. Supporting the latter interpretation, prospective studies showed that individuals with greater F2-isoprostane levels have a lower risk of diabetes and weight gain ²⁴. This inverse relationship acts as a protective factor for those at risk for T2DM. 2,3-dinor-iPF2 α -III (F2-isoP) is one F2-isoprostane isomer which showed the strongest inverse association with T2DM risk ²⁹. This association was even stronger among obese individuals. Therefore, the relationship between F2-isoprostanes,

specifically 2,3-dinor-iPF2 α -III (F2-isoP), and BMI as an index of adiposity is of interest in this study.

2.4 Conceptual framework

Adipose tissue is the main fatty acid storage unit, while skeletal and cardiac muscles are the most important tissues for fatty acid oxidation. Individuals differ in their ability to use fat as fuel and therefore in the intensity of fatty acid oxidation. Accumulation of fatty acids occur either with an increased uptake and/or decreased oxidation. There is evidence suggesting that accumulation of lipids within the skeletal muscle result from low capacity of fat oxidation ³⁴. In humans, a decrease in post absorptive fat oxidation was reported in obesity and T2DM after weight loss when compared with lean individuals ³⁴. Palmitate oxidation was 58% lower in skeletal muscle for extremely obese individuals when compared with normal-weight individuals and 83% lower in overweight/obese individuals ³⁸. Thus, impaired fatty oxidation is associated with both disorders – obesity and T2DM.

Metabolic adaptation is a concept stating that physiological response to positive (fat accumulation) and negative (fat loss) energy balance involves opposing changes in fatty acid oxidation. Increased levels of F2-isoprostanes have been found among individuals with obesity and diabetes in cross-sectional studies ¹⁹. According to the concept of metabolic adaptation, increase in systemic F2-isoprostanes in obesity might reflect increased mitochondrial fatty acid metabolism in response to fat accumulation. It has been hypothesized that F2-isoprostanes, in relation to BMI, captures the metabolic phenotype showing adaptation to increased adiposity through intensification of fat oxidation. This agrees with previous literature that slow fat oxidation promotes weight gain obesity-related deterioration of glucose homeostasis ¹⁹.

Chapter III: Methods

3.1 Study Population

The present analysis utilizes data from the Insulin Resistance Atherosclerosis Study (IRAS), a multicenter prospective cohort designed to study the relationships between insulin resistance, type 2 diabetes, cardiovascular disease risk factors and behaviors in a diverse population including non-Hispanic whites, African Americans, and Hispanics. Between October 1992 and April 1994 approximately 1625 participants, between 40-69 years of age at baseline, were recruited from four U.S. clinical centers located in San Antonio, TX; San Luis Valley, CO; Oakland, CA; Los Angeles, CA. Metabolic diversity was maintained and ensured by having equal number of individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes.

3.2 Diabetes status and covariates

Baseline glucose tolerance was measured through the oral glucose tolerance test, based on the criteria established by the World Health Organization (WHO). Age, gender, race/ethnicity was assessed through self-report and recorded through questionnaires. Body Mass Index (BMI) was calculated based on the measurements during the baseline examination as weight in kilograms divided by height in meters squared for each participant to represent adiposity.

3.3 Assessment of main exposure—2,3-dinor-iPF2 α -III

F2-isoprostane isomer, 2,3-dinor-iPF2 α -III (F2-isoP), was measured in morning spot urine samples collected at baseline examination; the urine samples were stored at -70° C. F2-isoP was quantified using liquid chromatography with tandem mass spectrometry detection and adjusted for urinary creatinine concentration as earlier described²¹. Creatinine was assayed using a fast electrospray ionization-tandem mass spectrometry method²¹.

3.4 Analytical Cohort

Our analytical cohort included those free of type 2 diabetes at baseline. A total of 1025 participants were identified as non-diabetic, NGT or IGT, at baseline; among them only 905

underwent the follow-up examination and had urine specimens available for measurements of F2-isoprostanes. F2-isoprostanes could be measured in 858 urine specimens. After excluding participants with missing values, 857 participants were included in the analysis; 140 participants were identified as those who had incident type 2 diabetes and 717 participants remained free of diabetes.

3.5 Statistical analysis

Descriptive statistics were examined using the baseline sample of 857 participants. Bivariate associations between diabetes status and all other participant characteristics (age, gender, race, BMI at baseline, F2-isoP, glucose tolerance status at baseline, and F2-isoP/BMI) were performed. All continuous variables were assessed using Wilcoxon-rank sum/ Kruskal-Wallis test and all categorical variables were assessed using Wald Chi-square test. We used Logistic regression to compare two models where F2-isoP and BMI are represented in an additive scale and multiplicative scale. In the additive scale model, F2-isoP and BMI are two separate variables to predict incident diabetes, whereas in the multiplicative scale, the F2-isoP/BMI ratio alone is used to predict incident diabetes. A p-value <0.05 was considered significant in the present analysis. All statistical analyses were performed using SAS statistical software, version 9.4.

Chapter IV: Results

The groups selected for this analysis were metabolically diverse among categories of gender and race/ethnicity which included NHW, NHB, Hispanics. The main variables of interest for this analysis were F2-isoP and F2-isoP/BMI ratio with regards to incident diabetes.

4.1 Baseline characteristics

Among the total number of participants included in this study (n=857), 16.33% developed T2DM by the follow-up examination (cases) and 83.66% remained free of diabetes (non-cases). Cases on average were approximately 2 years older than non-cases. The sex distribution did not differ between cases and non-cases. Ethnic distribution in this study population was 40.02%, 27.77% and 32.21% for non-Hispanic whites, non-Hispanic blacks, and Hispanics, respectively and did not vary between cases and non-cases. There was a significant difference in baseline BMI between cases and non-cases ($p < 0.0001$): the mean BMI at baseline measurement was 31.29 kg/m² for cases while it was 27.91 kg/m² for non-cases. The mean levels of F2-isoP were not significantly lower among cases as compared to non-cases: mean (median) values were 3.92 (3.43) among cases and 4.43 (3.77) among non-cases. Baseline glucose tolerance status was significantly different between cases and non-cases ($p < 0.0001$). Out of 140 cases, 32.14% had normal glucose tolerance (NGT) and 67.86% had impaired glucose tolerance (IGT) at baseline. Significant differences were found between cases and non-cases in the F2-isoP/BMI ratio. The mean (median) ratio was 0.126 (0.11) and 0.16 (0.14) for cases and non-cases, respectively ($p < 0.0001$).

Table 1. Distribution of demographic and baseline characteristics by diabetes status at follow-up.

<i>Participant Characteristics</i>	<i>Cases (N=140)</i>	<i>Non-Cases (N=717)</i>	<i>Total (N=857)</i>	<i>P-value*</i>
Age				
<i>Median (IQR)</i>	56 (13)	54 (15)	54 (143)	0.0187
<i>Mean (SD)</i>	56.07 (7.75)	54.26 (8.39)	54.56 (8.31)	
<i>Missing</i>	0			
Gender				
<i>Male N (%)</i>	56 (40.00)	307 (42.82)	363 (42.36)	0.5372
<i>Female N (%)</i>	84 (60.00)	410 (57.18)	494 (57.64)	
<i>Missing N (%)</i>	0			
Race/ethnicity				0.8368

<i>Non-Hispanic White N (%)</i>	53 (37.86)	290 (40.45)	343 (40.02)	
<i>Non-Hispanic Black N (%)</i>	41 (29.29)	197 (27.48)	238 (27.77)	
<i>Hispanic N (%)</i>	46 (32.86)	230 (32.08)	276 (32.21)	
<i>Missing N (%)</i>	0			
BMI				
<i>Median (IQR)</i>	28.99 (8.26)	26.98 (5.24)	27.29 (5.75)	
<i>Mean (SD)</i>	31.29 (6.43)	27.91 (5.32)	28.46 (5.66)	<0.0001
<i>Missing</i>		2		
2,3-dinor-iPF2α-III (ng/mg-cn)				
<i>Median (IQR)</i>	3.43 (2.76)	3.77 (2.74)	3.71 (2.74)	
<i>Mean (SD)</i>	3.92 (2.75)	4.43 (3.04)	4.35 (3.0)	0.0167
<i>Missing</i>	2	2	4	
Glucose tolerance status				
<i>NGT</i>	45 (32.14)	534 (74.48)	579 (67.56)	
<i>IGT</i>	95 (67.86)	183 (25.52)	278 (32.44)	<0.0001
<i>Missing</i>	0			
Ratio of 2,3-dinor-iPF2α-III to BMI				
<i>Median (IQR)</i>	0.11 (0.085)	0.14 (0.104)	0.132 (0.104)	<0.0001
<i>Mean (SD)</i>	0.126 (0.088)	0.16 (0.10)	0.154 (0.099)	
<i>Missing</i>	2	4	6	

NGT: normal glucose tolerance; IGT: impaired glucose tolerance; *Difference in the distribution of the continuous variables by cases status was assessed using Wilcoxon Rank Sum/Kruskal Wallis test and Chi Square test was used for categorical variables.

4.2 Crude associations between incident diabetes and participant characteristics

The strongest crude association of incidence diabetes was with IGT status (cOR=6.160, 95% CI: 4.160, 9.119). BMI was associated with increased diabetes risk, with the cOR for increase by 5 units, being 1.582 (95% CI: 1.364, 1.834). In this study population, no crude association was found between diabetes risk and either race/ethnicity or sex. F2-isoP and F2-isoP/BMI ratio were inversely associated with incidence diabetes, with cOR for 75-25 contrast of 0.818 (95% CI: 0.663, 1.010) and 0.591 (95% CI: 0.449, 0.776), respectively.

Table 2 Bivariate associations between baseline characteristics and incident diabetes and all participant characteristics.

<i>Participant Characteristics</i>	<i>cOR (95% CI)</i>	<i>Units</i>
<i>Age</i>	1.141 (1.022,1.274)	5

Gender		
<i>Males</i>	0.890 (0.616, 1.288)	N/A
<i>Females</i>	REF	
Race/ethnicity		
<i>Non-Hispanic White</i>	REF	N/A
<i>Non-Hispanic Black</i>	1.139 (0.729,1.779)	
<i>Hispanic</i>	1.094 (0.711,1.684)	
BMI	1.582 (1.364, 1.834)	5
2,3-dinor-iPF2α-III (ng/mg-cn)	0.818 (0.663, 1.010)	2.74*
Glucose tolerance		
<i>NGT</i>	REF	N/A
<i>IGT</i>	6.160 (4.160, 9.119)	
Ratio of 2,3-dinor-iPF2α-III (ng/mg-cn) to BMI	0.591 (0.449, 0.776)	0.1040*

*75th-25th percentile contrast

4.3 Additive and Multiplicative models

We compared two models in this analysis, additive and multiplicative (Table 3 and Table 4, respectively). The additive model included BMI at baseline, 2,3-dinor-iPF2 α -III (ng/mg-cn), and glucose tolerance status at baseline controlling for age, gender, race/ethnicity. The multiplicative model included 2,3-dinor-iPF2 α -III (ng/mg-cn)/BMI ratio and glucose tolerance status at baseline adjusting for age, gender, race/ethnicity.

Both models showed positive associations of T2DM risk with age and impaired glucose tolerance. The aOR for age in additive and multiplicative models were 1.091 (0.963, 1.237) and 1.063 (0.940, 1.202), respectively. Impaired glucose tolerance aOR for the additive model was 5.018 (3.306, 7.616) and 5.910 (3.940, 8.864) for the multiplicative model. In the multiplicative model, males have a lower aOR 0.734 (0.478, 1.128) compared to aOR of 0.822 (0.530, 1.274) in the additive model. No association was found between race/ethnicity in both models. In the additive model, Non-Hispanic blacks had an aOR of 0.846 (0.511, 1.400) and Hispanics had an aOR of 1.217 (0.754, 1.963) when compared to the referent group. In the multiplicative model, Non-Hispanic blacks had aOR of 0.907 (0.554,1.485) and Hispanics had an aOR of 1.267 (0.790, 2.032) compared to referent group.

Table 3 Adjusted odds ratios for incident diabetes measured at 5-year follow-up using additive model.

<i>Participant Characteristics</i>	<i>aOR (95% CI)</i>	<i>Units</i>
Age(years)	1.091 (0.963, 1.237)	5
Gender		
Male	0.822 (0.530, 1.274)	N/A
Female	REF	
Race/ethnicity		
Non-Hispanic White	REF	
Non-Hispanic Black	0.846 (0.511, 1.400)	N/A
Hispanic	1.217 (0.754, 1.963)	
BMI	1.557 (1.307, 1.854)	5
2,3-dinor-iPF2α-III (ng/mg-cn)	0.563 (0.427, 0.743)	2.74*
Glucose tolerance status		
NGT	REF	N/A
IGT	5.018 (3.306, 7.616)	

Full model includes only BMI at baseline, 2,3-dinor-iPF2 α -III (ng/mg-cn), and diabetes status at baseline adjusting for age, gender, race/ethnicity.

*75th-25th percentile contrast

Table 4 Adjusted odds ratios for incident diabetes measured at 5-year follow-up using multiplicative model.

<i>Participant Characteristics</i>	<i>aOR (95% CI)</i>	<i>Units</i>
Age(years)	1.063 (0.940, 1.202)	5
Gender		
Male	0.734 (0.478, 1.128)	N/A
Female	REF	
Race/ethnicity		
Non-Hispanic White	REF	
Non-Hispanic Black	0.907 (0.554, 1.485)	N/A
Hispanic	1.267 (0.790, 2.032)	
Glucose tolerance status		
NGT	REF	N/A
IGT	5.910 (3.940, 8.864)	
Ratio of 2,3-dinor-iPF2α-III (ng/mg-cn) to BMI	0.500 (0.364, 0.687)	0.1040*

Full model includes 2,3-dinor-iPF2 α -III (ng/mg-cn)/BMI ratio and diabetes status at baseline adjusting for age, gender, race/ethnicity.

*75th-25th percentile contrast

4.4 75%-25% contrast for the association of T2DM risk with F2-isoP and the ratio (F2-isoP/BMI) by gender

Association between T2DM and both variables of interest were stronger among females as compared to males. Males had an OR of 0.815 (0.486, 1.367) and females had an OR of 0.571 (0.425, 0.767) in the additive model. In the multiplicative model, males had an OR of 0.707 (0.412, 1.215) and females had an OR of 0.472 (0.332, 0.672). When looking at the additive and multiplicative relationship of 2,3-dinor-iPF2 α -III (ng/mg-cn) and BMI, males have a higher OR compared to females, thus, a stronger inverse relationship between T2DM and female gender was found.

Table 5 Association between T2Dm risk with F2-isoP and F2iso/BMI males and females.

OR (95% CI) FOR 75TH-25TH PERCENTILE CONTRAST			
	2,3-dinor-iPF2α-III (additive model)	2,3-dinor-iPF2α- III/BMI ratio (multiplicative model)	Cases/Non-cases
MALES	0.815 (0.486, 1.367)	0.707 (0.412, 1.215)	55/307
FEMALES	0.571 (0.425, 0.767)	0.472 (0.332, 0.672)	83/406

Chapter V: Discussion

T2DM is an important chronic disease linked to many other conditions including obesity and CVD. The primary purpose of this study was to examine the relationship between F2-isoP, BMI, and risk of T2DM in the IRAS cohort. The IRAS study provides valuable information on the relationships between insulin resistance, T2DM, CVD risk factors and behaviors. There are health disparities which exist between different race/ethnicity groups⁴⁵. It has been shown in various studies that individuals of African descent are at higher risk of developing metabolic disorders including obesity and T2DM^{24,45}. Higher risk of T2DM diagnosis was found in NHB (1.44 (95% CI: 1.18,1.75) and other races (1.58 (95% CI: 1.25, 2.00) compared to NHW (reference group)³⁰. The prevalence of T2DM diagnosis, in adults aged 20 years and older, was found to be highest among NHB (12.6%), Hispanics (11.8), and Native Americans/Alaska natives (16.1%). The prevalence of T2DM was higher among specific Hispanic populations including Mexican Americans (13.3%) and Puerto Rican Americans (13.8%)⁴⁵. Confirming that minorities have a higher risk in developing T2DM when compared to the major population⁴⁵. Overall levels of F2-isoP were found to be lower among NHB (mean=3.61 and standard deviation=2.1) when compared to NHW (mean=4.08 and standard deviation=2.30). Given that fat oxidation was found to be lower among NHB, this finding suggests that F2-isoP levels reflect the intensity of fat metabolism¹⁹. Biological factors such as genetics and non-biological factors such as socioeconomic status and access to care are all important when considering the health disparities between different race/ethnicity groups⁴⁵.

The study population used for this analysis was demographically and ethnically diverse. This diversity makes our results generalizable to the U.S. population. This study included three major ethnic groups: non-Hispanic whites, non-Hispanic blacks, and Hispanics. The United States Census Bureau population estimates, as of July 1st, 2018, show that there are approximately 76.6% are NHW, 13.4% NHB, and 18.1% Hispanics in the U.S.⁴³. The current analysis using the IRAS study cohort utilizes data from four different centers around the U.S. Participants from these four centers were selected if they were likely to have IGT or non-insulin dependent diabetes mellitus (NIDDM)⁴⁴. The researchers recruited participants to yield approximately the same proportion of NGT, IGT, and T2DM. In this study, only NGT and IGT groups were included in the

analytical cohort. However, the method of recruitment might have been a reason for the masked differences between race/ethnicity and risk of T2DM.

As expected, age, BMI and IGT were associated with T2DM risk in this study sample. The National Diabetes Statistics Report found that the highest incidence rate in 2015 was 10.9 per 1000 population in those aged 45-64 years. Those between 18-44 and ≥ 65 years of age had an incidence rate of 3.1 and 9.4 per 1000 people, respectively ¹¹. Individuals with IGT at baseline, had an OR of 9.06 of developing T2DM in a study conducted using the IRAS cohort ⁴⁶. Other studies have found that individuals with IGT (n=834) were aged approximately 55 years and BMI of 28.7 kg/m² when compared with healthy subjects ³⁵. They found a 30-fold increased risk in development of T2DM in participants who were obese, reported low levels of physical activity, and with impaired glucose regulation—compared to their healthy counterparts.

We compared two models with F2-isoP and F2-isoP/BMI ratio being two main variables of interest. We compared adjusted ORs for the analogous variables between the two models. Gender and race/ethnicity were not included in this comparison because they were not associated with T2DM. To assess whether the differences in ORs were meaningful, we calculated percent differences between analogous ORs in the additive and multiplicative models. We considered a commonly accepted measure of difference in the relative risk estimates of $>10\%$ to be indicative of meaningful change. The OR for age was similar in both models (difference $<10\%$). The difference in IGT was approximately 17.78%, the OR being higher in the multiplicative compared to the additive model. When comparing F2-isoP with F2-isoP/BMI ratio, there was an overall 11% difference between the additive and multiplicative models. The differences in the 75%-25% contrast odds ratios between males and females were 13.25% and 17.34%, respectively. Thus, multiplicative relationships between with F2-isoP and BMI (F2-isoP/BMI ratio) shows a stronger association with the risk of T2DM as compared for with F2-isoP adjusted for BMI as a covariate (additive model).

In conclusion, the results from our analysis (11% difference between the odds ratios derived from the additive and multiplicative model) show that the new variable, F2-isoP/BMI ratio, does not clearly indicate whether multiplicative model presents a better way to evaluate

the relationships between F2-isoP, BMI, and T2DM. However, IGT had an OR difference greater than 10%, the multiplicative model OR being higher than the additive model OR. This might be an indication that multiplicative relationships between F2-isoP and BMI strengthen the associations of other risk factors with T2DM. Evaluation of the additive and multiplicative models for outcomes—such as weight change, decrease in insulin resistance, blood pressure and others—might clarify whether the additive or multiplicative relationships, between F2-isoP and BMI, better predict these outcomes.

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