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Relationship Between F2 – Isoprostanes and Adult Metabolically Healthy Obesity

Tatiana Piccoli

RELASHIONSHIP BETWEEN F_2 – ISOPROSTANES AND ADULT METABOLICALLY HEALTHY OBESITY

By

Tatiana Victoria Piccoli

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Introduction: Obesity-related morbidity continues to increase worldwide. Obesity is one of the primary conditions for the development of metabolic syndrome which is characterized by a combination of different components, including metabolic, physiological, and biochemical factors that influence the development of cardiovascular disease, diabetes mellitus type two and contribute to all-cause mortality. The development of metabolic abnormalities is related to oxidative stress which increases among obese individuals. The obese population is not homogenous and represented by individuals with altered and with a normal metabolic profile, those with the normal metabolic profile are recognized as Metabolically Healthy Obese (MHO). The lipid peroxidation is the main hallmark of oxidative stress and this process can be evaluated via the measurement of the end products such as F_2 -Isoprostanes.

Aim: The goal of this study was to investigate the relationship between metabolically healthy and unhealthy obesity with markers of oxidative stress (urinary F_2 -Isoprostanes) and assess whether free radical-induced oxidative stress influences transition from metabolically healthy to the metabolically unhealthy group. Metabolic health was defined as blood pressure below 130/85 mmHg, fasting blood glucose level as below 126 mg/dl and HDL-cholesterol above 40 mg/dl for males and above 50 mg/dl for females.

Method: The cohort of 857 participants included Hispanic, non-Hispanic black, and non-Hispanic white from 40 to 69 years of age and had an obesity prevalence of 29% (n=244) at baseline. Among this group, 11.07% (n=27) were metabolically healthy and 88.93% (n=217) were metabolically unhealthy based on criteria for MHO. Among the MHO group, after 5-year follow-up, 37% remained metabolically stable and 63% developed metabolic abnormalities and among the MUO group, 5.6% became metabolically healthy and 94.4% remained metabolically unhealthy. The association between different types of metabolic health and F_2-Isoprostanes species was measured using Wilcoxon rank-sum test.

Result: The MUO status had a direct association with greater weight, Hispanic ethnicity, impaired glucose tolerance, decreased insulin sensitivity, and decreased fasting insulin level. No association was found between metabolic health status and levels of F_2-Isoprostanes at baseline and follow-up.

Conclusion: The result suggests that elevated levels of F_2-Isoprostanes do not promote the transformation of metabolically healthy obesity into metabolically unhealthy obesity.

Keywords: Obesity, metabolic health, metabolically healthy obesity, oxidative stress, F_2-Isoprostanes

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ABBREVIATIONS

BMI	Body Mass Index
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
F_2 -IsoPs	F_2 - Isoprostanes
HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance
IGT	Impaired Glucose Tolerance
IL	Interleukin
IRAS	Insulin Resistance and Atherosclerosis Study
LDL	Low-Density Lipoprotein
MetS	Metabolic Syndrome
MHO	Metabolically Healthy Obesity
MUO	Metabolically Unhealthy Obesity
NCEP/ATPIII	National Cholesterol Education Program Adult Treatment Panel III
NHANS	National Health and Nutrition Examination Survey
ox-LDL	Oxidized Low-Density Lipoprotein
P-value	Probability value (observed significant level)
ROS	Reactive Oxygen Species
TNF- α	Tumor necrosis factor alfa

CHAPTER I
INTRODUCTION

1.1 Background

Increase in obesity prevalence worldwide contributes to the growth of obesity-related morbidity (Landsberg et al., 2013). The study conducted by Finucane et al. (2011) investigated the obesity increase in 199 countries from 1980 to 2008. The result of the study demonstrated that 502 million adults had BMI above or equal to 30 kg/m ^{2} that allowed qualifying them as obese. Obesity is one of the primary conditions for the development of metabolic syndrome (Montague and O'rahilly, 2000, Furukawa et al., 2017), which is characterized by a combination of different components, including metabolic, physiological, and biochemical factors that influence the development of cardiovascular disease, diabetes mellitus type two and contribute to all-cause mortality (Kaur, 2014). The mechanism of development of metabolic syndrome is closely related to systemic oxidative stress (Keaney et al., 2003), which is more prominent among the obese population due to the tendency of adipose tissue to produce reactive oxygen species (ROS) in higher amount compared with other tissues (Furukawa et al., 2017). On one hand, the obesity has an association with diabetes, hypertension, cancer and other numerous health conditions, hence contributing to increase of the healthcare expenditure (Allen, Thorpe, and Joski,2015). On another hand, the health programs targeting this global health problem showed very little success on a population level (Ng et.al., 2014). Additionally, the expenditure of community-based programs targeting obesity can vary due to the kind of approach and variety of interventions included in this program. Finkelstein et al. (2008) showed that, on average,

medical spending on obese individual $1,429 higher per year compared with a person of normal weight

Nevertheless, the obese population is heterogeneous and consists of individuals with a variety of degree of obesity and metabolic abnormalities (Blüher,2014). Considering variability of individuals representing the obese population, it would be beneficial to allocate this population into groups according to their degree of metabolic abnormalities for being able to address their needs more specifically and cost-efficiently. Among the obese population, we can identify a group of individuals that do not have obesity-related comorbidities and do not demonstrate any evidence of metabolic abnormalities, including insulin sensitivity and lipid profile in the normal range and normal blood pressure, based on these criteria this subdivision is identified as metabolically healthy obese (MHO) (Primeau et al., 2011). However, it is not clear how healthy this group is and whether it progresses to metabolically unhealthy obesity (MUO). The large body of literature supports the evidence about beneficial inflammation profile of MHO individuals compared with metabolically unhealthy obese (MUO) population. Moreover, comparing MHO population with the non-obese group, no association was found between the MHO status and cardiovascular disease (CVD) prevalence and all-cause mortality (Calori et al.,2011, Hamer and Stamatakis, 2012, Iacobellis et al.,2007, Karelis et al., 2005). However, some researchers provide the evidence about the temporary condition of metabolic health among obese individuals who with time will develop metabolic abnormalities (Appleton et al., 2013). Additionally, some researchers found that although MHO population has not demonstrated any signs of metabolic abnormalities, their metabolic profile was inferior to the profile of non-obese individuals, including a lower level of HDL-cholesterol and higher non-HDL cholesterol (Manu et al., 2012). Furthermore, some studies demonstrated that this population undergoes subclinical

changes, such as early atherosclerosis (Oflaz et al.,2003). Based on contradicting results of several studies Denis and Obin (2013) suggested that MHO group should be viewed as a "cluster of traits" instead of as a category that can have a prognostic importance. Similarly, Plourde and Korelis (2014) questioned whether MHO is a permanent status or just a stage in the development of metabolic abnormalities accompanying obesity. Reviewing several longitudinal studies with controversial results, the authors concluded that future metabolic health of individuals belonging to MHO population cannot be guaranteed.

A level of influence of systemic oxidative stress in developing metabolic abnormalities among metabolically healthy obese can be assessed by measuring the association between the products of oxidative stress and MHO status. The lipid peroxidation is the main hallmark of oxidative stress, where free radicals activate the process. The degree of lipid peroxidation can be evaluated via the measurement of the end products of this process (Milne et al., 2005). The group of the secondary end products of lipid peroxidation includes prostaglandin-like products called F_2 – Isoprostanes (F_2 -IsoPs) that represent the result of peroxidation of arachidonic acid induced by free radicals. The F_2 -Isoprostanes species demonstrated a higher accuracy of the reflection of oxidative stress compared to other markers (Fam and Morrow, 2003).

1.2 Purpose of the study

The rich body of literature investigated the role of different lifestyle factors, such as physical activity and diet on metabolic health (Phillips et al., 2013), the association between a proinflammatory profile and metabolic status (Plourde and Korelis,2014), and F_2 -Isoprostanes as the markers of oxidative status (Morrow et al., 1995, Il`yasova et al., 2015). To the best of our knowledge, only one study, with a cross-sectional design, has been conducted to analyze the

association between different types of metabolic status of the overweight/obese population and the 8-epi-prostaglandins $F_{2\alpha}$, that represent the most common form of F_2 -Isoprostanes (Kim et al., 2013). But no study has been conducted to evaluate the association between four types of F_2 – Isoprostanes and metabolically healthy obesity (MHO).

The purpose of this study was to explore the relationship between the different metabolic profiles and level of four different forms of F_2 - Isoprostanes among the obese population, in a prospective cohort, and to determine whether the level of F_2 -Isoprostanes can predict the transformation from MHO into MUO. Additionally, to determine what health characteristics determining metabolic health have a correlation with the level of F_2 -Isoprostanes.

1.3 Research question and hypothesis

1). Is there a cross-sectional association between concentration of F_2-Isoprostanes and Metabolically Unhealthy Obesity (MUO) status?

Compared to Metabolically Unhealthy Obese (MUO) population the MHO population shows a lower level of F_2 -Isoprostanes

2). Is there an association between level of F_2 -Isoprostanes and transition from MHO to MUO?

Compared to MHO population that continue to stay MHO after 5 years of follow-up the MHO population that progresses into MUO has a higher level of F_2 -Isoprostanes.

1.4 Thesis Organization

This thesis is presented in five chapters. The first chapter contains an introduction with the description of the background information, purpose of the study, research questions and hypothesis. The literature review is presented in chapter two. The methods, sample, and measures described in chapter three. Next chapter presents the result of the study. The last chapter contents discussion, recommendations, strength and limitation of the study.

CHAPTER II

LITERATURE REVIEW

2.1 The relationship between metabolic syndrome and oxidative stress

The increasing prevalence of metabolic syndrome (MetS) worldwide and the related increase in CVD and diabetes type two prevalence became one of the main concerns of public health (Ceriello and Motz, 2004, Sjorgen, 2005). The changes in lifestyle, such as overnutrition and lack of physical activity, contribute to the development of the bundle of pathophysiological divergences that incorporate dyslipidemia, insulin resistance, high blood pressure, and impaired glucose tolerance. Additionally, all these changes are frequently associated with obesity (Eckel et al., 2005, Kahn et al.,2006, Xu et al.,2012). The mechanism of the development of metabolic syndrome is not completely understood (Bonomini et al., 2015). Even though obesity is one of the main drivers of MetS, there is a population of normal weight individuals with MetS (Ruderman et al., 1998, St-Onge et al., 2004). The study conducted by Park et al. (2003) evaluated the prevalence of metabolic syndrome among a multiethnic sample from the third National Health and Nutrition Examination Survey (NHANS) demonstrating that 4.6% of normal weight participants had metabolic syndrome with obese individuals showing a higher prevalence of metabolic syndrome compared to non-obese (59.6% vs 4.6%.) Throughout the last years, "metabolically triggered inflammation" is recognized as a characteristic of obesity and a contributor to the development of metabolic syndrome (Hotamisligil, 2006). Similarly, the development of metabolic abnormalities that lead to dyslipidemia (Zelzer et al.,2011), hypertension (Russo et al., 1998) and diabetes ((Keaney et al.,2003) is influenced by oxidative stress. Oxidative stress is considered a condition with a disproportion between reactive oxygen

species (ROS) and capacity of antioxidants (Roberts and Sindhu, 2009) that results in increased lipid peroxidation and damage to cellular structures (Yakes and Van Houten, 1997). The evidence about the relationship of metabolic syndrome and oxidative stress is supported by the decreased level of antioxidants among patients with MetS (Armutcu et al., 2008). Palmieri and Sblendorio 2006, showed the increased frequency of metabolic syndrome among postmenopausal women and thought to be due to the elevated free fatty acids (FFA) level and subsequent oxidative stress. Numerous studies demonstrated a positive association between metabolic status and markers of oxidative stress (Guilder et al., 2006, Hansel et al., 2004, Holvoet et al.,2004), confirming the hypothesis about a vital role of oxidative stress in the development of metabolic syndrome.

2.2 F_2-Isoprostanes as markers of oxidative stress

Throughout the last decades, the group of prostaglandin-like structures called F_2 – Isoprostanes became a well-recognized tool for assessing oxidative status (Basu, 2008, Il'yasova et al.,2012). Roberts and Milne (2009) stated that due to the stability of molecules of F_2 - Isoprostanes, their level the most accurately reflects the grade of oxidative injury in vivo. The circulation of free F_2 – Isoprostanes in plasma and excretion with the urine gives an opportunity to evaluate oxidative status in humans and animals (Li et al.,1999, Roberts and Morrow,2000). Moreover, the quantification of the level of F_2 – Isoprostanes in body fluids that reflect normal oxidative status allowed identifying the condition when lipid peroxidation exceeds the capacity of antioxidant defense and is recognized as oxidative stress (Milne et al.,2005). Analyzing the result of the study investigating the relationship between the level of F_2 - Isoprostanes and hypercholesterolemia, Milne et al. (2005) concluded that this positive association is likely due to oxidative stress among this population. Similarly, the elevated level of F_2 – Isoprostanes was

found among patients with diabetes. Comparing groups of patients with and without diabetes, Gopaul et al. (1995) showed that the group with diabetes had 3.3- fold elevation in F_2 – Isoprostanes level compared to the nondiabetic group. Another confirmation about the elevation of F_2 – Isoprostanes during oxidative stress was provided by Morrow et al. (1995) showing the increased level of F_2 – Isoprostanes among smokers compared to nonsmokers. The authors concluded that the negative effect of smoking can be attributed to oxidative injury caused by toxic products inhaled during the smoking process. An article by Milne et al. (2015) provided an overview in the field of F_2 – Isoprostanes research for 25 years since the discovery of F_2 – Isoprostanes by Morrow and Roberts. Since that time numerous studies have been conducted with the purpose of exploring the possibilities of using these compounds as biomarkers for illnesses. Several studies investigated the association between toxic agents causing oxidative damage and level of F_2 – Isoprostanes as markers of this injury and reported a positive relationship between these variables (Il'yasova et al.,2010, Kadiiska et al., 2005). Another study examining the levels of F_2 – Isoprostanes among the population of Inuit, showed an elevated level of these markers among the participants with metabolic syndrome compared to individuals with normal metabolic health (Alkazemi et al.,2012). A similar result was presented by the study conducted by Black et al. 2016, showing a positive association between F_2 – Isoprostanes and all constituents of metabolic syndrome including systolic blood pressure, triglycerides level, waist circumference, and LDL- level, but also surprisingly showing a positive correlation with HDL. Although the research conducted by Melton et al., (2017), did not show any association between F_2 – Isoprostanes index and hypertension. The authors concluded that free radical-induced oxidative stress did not play an important role in the development of hypertension.

2.3 MHO definition and prevalence

Despite the longtime existing knowledge of such phenomenon as metabolically healthy obesity (MHO), there is still no common adopted definition of MHO. Similar to variability in the definition of metabolic syndrome (Borch-Johnsen, 2007) variety of sets of criteria are used to define MHO. Presently, about 15 different sets of criteria have been used to describe MHO status (Plourde and Karelis, 2013). Many researchers agree to use lipid profile, glycemic status, and blood pressure as criteria to define metabolic health among the obese population, others claim that it is important to use the additional components (Velho et al., 2010). Based on different criteria, the prevalence of MHO among obese individuals varied from 6.8% to 36.6% (Phillips et al., 2013). Analyzing the results of several studies, Phillips (2013) stressed attention to MHO prevalence inconsistency across the studies that partially can be attributed to geographic, ethnic, age, and sample size differences, but mainly due to differences in criteria used for the definition of MHO. Furthermore, an even more significant variability of MHO prevalence from 6% to 75% in different studies was presented by Rey-Lopez et al. (2014). Although most studies incorporated the basic criteria of metabolic health such as the level of HDL-cholesterol, results of blood pressure measurements, blood glucose level, and triglycerides, also many of them used additional criteria, such as waist circumference, level of LDL-cholesterol, and others. The usage of these extra criteria can explain a variability in MHO prevalence (Rey-Lopez et al., 2014). Similarly, the result of the study conducted by Velho et al. (2010) showed that MHO prevalence varied from 3.3% to 32.1% among men and from 11.4% to 43.3% among women, depends on criteria used. This study investigated the prevalence of MHO among the same group of participants by using six different sets of criteria and allowed to demonstrate that a variability in

MHO prevalence was based on differences in used criteria. However, despite the utilization of a variety of criteria for MHO definition, many studies found a positive association between sex and MHO status, with women showing a higher prevalence of MHO, and age, with younger age being healthier (Rey-Lopez et al., 2014).

2.4 Relationship between inflammatory markers and markers of oxidative stress and MHO status.

Reviewing several studies Vincent and Taylor (2006) admitted the positive relationship between obesity and oxidative stress. Although the association of obesity and F_2 - Isoprostanes as markers of oxidative stress has been demonstrated in several studies (Il`yasova et al.,2005, Keaney et al., 2003, Stojiljkovic et al.,2002, Wu et al. 2009), literature on evaluation of oxidative stress and specifically level of F_2 - Isoprostanes among MHO population is limited. The results of prospective cohort study examining the relationship between MHO, MUO, markers of oxidative stress and development of cognitive impairment was presented by Farah et al. (2016). Researchers found the positive association between oxidative stress, mild cognitive deterioration and MUO status. This study did not use F_2 – Isoprostanes as markers of oxidative stress. Another study conducted by Bañuls et al. (2017) investigated the relationship between markers of oxidative stress, endoplasmic reticulum stress and status of metabolic health among the obese population. The result of the study showed a higher level of proinflammatory cytokines, such as IL-6 among MUO compared to MHO. Moreover, the production of ROS was lower among MHO compared to MUO, demonstrating a more significant level of oxidative stress among MUO population. Similarly, this study did not utilize F_2 – Isoprostanes as markers of oxidative injury. The result of the study investigating the relationship between markers of oxidative stress

and status of metabolic health among postmenopausal women was presented by Kim et al. (2013). The group of 1846 individuals was divided into four groups according to their body mass index (BMI), and metabolic health status. Participants with BMI≥25 kg/m^2 considered to be overweight/obese and were divided into two groups based on the criteria for metabolic health defined by the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATPIII). All participants with BMI<25 kg/m^2 were also divided into two groups with MetS and without MetS. For evaluating oxidative stress, researchers measured the level of inflammatory markers such as circulating oxidized LDL (ox-LDL) and 8-epi-prostaglandins $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$). The design of the study was cross-sectional, allowing to see the association between the variables but not the causation. The result of the study demonstrated that participants from overweight/obese group had less favorable metabolic profile compared to the normal weight group regardless of their metabolic health. Additionally, comparing subgroups with MetS and without of MetS, investigators saw a better metabolic profile among individuals without MetS regardless of their BMI. Overweight/obese individuals without MetS demonstrated a higher level of ox-LDL compared to the normal weight group without MetS, but lower than among the group with MetS. The level of 8-epi-$PRG_{2\alpha}$ was higher among participants with MetS compared to women without MetS. Researchers concluded that postmenopausal women with MetS with normal weight demonstrated a higher level of oxidative stress compared to overweight/obese with normal metabolic health. Additionally, between the markers of oxidative stress, no association was found that draw a conclusion about representation by these markers different stages of oxidative changes. The result of this study contradicts the result of the research conducted by Sjorgen et al. (2005). In this cross-sectional study, the sample was divided into three groups according to their metabolic risk factors, based on NCEP/ATPIII panel. One

group was presented by individuals without any risk factors for MetS, another with 1-2 risk factors, and the third group by participants with MetS. Examining the relationship between markers of oxidative stress, such as ox-LDL and 8-iso-$PGF_{2\alpha}$ with the metabolic status of otherwise healthy men (n=289), researchers did not find any association between these variables.

CHAPTER III

METHODOLOGY

3.1 Data source

The collected data from Insulin Resistance Atherosclerosis Study (IRAS) became the source of our secondary data analysis. This study was the first epidemiological study intended to evaluate the association between constituents of insulin resistance syndrome, CVD, and other risk factors (Wagenknecht et al., 1995). The study began in 1992 with a follow-up period of approximately five years and was approved by the Institutional Review Board of Wake Forest University School of Medicine (Melton at al., 2017)

3.2 Sample selection and participant observation

The study aimed to observe multiethnic cohort that was achieved with the recruitment of 1626 participants representing the Hispanic population (n=548), Non-Hispanic black (n=464), and Non-Hispanic white (n=614). Female population represented 56% of the sample. The participants were from 40 to 69 years of age. Aiming to have equal representation of individuals with a different level of glucose tolerance, the study oversampled nondiabetic individuals with an elevated fasting plasma glucose level and those who were previously identified as having impaired glucose tolerance (IGT). The participants were recruited at four clinical centers located in Los Angeles, California; Oakland, California; San Luis Valley, Colorado; and San Antonio, Texas (Wagenknecht et al., 1995). Each participant gave written informed consent. The complete

examination of each participant at the baseline was achieved during two visits, each lasted approximately four hours, with the variation of the interval between the appointments from one to thirty days. During these appointments, the participants were evaluated for glucose tolerance using a 75g glucose load and insulin resistance using an insulin injection. CVD and peripheral vascular disease were assessed using an interview, performed in their preferred language, and noninvasive testing, such as blood pressure measurement, electrocardiography, and ultrasonography. Additionally, the participants completed a questionnaire self-reporting their race/ethnicity, smoking, alcohol intake, physical activity, and nutrient intake. Anthropometric measurements were performed by trained medical personnel. Moreover, for examining F_2 – Isoprostanes level 901 enrollees provided a urine sample at the baseline. The examination of the participants began in October 1992 and was completed in April 1994. After approximately five years of the follow-up period, in 1997-1998, the study`s participants were examined again according to the protocol of the study. The participants free of diabetes mellitus (DM) type two at the baseline were included in the study analysis, that reduced the sample to 1125 participants, among those, 20 % were lost to follow-up.

3.3 Variable Measurement

World Health Organization criteria (WHO,1999) provided guidance for assessing glucose tolerance. A blood sample was taken before administering 75g of glucose and two hours after. Insulin resistance was measured after an injection of 50% glucose solution (0.3 g/kg) in 20 minutes followed by an injection of insulin (0.03 U/kg). During a three hours' period, blood was collected 12 times through another intravenous line and was evaluated for the concentration of glucose and insulin. The blood sample was drawn after fasting for 12 hours to assess the level of

lipids, fasting blood glucose, and components of the biochemical profile. At the baseline examination, morning urine samples were collected and kept at -70^{o} C until the analysis. A total number of 901 samples of urine were collected, among those 857 samples were satisfyingly measured for F_2 – Isoprostanes concentration. The measurement of F_2 – Isoprostanes was performed utilizing liquid chromatography/tandem mass spectrometry, the result was calibrated according to the urinary concentration of creatinine. Four different F_2 – Isoprostanes isomers were measured including iPF(2a)-III, 2,3-dinor-iPF(2a)-III, iPF(2a)-lV, 8,12-iso-iPF(2a)-lV. The F2 -Isopostanes index was created based on the result for four F_2 – Isoprostanes isomers and allowed to rank participants based on this calculation $[(X_{1i} - M_1)/SD_1 + (X_{2i} - M_2)/SD_2 + (X_{3i} - M_3)/SD_3 + (X_{4i} - M_4)/SD_4)/4$. In this formula, "i" is a code for a participant, X_{1-4} represent values of four F_2 – Isoprostanes isomers, M_{1-4} mean of these four isomers, and SD_{1-4} standard deviation values. The body mass index, as a measurement of general adiposity, was calculated as a relationship between body mass in kilograms divided by height in square meters.

3.4 The MHO phenotype definition

We identified two different groups of MHO based on two different definitions of this phenomenon. One of the most commonly used definitions of MHO status included the absence of diabetes, hypertension, and dyslipidemia (Muñoz-Garach et al.,2016). We used 126 mg/dl of fasting blood glucose level as a cut-off for diabetes. Enrollees with fasting plasma glucose level <126 mg/dl were considered nondiabetic. Individuals with systolic blood pressure equal or below 130 mmHg and diastolic blood pressure equal or below 85 mmHg and not taking blood pressure lowering medication were considered normotensive. Lipid profile was represented by the level of HDL-cholesterol, which was considered of normal value if its level was above or equal to 40mg/dl among males and above or equal 50mg/dl among females. All participants who met the

criteria for the first definition were united in the group named MHO1. Another definition of MHO status was based on modified criteria suggested by Wildman et al. (2008). This group included individuals with normotensive status (BP ≤130/85 mmHg) who did not undergo antihypertensive treatment. The absence of diabetes with fasting blood glucose level ≤ 100mg/dl was another criterion. Moreover, for being included in this MHO group additional criteria had to be met, such as homeostasis model assessment of insulin resistance (HOMA-IR) ≤ 5.1 and triglycerides HDL-cholesterol ratio ≤ 1.65 for male and ≤1.32 for female. This group was named MHO2.

3.5 Statistical analysis

Statistical analysis was performed using SAS statistical software (version 9.4: SAS Institute, Cary, NC). A total number of 857 nondiabetic participants were included in this analysis at the baseline. The descriptive statistical analysis was applied to the baseline sample. The individuals with BMI ≥ 30kg/m^2 were selected and the final sample was represented by 244 participants. At the follow-up visit, this sample reduced to 241 participants. The association between categorical variables, including a crude association between age category, sex, ethnicity and MHO1 and MHO2 groups, was measured using chi-square test. Univariate analysis was perfumed to identify a median and interquartile range for continuous variables. We used Wilcoxon rank-sum test to evaluate how different were continuous variables, including F_2 – Isoprostanes levels, between groups with MHO and MUO. We analyzed the differences between MHO and MUO groups at the baseline and at the follow-up appointment. Additionally, at the follow-up, we analyzed metabolic changes including how many MHO participants remained stable after five years of follow-up and how many demonstrated a decline in their metabolic

health and evaluated their association with oxidative stress markers among the participants. A p-value was considered statistically significant if it was less than 0.05

CHAPTER IV

RESULTS

4.1 Description of the study sample

The study population at the baseline included a slightly higher number of females (58%) compared to males (42%). Racial/ethnic diversity was represented by Hispanic (32%), Non-Hispanic black (28%) and Non-Hispanic white (40%). Among the age categories the highest representation was in the group from 50 to 59 years of age (36%), groups from 40 to 49 years of age and 60 to 69 years of age were represented equally 32% each. Almost half of the baseline population never smoked (46%), former smokers represented 40% of the population, and current smokers 14 %. Among the baseline population, 40% had hypertension and 60 % did not have increased blood pressure and did not take hypertensive medication. Approximately two-thirds of the baseline population (67.5%) belonged to the group with normal glucose tolerance and one-third (32.5%) had impaired glucose tolerance. The baseline sample did not include participants with diabetes. Prevalence of obesity (BMI$\geq$30kg/m^2) was 29%, (n=244) of the baseline population.

Table 1. Baseline characteristics of the entire IRAS nondiabetic cohort.

Continuous variables	**N (missing values)**	**Means (SD)**
Age (years)	857 (0)	54.6 (8.315)
BMI (kg/m^2)	855 (2)	28.5 (5.655)
Fasting Glucose (mg/dl)	857 (0)	98.6 (11.3)
Insulin sensitivity (S_I, $x10^{-4}minutes^{-1}/\mu Uml$)	795 (62)	2.20 (2.05)
Acute Insulin Response (microU/ml)	834 (23)	486.8 (494.3)
iPF(2a)-III (ng/mg CN)	853 (4)	0.249 (0.194)
2,3-dinor-iPF(2a)-III (ng/mg CN)	853 (4)	4.35 (2.10)
iPF(2a)-lV (ng/mg CN)	853 (4)	6.49 (4.16)
8,12-iso-iPF(2a)-lV (ng/mg CN)	853 (4)	4.15 (2.87)
F_2-IsoP Index*	853 (4)	-0.001 (0.821)
2-hour Glucose (mg/dl)	857 (0)	124.3 (33.5)
HDL (mg/dl)	854 (3)	47.11 (15.17)
Triglycerides (mg/dl)	853 (4)	130.7 (81.37)
Fasting Insulin (uU/ml)	856 (1)	15.69 (15.13)
Categorical variables	**N (missing values)**	**Percent**
Gender	855 (2)	100
Males	363	42.46
Females	492	57.54
Race/ethnicity	855 (2)	100
Hispanic	276	32.28
Non-Hispanic black	237	27.72
Non-Hispanic white	342	40.0
Smoking status	855 (2)	100
Never smoked	397	46.43
Former smoker	338	39.53
Current smoker	120	14.04
Hypertension	855 (2)	100
Yes	339	39.65
No	516	60.35
Obesity	244 (0)	100
MUO	217	88.93
MHO	27	11.07
Glucose tolerance status	855 (2)	100
NGT	577	67.49
IGT	278	32.51

- Index* -mean of 4 standardized F2-Isoprostanes.

4.2 Prevalence of metabolically healthy obesity among study population

Among the obese population, 27 individuals (11.07%) met criteria for MHO1 and 3 participants (1.23%) were recognized as metabolically healthy based on the criteria for MHO2 definition. All participants who met criteria for MHO2 definition also met the criteria for MHO1 definition. At follow-up, the sample from the obese population decreased (n=241) and included 22 participants (9.1%) belonging to the metabolically healthy (MH) group and 219 participants (90.9%) were metabolically unhealthy (MU). These 22 MHO individuals met the criteria for MHO1 definition. Additionally, 10 participants (4.1%) met criteria for MHO2 definition at follow-up. All participants who can be considered MHO2 also met the criteria for MHO1. Age category and sex did not show a statistically significant association with MHO status at baseline and follow-up. Smoking status did not demonstrate an association with MHO status at baseline, showing an equal prevalence of MHO among former smokers and participant who never smoked (44.4% each). At follow-up, this association increased with 45.4% of individuals who never smoked representing the MHO group of which 36.4% were former smokers, current smokers represented less than 20% of MHO population on both visits (P=.06). At baseline participants belonging to the Hispanic group demonstrated the lowest prevalence of MHO (14.8%) and the highest prevalence of MUO (37.3%) compared to two other racial/ethnic groups (P=.05). Although this trend continued to the follow-up, it became less statistically significant (P=.08). As anticipated, the MHO status was associated with glucose tolerance status, insulin sensitivity, and fasting insulin. Although an association between MHO status and BMI was not statistically significant at baseline, at follow-up there was a statistically significant association demonstrating higher median BMI among MUO compared to MHO (P=.01) (Table 2).

Table 2 Metabolically Healthy and Unhealthy Obesity status at baseline and follow-up.

	Baseline N= 244 (percent)		**p-value**	**Follow-up N=241 (percent)**		**p-value**
Categorical Variable*	**MHO** N=27	**MUO** N=217		**MH** N=22	**MU** N=219	
Age			0.07			0.5
40-49	10 (37.04)	71 (32.72)		9 (40.91)	70 (31.96)	
50-59	13 (48.15)	69 (31.80)		8 (36.36)	74 (33.79)	
60-69	4 (14.81)	77 (35.48)		5 (22.73)	75 (34.25)	
Gender			0.9			0.7
Male	9 (33.33)	70 (32.26)		8 (36.36)	71 (32.42)	
Female	18 (66.67)	147 (67.64)		14 (63.64)	148 (67.58)	
Ethnicity			0.05			0.08
Hispanic	4 (14.81)	81 (37.33)		3 (13.64)	81 (36.99)	
Non-Hispanic black	12 (44.44)	60 (27.65)		8 (36.36)	64 (29.22)	
Non-Hispanic white	11 (40.74)	76 (35.02)		11 (50.00)	74 (33.79)	
Smoking status			0.76			0.06
Never smoked	12 (44.4)	111 (51.2)		10 (45.4)	109 (50.7)	
Former smoker	12 (44.4)	81 (37.3)		8 (36.4)	95 (44.2)	
Current smoker	3 (11.2)	25 (11.5)		4 (18.2)	11 (5.1)	
Hypertension N, %			N/A			N/A
Yes	0	143 (65.90)		0	163 (74.43)	
No	27 (100.00)	74 (34.10)		22 (100.00)	56 (25.57)	
Glucose tolerance status N, %			0.01			< .001
NGT	20 (74.06)	103 (47.47)		19 (86.36)	82 (37.44)	
IGT	7 (25.94)	114 (52.53)		2 (9.09)	68 (31.05)	
Type 2 diabetes	N/A	N/A		1 (4.55)	69 (31.51)	
Continuous Variable	**Median ± IQR**	**Median ± IQR**		**Median ± IQR**	**Median ± IQR**	
BMI Kg/m^2	33.08 (31.23-36.33)	34.27 (31.89-37.705)	0.126	30.29 (29.02- 36.00)	35.45 (31.99- 38.53)	0.01
Fasting Glucose (mg/dl)	98.0 (91-104)	102.0 (95-111)	N/A	96.0 (90.0-104.5)	105.5 (95.5-121.5)	N/A
Insulin sensitivity	1.54	0.85	<.001	1.10	0.51	< .001

(S_I, x10^{-4}minutes^{-1}/µUml)	(1.28-2.62)	(0.370-1.54)		(0.96-2.50)	(0.0-0.98)	
Acute Insulin response (microU/ml)	565.0 (161.6-710.8)	417.6 (137.4-790.4)	0.552	428.5 (354.8- 735.8)	346.2 (98.00- 847.1)	0.19
2-hour Glucose (mg/dl)	116 (95-141)	142 (120-167)	<.001	108.0 (89.0-122.5)	155.5 (124.5- 213.7)	< .001
HDL (mg/dl)	55.0 (51.0-57.0)	40.0 (33.0-46.5)	N/A	58.5 (51.0-65.0)	43.0 (35.0-51.0)	N/A
Triglycerides (mg/dl)	104.0 (74.0-183.0)	139.0 (90.0-182.0)	N/A	76.0 (59.0-127.0)	124.0 (86.0-179.0)	N/A
Fasting Insulin (uU/ml)	14.0 (11.0-21.0)	19.0 (14.0-26.0)	0.005	15.0 (11.0-18.0)	23.0 (16.0-33.0)	< .001

- Variables value correspond to each time point. All categorical variables reported in N, %, p-value for categorical variables assessed using Chi-square. All continuous variables reported in median ± IQR assessed using univariate test, p-value for continuous variables assessed using Wilcoxon test.

4.3 Association between metabolically healthy obesity and F_2 – Isoprostanes

Each individual F_2 – Isoprostanes isomer demonstrated the variability of distribution. Examined association between MHO status and level of each isomer of F_2 – Isoprostanes was not statistically significant. Majority of F_2- Isoprostanes species demonstrated an inverse association with MHO status, such iPF (2a)-III, 2,3-dinor – iPF (2a)-III, 8,12-iso-iPF, and F_2 -Isoprostanes Index all were slightly higher among MUO compared with MHO group at baseline. Although iPF(2a)-lV had a direct association with MHO status being higher among MHO (median=7.53 ng/mg) compared to MUO (median= 5.93 ng/mg). None of the associations were statistically significant. Assessment of the association between levels of F_2 – Isoprostanes and MHO status on follow-up demonstrated an increase in the number of F_2 – Isoprostanes having direct associations with MHO status with only iPF(2a)-lll being slightly higher among MUO (0.214 ng/mg) compared with MHO (0.212 ng/mg).

The associations between F_2 – Isoprostanes isomers and MHO status on follow-up were not statistically significant (Table 3).

Table 3 Oxidative status of MHO and MUO

	Baseline N=244 (median, IQR)		**p-value**	**Follow-up N=241 (Median, IQR)**		**p-value**
variable	**MHO N=27**	**MUO N=217**		**MH N=22**	**MU N=219**	
iPF(2a)-lll (ng/mg)	0.178 (0.148- 0.418)	0.215 (0.127- 0.330)	0.795	0.212 (0.158- 0.336)	0.214 (0.127- 0.330)	0.66
2,3-dinor-iPF(2a)-lll (ng/mg)	4.322 (2.944- 6.689)	4.687 (2.954- 6.763)	0.659	4.804 (3.223- 7.435)	4.670 (2.930- 6.689)	0.87
iPF(2a)-lV (ng/mg)	7.53 (4.126- 9.037)	5.93 (4.017- 8.572)	0.215	6.559 (5.109- 10.327)	5.959 (3.931- 8.572)	0.15
8,12-iso-iPF (ng/mg)	3.125 (2.490- 5.860)	3.979 (2.729- 5.923)	0.212	4.224 (2.899- 7.167)	3.882 (2.637- 5.6905)	0.27
F_2-isoP Index*	-0.025 (-0.575-0.650)	-0.000 (-0.475-0.600)	0.950	0.100 (-0.300- 0.650)	-0.025 (-0.500- 0.572)	0.45

- All continuous variables reported in median ± IQR. Index*- mean of 4 standardized F2-isoPs.

Comparison of baseline and follow-up MHO status showed that from 27 individuals representing MHO at the baseline, 17 participants progressed into an unhealthy state and 10 participants remained stable metabolically healthy. Among those who were MUO (n=217) at baseline, 12 individuals demonstrated metabolic improvement and moved into a group of metabolically healthy and the rest of the group remained unhealthy. The association between level of F_2 – Isomers and metabolically stable state was not statistically significant with a median of F_2-isoP index being of the same value (-0.025ng/mg) among MHO stable and metabolically declined groups of individuals (Table4).

Table 4 Oxidative status at follow-up among MHO stable and MHO declined

variable	MHO stable (n=10)	MHO declined (n=17)	p-value
	Median (IQR)	Median (IQR)	
iPF(2a)-lll (ng/mg)	0.183 (0.149 - 0.419)	0.178 (0.148 - 0.310)	0.782
2,3-dinor-iPF(2a)-lll (ng/mg)	4.156 (2.944 - 7.963)	4.322 (3.550 - 6.3705)	0.744
iPF(2a)-lV (ng/mg)	7.1937 (4.221 - 8.554)	7.5345 (4.126- 9.037)	0.706
8,12-iso-iPF (ng/mg)	2.853 (2.273 - 8.704)	3.2885 (2.515 - 4.250)	0.860
F_2-isoP Index*	-0.025 (-0.600 - 0.650)	-0.025 (-0.550- 0.550)	0.920

- All continuous variables reported in median ± IQR. Index*- mean of 4 standardized F2-Isoprostanes.

CHAPTER V

DISCUSSION

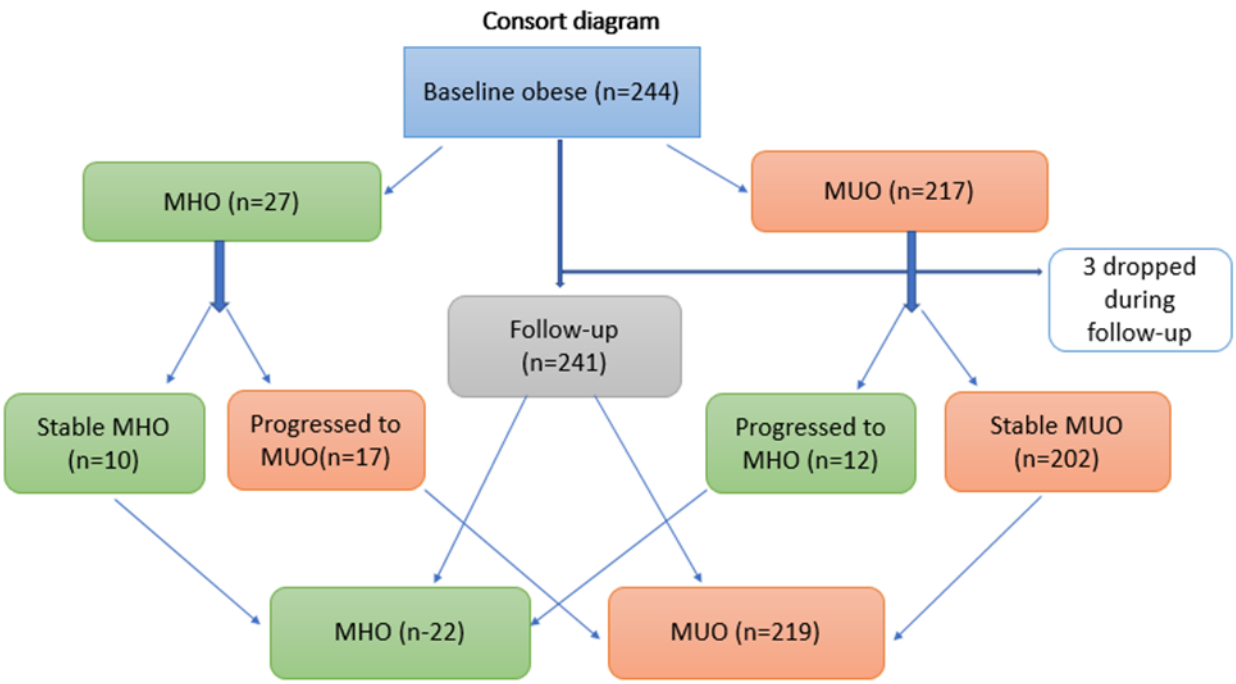
5.1 Discussion of Research Question

The purpose of the study was to examine whether increased oxidative status can promote a transition from metabolically healthy obesity (MHO) into metabolically unhealthy obesity (MUO). The increased mass of adipose tissue is characterized by the changed structure of adipocytes, including their hypertrophy and hyperplasia that affect the property of adipocytes, such as reaction to insulin due to decreased density of receptors for insulin (Fernández-Sánchez et al., 2011). The possibility of adipose tissue to produce certain bioactive molecules, including leptin and proinflammatory cytokines, such as IL-6 and tumor necrosis factor-alfa (TNF-α) promotes higher oxidative and inflammatory status of this tissue (BouloumiÉ et al.,1999, Perwez Hussain and Harris, 2007). This property of adipose tissue advances metabolic misbalance, including changed lipid profile and altered glucose metabolism. What contributes to a diversity of metabolic state among obese remained not well understood. Several studies identified an association between age, gender and MHO status (van Vliet-Ostaptchouk et al.,2014). Numerous conducted research provided conflicting data on the effect of lifestyle, diet, or behavior on metabolic health (Phillips, 2013). Some researchers provided evidence about the positive effect of physical exercise on metabolic health of the obese population (Phillips et al.,2013, Velho et al., 2010). Additionally, it has been shown that MHO population has a more favorable inflammatory profile compared to MUO (Phillips, 2013). We hypothesized that F_2-Isoprostanes as markers of free radical oxidative stress can become a predictor of deteriorating metabolic

health among still metabolically healthy obese individuals. Based on the result of our study, we did not identify any statistically significant association between level of F_2-Isoprostanes and MUO compared to MHO. Neither did we find any statistically significant difference in F_2-Isoprostanes association between the population who were MHO stable after five years of follow-up and those who progressed from MHO to MUO. The result of our study reflects the result of the study conducted by Sjorgen et al. (2005), where no association was found between markers of oxidative stress and different metabolic health status among obese men. The result of our study can be partially attributed to the small sample of metabolically healthy individuals, 27 participants at baseline and 22 at follow-up and can be considered as one of the limitations of our study. Another explanation for this result can be related to the criteria we used to identify MHO. There are a variety of criteria used to define metabolic health among obese and the debates about which combination of criteria is more correct are still ongoing (Phillips et al., 2013). The criteria we used for MHO1 were very basic and included the absence of hypertension, diabetes and healthy HDL-cholesterol level. It is possible to think that this MHO group already had underlying metabolic changes that had not been manifested yet in the form of diabetes or hypertension. Although criteria for MHO2 were stricter and incorporated HOMA, triglycerides/HDL-cholesterol ratio and lower level of fasting glucose, such as 100mg/dl as recommended by National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATPIII) (Lorenzo et al.,2007), the number of individuals who met these criteria was very small for drawing any conclusion about the association between F_2-Isoprostanes level and metabolic health of this population.

One of the main findings of the study was the identification of the changeable status of metabolic health among the obese population. After 5years of the follow-up period, some of the participants with initially healthy metabolic profile remained healthy and some developed metabolic abnormalities. Similarly, the group of obese participants who were metabolically unhealthy on the baseline demonstrated a bifurcation with some participants remained metabolically unhealthy and some demonstrated an improvement in metabolic health (Figure 1).

Figure 1. Changes in metabolic status among MHO and MUO populations



5.2 Study strengths and limitations

One of the strengths of this study is the utilization of prospective cohort data that allowed to see not only an association between variables but to determine a possible

causation. Moreover, it provided an opportunity to see the trend of metabolic changes among the obese population over a five-year period. Additionally, this study allowed to examine the relationship between four F_2 - Isoprostanes isomers and metabolic health of the nondiabetic obese population in a multiethnic cohort.

One of the limitations of the study was the small sample of the obese population (n=244) with a small group of MHO. Also, we did not analyze the changes in the diet, exercises, and weight in this initially obese cohort group that could affect the transition from one category of metabolic health into another, but it was not the purpose of this study.

5.3 Conclusions and recommendations

Based on the result of our study we did not identify any causal relationship between free radical oxidative stress and development of metabolic abnormalities among the metabolically healthy obese adult population. The difference in the level of oxidative stress between MHO and MUO was statistically insignificant and at the same time, both groups of obese population demonstrated a higher level of oxidative stress compared to non-obese. We can conclude that MHO population has invisible pathological processes that in the future can manifest as metabolic abnormalities. Considering this result, we recommend including MHO group in all programs targeting obesity that can provide benefits to the health of obese population regardless of their metabolic profile. Future research is needed to investigate what factors can trigger metabolic changes or prevent clinical manifestation of oxidative stress among MHO population.

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Table 2 Metabolically Healthy and Unhealthy Obesity status at baseline and follow-up.

	Baseline N= 244 (percent)		**p-value**	**Follow-up N=241 (percent)**		**p-value**
Categorical Variable*	**MHO** N=27	**MUO** N=217		**MH** N=22	**MU** N=219	
Age			0.07			0.5
40-49	10 (37.04)	71 (32.72)		9 (40.91)	70 (31.96)	
50-59	13 (48.15)	69 (31.80)		8 (36.36)	74 (33.79)	
60-69	4 (14.81)	77 (35.48)		5 (22.73)	75 (34.25)	
Gender			0.9			0.7
Male	9 (33.33)	70 (32.26)		8 (36.36)	71 (32.42)	
Female	18 (66.67)	147 (67.64)		14 (63.64)	148 (67.58)	
Ethnicity			0.05			0.08
Hispanic	4 (14.81)	81 (37.33)		3 (13.64)	81 (36.99)	
Non-Hispanic black	12 (44.44)	60 (27.65)		8 (36.36)	64 (29.22)	
Non-Hispanic white	11 (40.74)	76 (35.02)		11 (50.00)	74 (33.79)	
Smoking status			0.76			0.06
Never smoked	12 (44.4)	111 (51.2)		10 (45.4)	109 (50.7)	
Former smoker	12 (44.4)	81 (37.3)		8 (36.4)	95 (44.2)	
Current smoker	3 (11.2)	25 (11.5)		4 (18.2)	11 (5.1)	
Hypertension N, %			N/A			N/A
Yes	0	143 (65.90)		0	163 (74.43)	
No	27 (100.00)	74 (34.10)		22 (100.00)	56 (25.57)	
Glucose tolerance status N, %			0.01			< .001
NGT	20 (74.06)	103 (47.47)		19 (86.36)	82 (37.44)	
IGT	7 (25.94)	114 (52.53)		2 (9.09)	68 (31.05)	
Type 2 diabetes	N/A	N/A		1 (4.55)	69 (31.51)	
Continuous Variable	**Median ± IQR**	**Median ± IQR**		**Median ± IQR**	**Median ± IQR**	
BMI Kg/m^2	33.08 (31.23-36.33)	34.27 (31.89-37.705)	0.126	30.29 (29.02- 36.00)	35.45 (31.99- 38.53)	0.01
Fasting Glucose (mg/dl)	98.0 (91-104)	102.0 (95-111)	N/A	96.0 (90.0-104.5)	105.5 (95.5-121.5)	N/A
Insulin sensitivity	1.54	0.85	<.001	1.10	0.51	< .001

(S_I, $x10^{-4}minutes^{-1}/\mu Uml$)	(1.28-2.62)	(0.370-1.54)		(0.96-2.50)	(0.0-0.98)	
Acute Insulin response (microU/ml)	565.0 (161.6-710.8)	417.6 (137.4-790.4)	0.552	428.5 (354.8- 735.8)	346.2 (98.00- 847.1)	0.19
2-hour Glucose (mg/dl)	116 (95-141)	142 (120-167)	<.001	108.0 (89.0-122.5)	155.5 (124.5- 213.7)	< .001
HDL (mg/dl)	55.0 (51.0-57.0)	40.0 (33.0-46.5)	N/A	58.5 (51.0-65.0)	43.0 (35.0-51.0)	N/A
Triglycerides (mg/dl)	104.0 (74.0-183.0)	139.0 (90.0-182.0)	N/A	76.0 (59.0-127.0)	124.0 (86.0-179.0)	N/A
Fasting Insulin (uU/ml)	14.0 (11.0-21.0)	19.0 (14.0-26.0)	0.005	15.0 (11.0-18.0)	23.0 (16.0-33.0)	< .001

- Variables value correspond to each time point. All categorical variables reported in N, %, p-value for categorical variables assessed using Chi-square. All continuous variables reported in median ± IQR assessed using univariate test, p-value for continuous variables assessed using Wilcoxon test.