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Joint Associations of Oxidative Stress, Central Obesity, and Cardiorespiratory Fitness with Diabetes and Pre-Diabetes: NHANES 1999-2004

By

Jennifer Gao
Degree to be awarded: MPH

Epidemiology

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Joint Associations of Oxidative Stress, Central Obesity, and Cardiorespiratory Fitness with Diabetes and Pre-Diabetes: NHANES 1999-2004

By

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Bachelor of Science
Boston University
2012

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An abstract of
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ABSTRACT

PURPOSE: Obesity and central adiposity are well-known risk factors for cardiometabolic conditions including diabetes (DM), but little is known about the contribution of oxidative stress (OS) and cardiorespiratory fitness (CRF). This study will assess the individual and joint associations of central obesity, OS, and CRF with glycemic status using data from the National Health and Nutrition Examination Survey (NHANES) 1999-2004.

METHODS: This analysis was restricted to adults 20 years of age and older (n=12,662). We used polytomous logistic regression to assess the associations between waist-to-height ratio (WHtR) and oxidative stress score (OSS) with glycemic status outcomes (DM and pre-DM), adjusting for sociodemographics, family history of DM, modifiable risk factors, and co-morbidities. We conducted a sub-analysis (n=3,141; ages 20-49) of these associations with the addition of CRF as our third main exposure. Glycemic status was defined using self-reported diagnoses, use of insulin or other glucose-lowering medications, measured HbA1c, or fasting plasma glucose. OSS was calculated for each participant and divided into quartiles for the analyses using seven equally weighted biomarkers. WHtR ≥ 0.65 was used as a measure of central obesity. CRF was measured using submaximal treadmill testing, where the lowest 20th percentile of estimated VO2max represented low fitness.

RESULTS: 43.1% and 28.2% of adults with DM and pre-DM, respectively, had a WHtR ≥ 0.65. The mean OSS for those with DM and pre-DM was 1.01 and 0.45, respectively. An increased WHtR was significantly associated with pre-DM (OR=1.79, 95% CI: 1.52, 2.12) and DM (OR=3.42, 95% CI: 2.73, 4.29). The highest quartile of OSS was significantly associated with pre-DM (OR=2.34, 95% CI: 1.78, 3.07) and DM (OR=2.15, 95% CI: 1.53, 3.03). The sub-analysis showed that low CRF was significantly associated with pre-DM (OR=1.27, 95% CI: 1.07, 1.51) and DM (OR=2.86, 95% CI: 1.73, 4.70). The highest risk group (high central obesity, high OS, low CRF) had the strongest associations with pre-DM (OR=2.84; 95% CI: 2.51, 3.22) and DM (OR=7.57; 95% CI: 6.24, 9.19).

CONCLUSIONS: This study highlights the importance of central obesity, elevated OS, and low CRF in hyperglycemic individuals. These risk factors are all significantly associated with the odds of DM and pre-DM.

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List of Abbreviations

AT	Alpha-Tocopherol
AUC	Area Under the Curve
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
CI	Confidence Interval
CRF	Cardiorespiratory Fitness
CRP	C-reactive Protein
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
FPG	Fasting Plasma Glucose
GGT	Gamma-Glutamyl Transferase
HbA1c	Glycohemoglobin
HTN	Hypertension
MI	Myocardial Infarction
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examinations Survey
OR	Odds Ratio
OS	Oxidative Stress
OSS	Oxidative Stress Score
PA	Physical Activity
PIR	Poverty Income Ratio
ROS	Reactive Oxygen Species
TAC	Total Antioxidant Capacity
UA	Uric Acid
WHtR	Waist-to-Height Ratio

CHAPTER I: BACKGROUND

Diabetes Mellitus

Diabetes mellitus (DM) is the seventh leading cause of death in the United States and is a major health threat that can cause other complications, including cardiovascular disease, kidney disease, lower limb amputations, and adult-onset blindness [1]. The prevalence of DM in the United States has increased over time and it is estimated that 29.1 individuals (9.3% of the population) are affected by diabetes, with healthcare expenditures and lost productivity costing the country close to $245 billion annually [1]. The number of DM cases in the United States is expected to reach upwards of 50 million by 2050 [2].

Type 2 DM, also known as non-insulin-dependent diabetes or adult-onset diabetes, accounts for 90 to 95% of all cases of diabetes. Other types of diabetes include type 1 diabetes (insulin-dependent DM), which accounts for about 5% of all cases, and gestational diabetes, which develops in 2-10% of all pregnancies [3]. The most studied and well-known independent risk factors for diabetes include: older age, obesity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance, physical inactivity, and race/ethnicity [3]. Some individuals are also more genetically predisposed to developing diabetes, but the exact genes that play in a role in this disease are still being studied.

Oxidative Stress and Diabetes

Oxidative stress (OS) is a general term to describe the state of damage in the body's tissues caused by reactive oxygen species (ROS) [4]. OS is determined by the balance between the rates at which pro-oxidants are produced and at which they are removed by antioxidant mechanisms [4]. OS plays an important role in the mechanisms of obesity and various diseases, including DM and cardiovascular disease [5, 6] and acts as a mediator of insulin resistance and its progression to glucose tolerance and DM [7, 8]. DM is usually accompanied by an increased production of free radicals (pro-oxidant) and/or impaired antioxidant defenses, i.e., a net increase in oxidative stress [9]. OS impairs glucose uptake in muscle and fat in diabetic conditions and decreases insulin secretion from pancreatic beta cells [10]. Studies have shown that oxidative stress is provoked under hyperglycemic conditions [5], as it is likely that oxidative stress is involved in beta-cell deterioration. One study that investigated the association between endothelial dysfunction and oxidative stress in individuals with pre-diabetes showed that increased oxidative stress was present even at early stages of diabetes [11].

The role and associations between individual biomarkers that contribute to oxidative stress with DM have been studied. However, few studies have examined the combined associations between multiple biomarkers with prevalent DM. One such longitudinal study assessed the reduction in oxidative stress in diabetic patients and used a Z-score calculation method to combine 12 different parameters [12]. The researchers found that elevated levels of oxidative stress in diabetic patients were associated with reduced beta-cell function [12]. Furthermore, no studies have looked at

how combined oxidative stress interacts with central obesity and cardiorespiratory fitness in its associations with diabetes.

The following surrogate oxidative stress markers and their associations with diabetes have been examined in the literature: bilirubin, gamma-glutamyl transferase (GGT), uric acid, C-reactive protein (CRP), albumin, creatinine, and vitamin E.

Bilirubin

Bilirubin is the end product of heme catabolism in the systematic circulation, and plays an important role in metabolic pathways. It has been recognized in previous studies that high levels of bilirubin can be a powerful antioxidant agent, and can be protective against diabetes and other inflammatory diseases [13]. Specifically in diabetes, high bilirubin levels are inversely associated with DM [14].

GGT

GGT is an enzyme responsible for the extracellular catabolism of glutathione, and many studies have shown that elevated levels of GGT are associated with DM and greater oxidative stress [15-17]. A previous analysis of data from the National Health and Nutrition Examinations Survey (NHANES) from 1988-1994 in the United States showed that the association between obesity and prevalent DM varied with serum GGT levels [18], where BMI, as a measure of obesity, was not associated with DM among individuals with low GGT levels. This indicates that obesity itself may not be a sufficient risk factor for DM, and other factors can also contribute to the development of DM. A more recent analysis of data from the NHANES 1999-2002 cycles showed higher serum GGT levels were positively associated with DM [17]. While GGT may be a marker of

oxidative stress due to other causes, it may also produce oxidative stress in an individual [16]. Increases in GGT may be a response to oxidative stress, but it is especially important to note that oxidative stress not only contributes to DM, but also the pathogenesis of secondary diabetic complications [6].

Uric Acid

Uric acid (UA) is the metabolic end product of purine (from nucleic acids like DNA and RNA) metabolism and is freely eliminated in the urine. UA can induce oxidative stress in smooth muscle cells and adipocytes [19]. High levels of UA have been associated with high risk of cardiovascular diseases (CVD), insulin resistance, and metabolic syndrome, but have been less studied in association with the risk of incident DM [19].

C-Reactive Protein (CRP)

CRP is a marker of systemic inflammation and is an independent risk factor for CVD [20]. Higher CRP levels are present in those with DM compared to those without DM [20], however there has been limited research on the presence of CRP with other oxidative stress markers in patients with DM and pre-DM. One study found that CRP and GGT levels were significantly increased in DM patients, and these levels are even further increased with diabetic complications and poor glycemic control [6], but these are only two of many oxidative stress markers.

Albumin and Creatinine

Albumin and creatinine are important clinical measures of kidney function that are inexpensive to measure and can be used to screen people with chronic conditions,

including DM, hypertension, and kidney disease. Therefore, both urinary albumin and creatinine have been included in the oxidative stress score for this analysis.

Vitamin E

Vitamin E refers to a group of eight different compounds, including tocopherol, where alpha-tocopherol (AT) especially has been shown to have anti-inflammatory effects [21, 22]. AT is the most abundant and biologically active among the tocopherols, and a systematic review of prospective cohort studies reported that the consumption of vitamin E was associated with a 13% reduction in the risk of DM [22].

Oxidative Stress Score

The use of an oxidative stress score that encompasses multiple biomarkers that are associated with increased inflammation and cardiometabolic outcomes, such as pre-DM and DM, is a strong measure that can more accurately capture an individual's true oxidative stress level.

Waist-to-height ratio and Diabetes

Waist-to-height ratio (WHtR) has been increasingly used in place of body mass index (BMI) as an anthropometric measure of obesity and cardiometabolic risk, as BMI does not take into account fat distribution within the body nor the balance of lean mass and fat that comprise weight [23]. WHtR is also a simple and practical screening tool. An individual with more centrally distributed fat has excessive intra-abdominal fat, and subsequently a greater risk of many diseases, especially cardio-metabolic conditions.

WHtR is an anthropometric measure that more accurately captures CVD risk [24]. Studies have shown that WHtR is strongly associated with DM, more so than BMI

or other anthropometric measures [25], since it accounts for central obesity. Studies have shown that the association of an increased WHtR with undiagnosed DM was stronger than the association with BMI or waist-to-hip ratio [25]. One study used 0.65 as the cutoff to indicate central obesity, where adolescents and young adults with WHtR>0.65 were at increased risk of mortality associated with cardiometabolic risk factors [26].

A meta-analysis that included data on more than 300,000 individuals from diverse populations around the world has shown that WHtR is a superior measure compared to BMI or waist circumference. Using area under the curve (AUC) analyses for different anthropometric measures found that WHtR provided 75% and 71% discrimination for DM status for women and men, respectively, compared to 70% and 66% for BMI [27]. Ashwell and Hsieh (2005) propose several strong arguments as to why WHtR is a valid global indicator for increased health risk: it is more sensitive than BMI, cheaper and easier to measure and calculate. Furthermore, a boundary of 0.5 indicates increased risk in both men and women and people of different ethnicities, and it may be used in both children and adults [28].

Cardiorespiratory Fitness

Cardiorespiratory fitness (CRF) is defined as the body's ability to take up, transport, and utilize oxygen. Studies have shown that CRF is inversely associated with impaired glucose tolerance or DM [29], and that among individuals with diabetes, lower levels of physical activity and higher BMI correlated with lower levels of CRF [30]. It is well known that physical activity can be protective against cardiometabolic conditions

such as DM, and increasing physical activity can improve an individual's CRF [31]. Previous NHANES analyses have shown that US workers with metabolic syndrome had lower CRF compared to those without metabolic syndrome [32], but there have been no studies assessing the associations with dysglycemia and any potential interactions with oxidative stress.

Physical activity (PA) is defined as any bodily movement produced by skeletal muscles that results in energy expenditure [33]. Regular PA is beneficial for DM prevention because single bouts of exercise release free oxygen radicals as a byproduct and stimulate an antioxidant response by cells to counteract oxidative stress. Repeated bouts of PA allow the body to adjust to regular levels of OS so that it can efficiently eliminate or reduce the effects of chronically high levels of OS in an individual [4]. Therefore, individuals who regularly exercise have a better total antioxidant capacity (TAC) [4, 34] and net lower OS. PA and fitness are associated, where individuals with high PA likely also have high fitness levels. While the two are associated, PA relates more to the movements that people perform, and fitness refers to a measurable component of an individual's ability to utilize oxygen [33]. Physical activity is a complex behavior that can be difficult to assess based on self-report, therefore using CRF is an objective and highly reliable measure that minimizes misclassification bias [35].

While several studies have reported single associations between fitness or obesity with glycemic status, the relative contributions of each of those factors remains controversial. One study assessed the joint association of fitness and obesity measures with the risk of DM and found a significant dose-response relationship of fitness and a

positive association of obesity measures with the risk of pre-DM and DM [36]. Another study showed that while obesity and low fitness are both strong risk factors for DM, there is a strong independent association between fitness and DM, and obesity alone does not fully account for the association [37]. Cardiorespiratory fitness (CRF) has been shown to modify the relationship between adiposity and mortality in individuals with pre-DM [38]. Several studies have shown that the risk of developing DM is lower among those with higher fitness levels among different subpopulations [39, 40]. A longitudinal study of men with DM showed that patients with low fitness levels and who were physically inactive had higher mortality rates compared to men who were active and fit [41].

The gold standard method of estimating CRF involves using submaximal treadmill testing. This test is performed in some NHANES surveys cycles. More details about the testing procedures can be found in the NHANES Procedure Manual [42]. Participants were excluded from performing the fitness test if they had specific medical conditions that would risk their safety or if they were greater than 12 weeks pregnant. Medical conditions that excluded participants from performing the treadmill testing included previous myocardial infarction, coronary heart disease, stroke, emphysema, and certain physical function or development problems. Participants taking medications such as calcium channel blockers, anti-arrythmics, beta-blockers, nitrates, nitroglycerin, and digitalis were also excluded from the CV fitness component. A formula is used to determine the predicted VO2 max for a given participant, based on age, BMI, and sex.

Problem Statement

In general, the studies that have examined oxidative stress markers and diabetes risk have been small or are currently outdated. While each of the main exposures described (oxidative stress, obesity, CRF) have been shown to be associated with dysglycemia (DM and pre-DM) individually, there have been few studies examining joint associations, where the exposures are permitted to interact with each other. Furthermore, few studies have examined the combined exposure to multiple oxidative stress markers (in a score) and its relationship with DM and pre-DM. This analysis will describe the associations between an oxidative stress score and waist-to-height ratio with diabetes and prediabetes. In a sub-analysis, we will include CRF as a third exposure.

Study Hypotheses

1. Higher levels of oxidative stress, as indicated by a higher oxidative stress score, will be positively associated with higher odds of diabetes and pre-diabetes.
2. An elevated WHtR will be positively associated with diabetes and pre-diabetes.
3. Low CRF is positively associated with DM and pre-DM.
4. There is a joint association between high oxidative stress, high WHtR, and low CRF and greater odds of diabetes and pre-diabetes.

Specific Aims

1. Investigate the joint associations of oxidative stress and central obesity (WHtR) with diabetes and pre-diabetes
2. Conduct a sub-analysis with the combined associations between oxidative stress, central obesity, and CRF with diabetes and pre-diabetes

CHAPTER II: METHODS

Study Design

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional survey designed to assess the health and nutritional status of resident civilian non-institutionalized adults and children in the United States. The survey is conducted in two-year cycles by the National Center for Health Statistics (NCHS), which is part of the Centers for Disease Control and Prevention (CDC).

NHANES uses a complex, multistage, probability sampling design to recruit a nationally representative sample of the U.S. population. A majority of the survey data for each cycle is publicly available. For particular subgroups of public health interest, oversampling was used for more precise estimates. The oversampled subgroups for 1999-2004 include non-Hispanic black persons, Mexican-American persons, low-income white persons, persons aged 70 and older, and adolescents aged 12-19. Overall response rates from each of the three cycles of participants examined were 76%, 80%, and 76%, respectively [43].

Potential sample participants were informed of the survey process and their rights as a participant and then had the opportunity to consent or decline, as participation was voluntary. Participants who agreed to participate signed an informed consent form for each part of the two phases, if they were involved in the home interview and/or the health examination phase.

NHANES surveys collect information on participant demographics, dietary habits, health and behaviors, medical conditions, biomarkers, and body size using

questionnaires, examinations, and specimen collection. Variables for this analysis were selected based on their relevance to the main study question and all variables were verified that they were measured in the same fashion from 1999-2004.

Study Population

Data from three cycles of NHANES from 1999-2004 were used for this analysis (1999-2000, 2001-2002, 2003-2004) in order to ensure adequate sample size and power. The analyses were restricted to participants 20 years of age and older. Restricting the dataset to individuals older than 20 years old minimizes the number of potential type 1 DM patients in our dataset.

Outcome Measures

The outcomes of interest in this study are diabetes (DM) and pre-diabetes (pre-DM). A participant was considered to have DM if they met at least one of the following criteria: participant reported physician-diagnosed diabetes, use of insulin, use of medications to lower blood sugar, or had a measured glycohemoglobin (HbA1c) ≥ 6.5% or fasting plasma glucose ≥126 mg/dL. Pre-DM was defined by having one of the following criteria: participant reported having physician-diagnosed "borderline diabetes," or measured Glycohemoglobin of 5.7-6.4% or a fasting plasma glucose of 100-125 mg/dL. Cutoff criteria for determining DM and pre-DM statuses were obtained from the American Diabetes Association. Both groups of participants with DM and pre-DM were compared to a referent group that was considered to be normoglycemic, meaning that they reported no doctor-diagnosed diabetes or borderline diabetes, Glycohemoglobin < 5.7%, and fasting plasma glucose < 100 mg/dL (Table 1).

Table 1. Criteria used to define diabetes, pre-diabetes, and normoglycemia.

	Self-reported condition*	HbA1c (%) §	Fasting Plasma Glucose (mg/dL)¶
Diabetes	Diabetes, use of insulin, or use of glucose-lowering medications	≥6.5	≥126
Pre-Diabetes	Borderline diabetes	5.7-6.4	100-125
Normal	Neither	<5.7	<100

* Self-reported conditions were based on the questions "Have you ever been told by a doctor or health professional that you have diabetes?" and "Are you taking insulin now?" and "Are you taking diabetic pills to lower your blood sugar?"
§ HbA1c: the glycohemoglobin measure was available for all participants over the age of 12. HbA1c is a standard diabetes test that reflects plasma glucose levels over the past 120 days.
¶ fasting plasma glucose was measured for the morning examination session only for all eligible participants over the age of 12 and who fasted for at least eight hours

Main Exposures

Waist-to-Height Ratio

WHtR was calculated for each participant in the survey as a measure of adiposity in both men and women. Trained study personnel measured waist circumference to the nearest millimeter. Standing height was measured using a stadiometer to the nearest centimeter. WHtR was calculated by dividing the measured waist circumference by the standing height. Based on the distribution, WHtR as our main exposure in this analysis was dichotomized, where WHtR≥0.65 indicated central obesity and WHtR<0.65 indicated no central obesity (Figure 1).

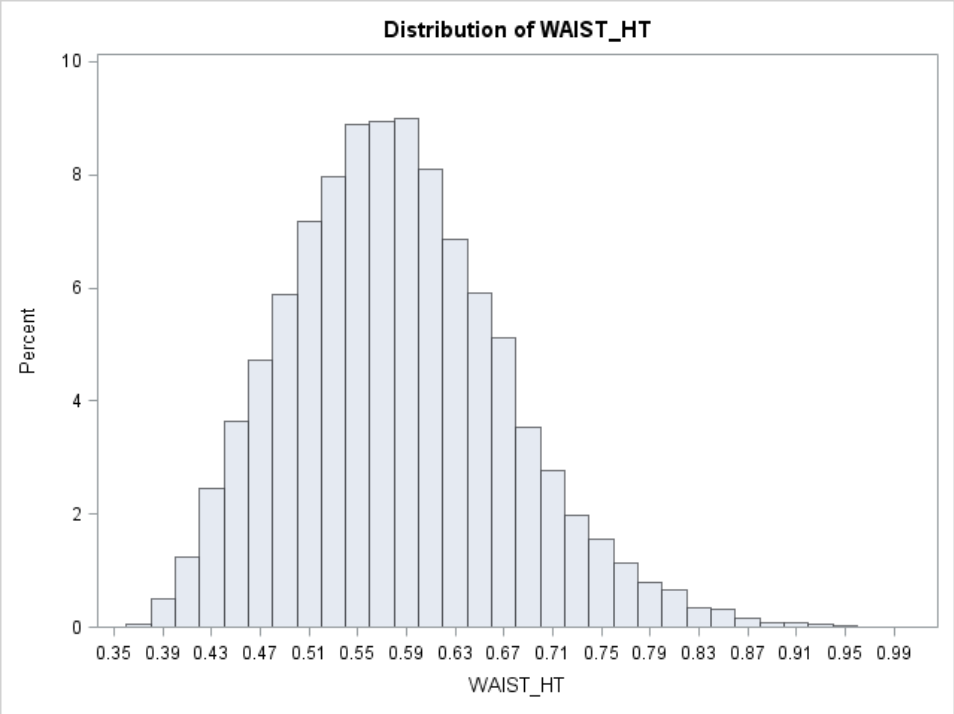

Figure 1. **Distribution of waist-to-height ratio in NHANES 1999-2004 population (n=12,662).**
Waist-to-height ratio was calculated for each study participant based on the measured waist circumference and standing height at the time of the survey. Dividing the waist circumference by the height produced the waist-to-height ratio.

Oxidative Stress Score

An oxidative stress score (OSS) was calculated for each participant in the analysis by creating a Z-score for each individual component of OSS and adding up the scores to obtain a sum of individual scores. Using a Z-score standardizes all of the laboratory values, and allows us to compare scores from different distributions. Mean values and standard deviations were calculated for each of the seven proposed primary oxidative stress markers (GGT, bilirubin, uric acid, CRP, albumin, creatinine, and vitamin E). Each of the markers was assessed for normality, and those that were heavily skewed were log-transformed in order to generate a more normal distribution. Log-transformed means and standard deviations were used in the OSS calculation. OSS was

categorized based on the quartiles of the distribution: <-2.13, -2.13 to -0.005, -0.005 to 2.10, ≥ 2.10 (Figure 2).

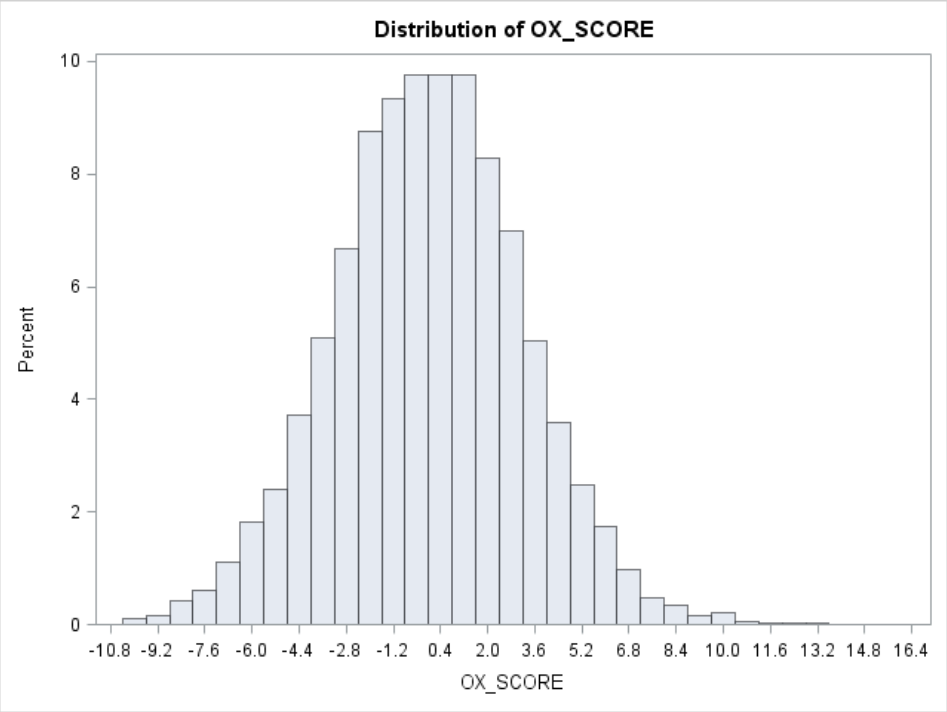

Figure 2. Distribution of oxidative stress score in NHANES 1999-2004 population (n=12,662).
The oxidative stress score was calculated for each individual based on the individual contributions of seven separate oxidative stress markers: GGT, bilirubin, uric acid, CRP, albumin, creatinine, and vitamin E.

Cardiorespiratory Fitness

In each of the three survey cycles, individuals aged 12-49 years old were eligible to participate in treadmill testing and between 2,561 to 2,809 individuals had an estimated VO2max recorded from this assessment. After restricting the analysis to only individuals older than 20 years of age, this decreased the sample size for individuals with data for cardiorespiratory fitness. For our analysis, this meant that out of 12,662 observations in the study sample, 3,141 individuals had valid data with regards to estimated VO2 max. Estimated VO2max was dichotomized into low fitness (bottom 20th

percentile) and normal fitness (above the 20th percentile) (Figure 3). Previous studies have showed that individuals who were in the lowest 20th percentile of cardiorespiratory fitness were at the highest risk [44-46], and therefore this approach was used for this analysis.

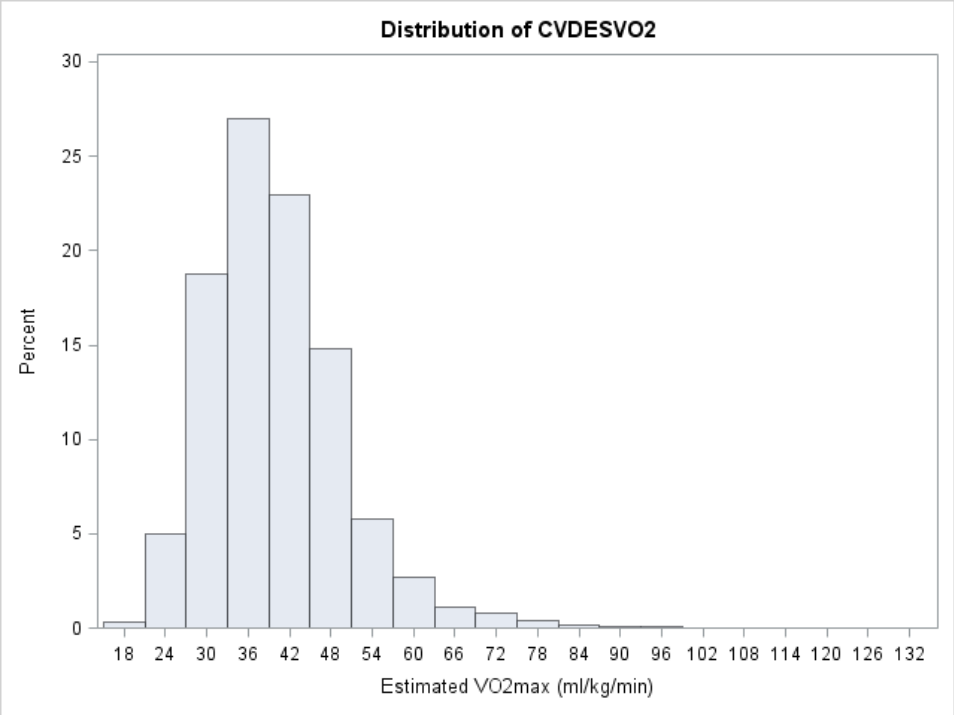

Figure 3. Distribution of estimated VO2max in participants with valid CRF data in NHANES 1999-2004 (n=3,141).
CRF was measured in eligible survey participants aged 12-49 years old. A submaximal exercise test was conducted using eight different treadmill test protocols depending on an individual's gender, age, body mass index, and self-reported level of physical activity. Estimated maximal oxygen uptake (VO2max) was derived for each participant who completed the test.

Covariates

We considered variables that were associated with oxidative stress, WHtR, and prediabetes/diabetes as potential covariates (Figure 7). The covariates included in this analysis were: age, gender, race/ethnicity, education level, marital status, poverty income ratio, smoking status, binge alcohol consumption, physical activity, family history of diabetes, and co-morbidities (coronary heart disease, hypertension,

congestive heart failure, and history of myocardial infarction). All covariates were based on self-report.

The sociodemographic variables collected were age (20-39, 40-59, 60+), gender (male or female), race/ethnicity (Mexican-American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), education level (high school graduate/GED or less, some college, or college graduate and above), marital status (married or not married; "not married" was collapsed from widowed, divorced, separated, never married, living with partner), and poverty income ratio (based on tertiles of the distribution, where lower PIR indicated higher poverty).

Modifiable risk factors that were assessed include smoking status, alcohol consumption, and physical activity. Smoking status was defined as never, ever, or current. This was based off of two survey questions: "Have you smoked at least 100 cigarettes in your entire life?" and "Do you now smoke cigarettes?" If a participant answered "no" to both questions, they were categorized as a never smoker. An ever smoker was categorized as answering "yes" to the first question and "no" to the second question. A participant who answered "yes" to both questions was considered a current smoker. A binge drinker was considered an individual who drank five or more alcoholic beverages almost every day. Physical activity was derived from household/yard activity, transportation physical activity, and moderate and vigorous leisure-time physical activity. Total minutes and METs per week were calculated and weighted appropriately to determine whether or not participants were adhering to the physical activity guidelines (150 minutes of moderate-intensity physical activity per week) [47]. If an

individual achieved less than 150 minutes per week, guideline adherence was not met. If an individual achieved between 150-300 minutes per week, guideline adherence was met. If weekly physical activity exceeded 300 minutes, an individual exceeded the guideline requirements.

Family history of diabetes was dichotomized based on whether an individual reported having blood relatives with a history of diabetes. All co-morbidities (coronary heart disease, hypertension, congestive heart failure, and history of myocardial infarction) were based on yes/no self-report questions directly from the Medical Conditions Questionnaire.

Analytic Sample

The NHANES 1999-2004 population consisted of 31,126 observations. After restricting the study population to those 20 years of age or older, 15,332 participants remained. Participants with unknown diabetes status were additionally excluded and those who were interviewed only, and not interviewed and examined were also excluded. Of the remaining 14,212 observations, 1,550 were excluded for missing WHtR, GGT, bilirubin, or uric acid. The final analytic dataset consisted of 12,662 observations and participants were categorized as having DM, pre-DM, or neither. 1,611 (12.72%) participants were classified as having DM, 2,891 (22.83%) were classified as having pre-DM, and 8,160 (64.44%) had neither DM nor pre-DM.

The calculation of the OSS involved all observations with complete data for GGT, bilirubin, uric acid, CRP, albumin, creatinine and vitamin E. The OSS used 12,487 of the 12,662 observations in the calculation. All oxidative stress markers are assumed to

contribute equally to an individual's oxidative stress score. A higher OSS indicates higher levels of oxidative stress based on the proposed markers. Below are the variables that were included in calculation of this score:

$$\textit{Oxidative Stress Score} = \log(\text{CRP}) + \log(\text{GGT}) + \log(\text{BILIRUBIN}) + \text{URIC ACID} + \log(\text{VITAMIN E}) + \log(\text{ALBUMIN}) + \log(\text{CREATININE})$$

Statistical Analysis

All data were analyzed using SAS 9.4 (SAS Institute, Inc., Cary, NC). After downloading the relevant survey components for each of the three cycles, data files were merged and cleaned into a final analytic dataset. Sample weights were calculated for each survey respondent to produce nationally representative data. Survey weights take into account non-response, over-sampling, post-stratification, and sampling error. All continuous variables were tested for normality using PROC UNIVARIATE procedures.

Descriptive statistics were generated using SAS SURVEYMEANS and SURVEYFREQ procedures for continuous and categorical predictors, respectively. The data were stratified by glycemic status to compare the distribution of covariates among those with DM, pre-DM, and neither DM nor pre-DM. Rao-Scott chi-squared (X^2) tests were performed to compare categorical variables and t-tests were used to compare continuous variables.

Univariate and bivariate associations were used to obtain unadjusted odds ratios, showing the associations between each individual covariate and the outcome of the study. Polytomous regression models were fit using SAS SURVEYLOGISTIC procedures to obtain adjusted odds ratios, as there are three levels of the outcome –

DM, pre-DM, or normoglycemia. Ordinal logistic regression was not used since the data did not meet the proportional odds assumption. All potential confounders were included in the model, and forward selection procedures were used to determine the final model. Variables were added sequentially, while ensuring significance with each addition.

Six models were fit for the data. Each model contains both of the main exposures: WHtR and OSS. The initial unadjusted model included the main exposures, WHtR and OSS. Model 1 adjusted for demographics (age, sex, race/ethnicity, education, marital status, and poverty income ratio). Marital status was not significant, but was kept in the model as an important covariate based on *a priori* information. Model 2 additionally adjusted for family history of diabetes. Model 3 adjusted for modifiable risk factors: physical activity, smoking, and history of binge drinking. Smoking and binge drinking were not significant in this model, and were therefore dropped from the model. Model 4 additionally adjusted for co-morbidities: CHD, HTN, CHF, and MI. Only CHD and HTN were significant and remained in the model. Model 5 was the gold standard model that included all of the potential covariates, and was fit separately from Models 1-4. All covariates, both significant and non-significant, were included in this model. Partial Pearson correlation matrices adjusted for age were constructed, and the low correlation coefficients indicated that multicollinearity did not pose a threat on the analysis. In each candidate model, interaction between the exposure and all of the covariates was assessed. A two-sided p-value of 0.05 was used to determine statistical significance.

A sub-analysis was conducted with a restricted sample of study participants who had valid CRF data. CRF was not used in the primary analysis, given the large extent of missing data. A similar set of six polytomous logistic regression models were fit using estimated VO2max as a third main exposure in each model. VO2max was categorized into normal fitness (above the 20^{th} percentile) and low fitness (lowest 20^{th} percentile). Models were selected using identical forward selection procedures, assessing for the same covariates in each of the six models as in the primary analysis.

CHAPTER III: RESULTS

There were a total of 31,126 eligible individuals in NHANES 1999-2004, 12,662 of whom were eligible for this analysis. **Table 1** shows the criteria that were used to define each of the three levels of the outcome – DM, pre-DM, and normoglycemia. **Table 2** compares participant characteristics by glycemic status (DM, pre-DM, no DM). There were statistically significant differences across the three groups. Of the 12,662 individuals, 1,611 had DM (12.7%), 2,891 had pre-DM (22.8%), and 8,160 had neither DM nor pre-DM (64.4%). Individuals with DM were slightly older than those with pre-DM (58.3 vs. 53.1 years old, respectively) and most of the study population was male (52.5% of those with DM and 55.1% of those with pre-DM). Only 10.8% and 16.0% of those with DM and pre-DM, respectively, had a college degree or higher, more than half of both populations were married, and as a whole, individuals with DM experienced greater poverty than those with pre-DM, based on poverty income ratio (PIR) (37.5% and 30.6%, respectively).

The distributions of our three primary exposures are shown in **Table 3.** Means and standard deviations of each of the individual oxidative stress markers are reported along with their reference ranges, and the overall oxidative stress score for each outcome group is reported as well. Individuals with DM had the highest OSS (mean OSS=1.01), compared to those with pre-DM (mean OSS=0.45) and normoglycemic individuals (mean OSS=-0.72) **(Table 3).** Individuals with DM had higher mean WHtRs compared to those with pre-DM and normoglycemia (0.65, 0.60, and 0.55, respectively). Those with DM had the lowest mean CRF levels (est. VO2max=35.27 ml/min/kg)

compared to individuals with pre-DM (est. VO2max=39.66) and normoglycemia (est. VO2max=40.15) (**Table 3**).

Table 4 shows the partial correlation between all exposure variables in both the main analysis and the sub-analysis adjusted for age. OSS was significantly correlated with central obesity (WHtR) (correlation=0.42, p<0.0001) and with CRF (correlation=-0.04, p=0.04). Each of the individual components of OSS was also assessed for correlation with both WHtR and CRF. All OSS components were significantly correlated with WHtR except for albumin (correlation=0.03, p=0.07) and vitamin E (correlation=0.19, p=0.19). Similarly, all OSS components were significantly correlated with CRF with the exception of albumin (correlation=0.01, p=0.47) and vitamin E (correlation=0.01, p=0.60). Central obesity (WHtR) and CRF were also significantly correlated (correlation=-0.19, p<0.0001).

All bivariate associations between the main exposures (WHtR, OSS, individual oxidative stress markers, CRF) and glycemic status are shown in **Table 5**, and bivariate associations between covariates and glycemic status are shown in **Table 6**. Compared to individuals with WHtR<0.65, those with central obesity had 2.74 and 5.79 times higher odds of having pre-DM (95% CI: 2.39, 3.14) and DM (95% CI: 4.96, 6.75), respectively. OSS, as a continuous predictor, was positively associated with diabetes and pre-diabetes (OR=1.19 and 1.13, respectively). When categorizing OSS into quartiles, all higher quartiles were significantly associated with DM and pre-DM, with a dose-response relationship between higher quartile of oxidative stress and diabetes, where the lowest quartile was the referent group. Individually, lower levels of CRF were

associated with lower odds of DM in the bivariate analyses (OR=0.93, 95% CI: 0.90, 0.97), and showed a null association with pre-DM (OR=1.00, 95% CI: 0.98, 1.01) (**Table 5**).

The adjusted odds ratios from the polytomous logistic regression models for the primary analysis are shown in **Tables 7a and 7b**, for individuals with pre-DM and DM respectively. Model 0 is the unadjusted model that only contains the main exposures, WHtR and OSS, to show the joint associations of those two exposures without controlling for any other covariates. Compared to those with WHtR<0.65, those with central obesity had a 2.30 and 4.91 times higher odds of pre-DM (95% CI: 2.01, 2.63) and DM (95% CI: 4.23, 5.71), respectively. Compared to the lowest OSS quartile (reference group), the third and fourth quartiles of OSS were significantly associated with DM. Compared to the lowest OSS quartile, all higher quartiles were significantly associated with pre-DM.

Model 5 was the fully adjusted model that included all potential covariates. When including all potential covariates, those with central obesity had 1.79 and 3.42 times higher odds of pre-DM (95% CI: 1.52, 2.12) and DM (95% CI: 2.73, 4.29), respectively, compared to those with WHtR<0.65,. In the fully adjusted models for both pre-DM and DM, only the top two quartiles of OSS showed a significant association with glycemic status outcomes. **Table 8** provides a summary of the covariates that were included in each of the six models.

Results of the sub-analysis are shown in **Tables 9a and 9b** for individuals with pre-DM and DM, respectively. In an unadjusted model (Model 0) that only included the

main exposures (WHtR, OSS, and CRF), compared to those with WHtR<0.65, those with central obesity had a 2.08 and 4.10 times higher odds of pre-DM (95% CI: 1.81, 2.38) and DM (95% CI: 3.52, 4.77), respectively. The ORs comparing the associations between OSS and pre-DM and DM showed a dose-response relationship, where higher quartiles of OSS were associated with higher odds of glycemic status outcomes, when the lowest OSS quartile was used as the referent group. Compared to those with normal CRF levels (greater than the 20th percentile), those with low CRF had a 2.17 and 7.71 times higher odds of pre-DM (95% CI: 1.90, 2.47) and DM (95% CI: 5.63, 10.57), respectively. Low CRF was significantly associated with both DM and pre-DM. **Table 10** provides a summary of the covariates that were included in each of the six models as part of the sub-analysis.

Figure 6 shows the joint associations of central obesity, oxidative stress, and CRF – the three main exposures considered in both the primary and secondary analyses. The reference group was the group that had low central obesity (low WHtR), low oxidative stress (low OSS), and normal CRF (above the 20th percentile). The highest risk group (high central obesity, high oxidative stress, low fitness) exhibited the highest odds of both DM (OR=7.57; 95% CI: 6.24, 9.19) and pre-DM (OR=2.84; 95% CI: 2.51, 3.22). Significant differences were observed between these risk factors in those with DM and pre-DM. Individuals with two out of the three risk factors (**Figure 6** – Combinations 2-4) had lower odds of disease, but the associations were still significant. Those with one of the three risk factors (**Figure 6** – Combinations 5-7) had lower odds, with all associations still remaining significant.

CHAPTER IV: DISCUSSION

The findings from this study suggest that central obesity, oxidative stress, and CRF are all important factors that contribute to an individual's susceptibility to DM or pre-DM. This association was most pronounced in individuals with the highest quartiles of cumulative oxidative stress, as indicated by the oxidative stress score. Individually, those with central obesity had nearly six times the odds of DM and nearly three times the odds of pre-DM, compared to those without central obesity. These associations were attenuated when adjusting for each other and other covariates, but there was still a strongly significant association for both diabetes and pre-diabetes.

Central obesity and fitness are both strong predictors of diabetes, but substantial evidence has suggested that fitness can significantly ameliorate the adverse effects of body fatness on diabetes [48]. Our analysis showed similar results, in that low CRF was significantly associated with DM and pre-DM in the US population. Our analyses might even contribute to discussions regarding the "obesity paradox", where being obese or overweight are associated with lower risk of mortality, especially if metabolically healthy [49]. Conversely, individuals who fall within normal weight ranges may have higher mortality rates due to metabolic abnormalities such as DM. In one longitudinal study, participants who were of normal weight at the time of incident DM experienced higher mortality compared to those who were overweight or obese [50]. Since we cannot determine temporality in NHANES, we cannot conclude whether this paradox was observed in our study population. One study showed that obese individuals who

increase their cardiorespiratory fitness would be of great benefit even if they remain overweight [51].

In the primary analysis, the associations between OSS and both DM and pre-DM were strongest in the highest quartile of OSS (Tables 7a and 7b). Obesity alone is not a sufficient risk factor for DM. A cross-sectional study that assessed the use of multiple obesity indices (including WHtR and BMI) found that WHtR was the best measure of obesity and high WHtR was significantly associated with higher DM (OR=2.05, 95% CI: 1.37, 3.06), which was similar to the associations observed in our study [52]. Additionally, several studies have confirmed that the presence of inflammation predicts the development of DM [53-55]. This study allowed us to assess the joint effects of several known risk factors. This highlights the importance of gaining a better understanding of the multifactorial mechanisms that are involved with DM.

Among the sub-analysis of individuals with CRF data, the associations between central obesity and glycemic status remained significantly strong, where a higher WHtR was significantly associated with both DM and pre-DM, and low CRF showed strong associations with DM and pre-DM (Tables 9a and 9b). Individuals with a high WHtR (indicating high central obesity), a high OSS (indicating elevated levels of oxidative stress), and low CRF had the highest odds of both DM and pre-DM. Those with a low WHtR, low OSS, and normal CRF had the lowest odds of both DM and pre-DM compared to normoglycemic individuals, which was expected in our analysis. Individuals with any two of the risk factors also had higher odds of disease, but this association was more pronounced in those with DM. A combination of central obesity and low CRF were the

two strongest risk factors associated with 2.18 times the odds of pre-DM and 5.80 times the odds of DM. When assessing the odds of disease in individuals with one of the three risk factors, low CRF showed the strongest association with higher odds of both pre-DM and DM (Figure 6).

One of the major strengths of this analysis is that it is nationally representative, given the complex, multi-stage, probability cluster design. The oversampling design is used to sample larger numbers of subpopulations of particular public health interest, and this increases the reliability and precision of our estimates. Nationally representative populations in NHANES allow us to make generalizable results to the US population. Using six years of survey data provides stronger power than only using one cycle of survey data.

Secondly, the primary exposures and outcomes in our study are unlikely to be biased by self-report. WHtR was based on standardized measurements of waist circumference and standing height at the time of the survey, OSS was calculated based on biomarkers analyzed in the blood samples taken at the time of the survey, and CRF was measured using standardized treadmill procedures to estimate VO2max. DM was defined based on meeting any one of the following criteria: self-report of diabetes, use of insulin or oral medication, HbA1c, or fasting plasma glucose. NHANES uses standardized and automated data collection procedures, and laboratory estimates are considered highly reliable given this standardized process. It is highly likely that we correctly classified individuals with DM and pre-DM, given the multiple criteria that were used.

Lastly, this analysis provided an innovative approach at quantifying oxidative stress. All of the individual oxidative stress markers that were included in this analysis were individually known to contribute to oxidative stress and were also associated with DM, so the use of a standardized z-score allowed us to create a score to compare oxidative stress levels among individuals with DM and pre-DM, compared to normoglycemic individuals as the referent group.

There are several limitations to be addressed with this study. Firstly, given that NHANES is a cross-sectional survey, temporality cannot be determined to draw causal inferences between exposures and outcomes. However, despite this limitation, the associations seen in this study are highly representative of the US population and contribute to the better understanding of oxidative stress and its associations with hyperglycemia. Secondly, a majority of the covariates included in this analysis were based on self-report. Therefore, there is the possibility for bias on the accuracy of the reported information. This type of response bias is likely to over-report variables such as physical activity, and likely to under-report variables such as socially undesirable behaviors, including smoking and alcohol consumption. Individuals who are overweight or obese are more likely to overestimate their amount of total exercise [56]. However, this bias is not likely to differ amongst the different outcome groups (DM, pre-DM, no DM). Diet is a component that contributes to both oxidative stress and diabetes, however it was not adjusted for in this analysis. Future studies would benefit from including diet as a covariate. However, given this limitation, this analysis still showed strong significant associations between oxidative stress and diabetes, given the strength

of our other covariates that were adjusted for in this study. Given the cross-sectional design, the values for the biomarkers may not necessarily represent an individual's baseline characteristics, if the individual is ill, for example. However, given our large sample size, this is not likely to be an important issue.

Using NHANES data does not allow us to definitively differentiate between type I and type II DM, however restricting our analysis to individuals over the age of 20 is likely to minimize the number of type I diabetics that would have been included. Lastly, the oxidative stress score calculation was based on individual biomarkers that contribute to oxidative stress. All biomarkers are assumed to contribute equally to an individual's oxidative stress levels. However, combining the individual oxidative stress markers could potentially mask some of the individual associations of the biomarkers with disease state. Therefore, in addition to providing the associations with the oxidative stress score and diabetes status, the individual associations between each of the seven biomarkers was also reported in this analysis. Vitamin C was proposed to be included as an important antioxidant marker to reduce oxidative stress, however vitamin C was only measured in one of the three cycles of survey data in this analysis, and therefore was not included.

Future Directions

This study showed that waist-to-height ratio, oxidative stress, and cardiorespiratory fitness are all strongly associated with both pre-DM and DM. The odds of DM are highest compared to the odds of pre-DM when all risk factors (WHtR ≥ 0.65, high OSS, low CRF) are present. This analysis significantly contributes to the

existing literature to better understand the joint associations of central obesity, oxidative stress, and fitness with pre-DM and DM. Future studies should be conducted longitudinally in order to assess trends in central obesity, oxidative stress, and fitness over time in individuals with pre-DM and DM. Additionally, assessing more recent data would be beneficial to assess whether the associations of central obesity, oxidative stress, and CRF remained among individuals with pre-DM and DM in the US. In conclusion, the results of this analysis magnify the significant associations between central obesity, oxidative stress, and CRF with DM and pre-DM. These analyses warrant further research to standardize the use of WHtR and OSS in individuals with DM and pre-DM.

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TABLES

Table 2. Characteristics of study participants by glycemic status, NHANES 1999-2004 population (n=12,662).

	Mean ± SD or n (%)			
	DM (n=1,611)	**Pre-DM (n=2,891)**	**No DM (n=8,160)**	**P-value**
Age (years)	58.3 ± 0.6	53.1 ± 0.4	42.1 ± 0.3	<0.0001
20-39	107 (6.6)	526 (18.2)	3,951 (48.4)	<0.0001
40-59	470 (29.2)	944 (32.7)	2,392 (29.3)	
60+	1,034 (64.2)	1,421 (49.2)	1,817 (22.3)	
Gender				<0.0001
Male (ref=Female)	846 (52.5)	1,594 (55.1)	3,606 (44.2)	
Race/Ethnicity				<0.0001
Mexican American	466 (28.9)	669 (23.1)	1,777 (21.8)	
Other Hispanic	76 (4.7)	139 (4.8)	364 (4.5)	
Non-Hispanic White	637 (39.5)	1,431 (49.5)	4,336 (53.1)	
Non-Hispanic Black	366 (22.7)	555 (19.2)	1,396 (17.1)	
Other (Including multi-racial)	66 (4.1)	97 (3.4)	287 (3.5)	
Education Level				<0.0001
College graduate or above	174 (10.8)	462 (16.0)	1,727 (21.2)	
Some college	336 (20.9)	669 (23.1)	2,260 (27.7)	
HS grad or less	1,099 (68.2)	1,752 (60.6)	4,164 (51.0)	
Missing	2 (0.1)	8 (0.3)	9 (0.1)	
Marital Status				0.0003
Married	913 (56.7)	1,693 (58.6)	4,378 (53.7)	
Not Married	650 (40.3)	1,121 (38.8)	3,467 (42.5)	
Missing	48 (3.0)	77 (2.7)	315 (3.9)	
Poverty Income Ratio*				<0.0001
Least Poverty (>3.48)	344 (21.4)	839 (29.0)	2,679 (32.8)	
Moderate Poverty (1.50-3.48)	507 (31.5)	928 (32.1)	2,468 (30.2)	
More Poverty (<1.50)	604 (37.5)	886 (30.6)	2,350 (28.8)	
Missing	156 (9.7)	238 (8.2)	663 (8.1)	
Smoking Status				<0.0001
Never	739 (45.9)	1,386 (47.9)	4,362 (53.5)	
Ever	586 (36.4)	919 (31.8)	1,875 (23.0)	
Current	284 (17.6)	582 (20.1)	1,915 (23.5)	
Missing	2 (0.1)	4 (0.1)	8 (<0.1)	
Ever Binge Drinker				<0.0001
Yes	280 (17.4)	464 (16.0)	976 (12.0)	
No	973 (60.4)	1,896 (65.6)	5,637 (69.1)	
Missing	358 (22.2)	531 (18.4)	1,547 (19.0)	
Body Mass Index (kg/m^2)				<0.0001
>30	796 (49.4)	1,144 (39.6)	2,090 (25.6)	
25-29.9	561 (34.8)	1,091 (37.7)	2,926 (35.9)	
18.5-24.9	242 (15.0)	623 (21.5)	2,966 (36.3)	

<18.5	5 (0.3)	24 (0.8)	169 (2.1)	
Missing	7 (0.4)	9 (0.3)	5 (<0.1)	
Physical Activity (min./wk)				<0.0001
<150	401 (24.9)	711 (24.6)	1,898 (23.3)	
150-299	193 (12.0)	376 (13.0)	1,087 (13.3)	
300+	460 (28.6)	1,045 (36.1)	3,437 (42.1)	
Missing	557 (34.6)	759 (26.3)	1,738 (21.3)	
HbA1c (%)	7.44 ± 0.06	5.59 ± 0.01	5.17 ± 0.01	<0.0001
Fasting plasma glucose (mg/dL)	161.82 ± 3.11	104.90 ± 0.24	90.09 ± 0.17	<0.0001
Family History of Diabetes				<0.0001
Yes	1,125 (69.8)	1,381 (47.8)	3,661 (44.9)	
No	448 (27.8)	1,459 (50.5)	4,338 (53.2)	
Missing	38 (2.4)	51 (1.8)	161 (2.0)	
Congestive Heart Failure				<0.0001
Yes	137 (8.5)	94 (3.3)	133 (1.6)	
No	1,456 (90.4)	2,781 (96.2)	8,013 (98.2)	
Missing	18 (1.1)	16 (0.6)	14 (0.2)	
Coronary Heart Disease				<0.0001
Yes	171 (10.6)	155 (5.4)	215 (2.6)	
No	1,416 (87.9)	2,718 (94.0)	7,919 (97.0)	
Missing	24 (1.5)	18 (0.6)	26 (0.3)	
Heart Attack				<0.0001
Yes	179 (11.1)	148 (5.1)	222 (2.7)	
No	1,425 (88.5)	2,738 (94.7)	7,931 (97.2)	
Missing	7 (0.4)	5 (0.2)	7 (<0.1)	
Hypertension				<0.0001
Yes	990 (61.5)	1,138 (39.4)	1,766 (21.6)	
No	613 (38.1)	1,725 (59.7)	6,303 (77.2)	
Missing	8 (0.5)	28 (0.1)	91 (1.1)	

Abbreviations: NHANES, National Health and Nutrition Examination Survey; DM, Diabetes mellitus; Pre-DM, Pre-diabetes mellitus; HbA1c, glycosylated hemoglobin

Data Source: NHANES 1999-2004 (CDC)

* Poverty Income Ratio (PIR): higher PIR indicates less poverty, lower PIR indicates more poverty

Table 3. Main exposures of interest.

	Mean ± SD			
Oxidative Stress Markers	**DM (n=1,611)**	**Pre-DM (n=2,891)**	**No DM (n=8,160)**	**Reference Range**
GGT (U/L)	41.89 ± 2.48	33.22 ± 0.95	27.13 ± 0.44	8-65
Bilirubin (mg/dL)	0.68 ± 0.01	0.70 ± 0.01	0.71 ± 0.01	0.3-1.9
Uric acid (mg/dL)	5.65 ± 0.06	5.82 ± 0.03	5.18 ± 0.02	M: 3.4-7.2 F: 1.9-7.5
CRP (mg/dL)	0.65 ± 0.03	0.52 ± 0.02	0.36 ± 0.01	0-10
Albumin (ug/mL)	145.96 ± 20.89	27.15 ± 2.48	20.38 ± 1.85	Less than 30
Creatinine (mg/dL)	119.38 ± 2.52	132.59 ± 2.83	130.32 ± 1.62	M: 20-25† F: 15-20†
Vitamin E (ug/dL)	1,571.55 ± 27.68	1,458.43 ± 20.12	1,286.19 ± 13.29	50-200 ug/dL
OSS	1.01 ± 0.15	0.45 ± 0.11	-0.72 ± 0.07	
Additional Labs				
C-peptide (nmol/L)	1.22 ± 0.04	0.96 ± 0.01	0.70 ± 0.01	0.26-1.03
Insulin (uU/mL)	24.49 ± 1.53	13.88 ± 0.29	9.50 ± 0.18	
Total Cholesterol (mg/dL)	207.00 ± 1.82	210.56 ± 1.20	199.96 ± 0.67	Less than 200
HDL (mg/dL)	46.83 ± 0.47	49.01 ± 0.42	53.89 ± 0.31	Greater than 60
LDL (mg/dL)	116.69 ± 1.46	127.44 ± 1.16	118.71 ± 0.79	Less than 100
Triglycerides	214.37 ± 11.58	169.63 ± 4.57	128.46 ± 2.78	Less than 150
WHtR	0.65 ± 0.004	0.60 ± 0.002	0.55 ± 0.001	
≥0.65	694 (43.1%)	815 (28.2%)	1,201 (14.7%)	
<0.65	917 (56.9%)	2,076 (71.8%)	6,959 (85.3%)	
CV Fitness*				
Est. VO2 max(ml/min/kg)	35.27 ± 0.82	39.66 ± 0.52	40.15 ± 0.29	

Abbreviations: GGT, gamma-glutamyl transferase; CRP, C-reactive protein; OSS, oxidative stress score; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WHtR, waist-to-height ratio; CV fitness: cardiovascular fitness; est. VO2 max = estimated VO2 max; M, male; F, female
Data Source: NHANES 1999-2004 (CDC)
** CV fitness was included as a main exposure for the sub-analysis due to the limited sample size (n=3,141)*
† Reference range units for creatinine are expressed in mg/kg/day depending on individual's body weight

Table 4. Partial correlation coefficients between exposure variables: OSS, WHtR, estimated VO2max.

	OSS	p-value	**WHtR**	p-value	**Est. VO2max**	p-value
OSS *	1.00	--	0.42	<0.0001	-0.04	0.04
GGT			0.13	<0.0001	0.06	0.0009
Bilirubin			-0.18	<0.0001	0.15	<0.0001
Uric acid			0.23	<0.0001	0.17	<0.0001
CRP			0.26	<0.0001	-0.12	<0.0001
Albumin			0.03	0.07	0.01	0.47
Creatinine			0.04	0.01	0.06	0.001
Vitamin E			0.02	0.19	0.01	0.60
WHtR †			1.00	--	-0.19	<0.0001
Est. VO2 max					1.00	--

Pearson correlation matrices were created to assess the correlations between all main exposures in this analysis. All exposure variables were used as continuous measures. Correlation between individual components of OSS were also assessed with WHtR and est. VO2max.

* OSS: oxidative stress score; † WHtR: waist-to-height ratio; § Est. VO2 max: estimated maximal oxygen uptake (VO2)

Table 5. Summary of bivariate associations between exposures and diabetes status.

	OR (95% CI)	
	DM	**Pre-DM**
WHtR		
<0.65	1.00 (ref.)	1.00 (ref.)
≥0.65	5.79 (4.96, 6.75)	2.74 (2.39, 3.14)
OSS		
<-2.13	1.00 (ref.)	1.00 (ref.)
-2.13 to -0.005	1.38 (1.07, 1.79)	1.38 (1.15, 1.66)
-0.005 to 2.10	1.83 (1.46, 2.30)	1.81 (1.46, 2.25)
≥2.10	3.70 (2.86, 4.78)	2.73 (2.27, 3.28)
Continuous	1.19 (1.16, 1.23)	1.13 (1.10, 1.15)
GGT (U/L)		
<15	1.00 (ref.)	1.00 (ref.)
15-21	1.68 (1.29, 2.20)	1.79 (1.47, 2.17)
21-32	3.31 (2.52, 4.35)	2.90 (2.43, 3.45)
≥32	4.49 (3.46, 5.82)	2.94 (2.38, 3.63)
Continuous	1.01 (1.00, 1.01)	1.01 (1.00, 1.01)
Bilirubin (mg/dL)		
<0.5	1.00 (ref.)	1.00 (ref.)
0.5-0.7	1.34 (0.99, 1.81)	1.09 (0.94, 1.27)
0.7-0.8	1.22 (0.89, 1.67)	1.08 (0.89, 1.30)
≥0.8	0.97 (0.71, 1.31)	1.02 (0.87, 1.21)
Continuous	0.71 (0.54, 0.93)	0.89 (0.78, 1.00)
Uric acid (mg/dL)		
<4.4	1.00 (ref.)	1.00 (ref.)

4.4-5.3	1.41 (1.16, 1.72)	2.24 (1.87, 2.68)
5.4-6.3	1.50 (1.19, 1.91)	3.11 (2.58, 3.74)
≥6.4	2.17 (1.77, 2.66)	3.74 (3.15, 4.46)
Continuous	1.26 (1.20, 1.33)	1.37 (1.32, 1.41)
CRP (mg/dL)		
<0.10	1.00 (ref.)	1.00 (ref.)
0.10-0.23	1.85 (1.43, 2.40)	1.76 (1.51, 2.06)
0.24-0.52	2.95 (2.37, 3.67)	2.23 (1.93, 2.58)
≥0.53	4.23 (3.34, 5.34)	2.73 (2.36, 3.17)
Continuous	1.48 (1.36, 1.60)	1.36 (1.26, 1.46)
Albumin (ug/mL)		
<4.1	1.00 (ref.)	1.00 (ref.)
4.1-7.9	1.21 (0.92, 1.58)	1.27 (1.10, 1.45)
8.0-16.9	1.46 (1.13, 1.87)	1.47 (1.27, 1.71)
≥17.0	4.91 (4.05, 5.95)	1.91 (1.63, 2.26)
Continuous	1.00 (1.00, 1.00)	1.00 (1.00, 1.00
Creatinine		
<68	1.00 (ref.)	1.00 (ref.)
68-118	1.28 (1.07, 1.54)	1.30 (1.13, 1.48)
119-176	0.98 (0.79, 1.21)	1.27 (1.06, 1.52)
≥177	0.73 (0.60, 0.90)	1.20 (1.01, 1.43)
Continuous	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Vitamin E		
<933	1.00 (ref.)	1.00 (ref.)
933-1183	1.54 (1.27, 1.87)	1.34 (1.14, 1.58)
1184-1571	1.93 (1.58, 2.35)	1.66 (1.39, 1.98)
≥1572	2.95 (2.45, 3.54)	2.17 (1.85, 2.54)
Continuous	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Estimated VO2max*		
<33.23	4.66 (2.51, 8.65)	1.44 (1.07, 1.93)
33.23-38.65	2.70 (1.40, 5.18)	1.32 (0.93, 1.89)
38.66-45.19	1.65 (0.75, 3.63)	1.27 (0.91, 1.79)
≥45.20	1.00 (ref.)	1.00 (ref.)
Continuous	0.93 (0.90, 0.97)	1.00 (0.98, 1.01)

Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; GGT, gamma-glutamyl transferase; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WHtR, waist-to-height ratio; estimated VO2max: measure of cardiovascular fitness

* Estimated VO2max was only available for 3,141 individuals and therefore was only included in the secondary sub-analysis

Table 6. Summary of bivariate associations between covariates and diabetes status.

	OR (95% CI)	
Age	1.06 (1.06, 1.07)	1.04 (1.04, 1.05)
Gender		
Female	1.00 (ref.)	1.00 (ref.)
Male	1.30 (1.18, 1.44)	1.43 (1.29, 1.60)
Race/Ethnicity		
Non-Hispanic White	1.00 (ref.)	1.00 (ref.)
Mexican American	1.20 (0.93, 1.55)	1.05 (0.89, 1.23)
Other Hispanic	1.40 (0.96, 2.05)	1.21 (0.98, 1.50)
Non-Hispanic Black	1.73 (1.48, 2.02)	1.19 (1.04, 1.37)
Other	2.03 (1.45, 2.83)	1.18 (0.92, 1.52)
Education		
High school grad or less	1.00 (ref.)	1.00 (ref.)
Some college	0.61 (0.50, 0.74)	0.69 (0.61, 0.78)
College grad or above	0.41 (0.34, 0.51)	0.61 (0.51, 0.72)
Marital Status		
Not married	1.00 (ref.)	1.00 (ref.)
Married	1.08 (0.90, 1.29)	1.27 (1.14, 1.42)
Poverty Income Ratio		
>3.48 (Lowest poverty)	1.00 (ref.)	1.00 (ref.)
1.50-3.48	1.57 (1.29, 1.92)	1.16 (1.00, 1.36)
<1.50 (Highest poverty)	1.93 (1.59, 2.35)	1.23 (1.05, 1.44)
Smoking		
Never	1.00 (ref.)	1.00 (ref.)
Current	0.88 (0.76, 1.02)	0.96 (0.86, 1.07)
Ever	1.75 (1.50, 2.03)	1.52 (1.35, 1.71)
History of Binge Drinking		
No	1.00 (ref.)	1.00 (ref.)
Yes	1.47 (1.26, 1.70)	1.24 (1.09, 1.43)
BMI		
Normal	1.00 (ref.)	1.00 (ref.)
Underweight	0.24 (0.08, 0.72)	0.73 (0.43, 1.23)
Overweight	2.34 (1.84, 2.99)	2.02 (1.75, 2.32)
Obese	5.73 (4.40, 7.46)	3.17 (2.70, 3.71)
Physical Activity		
<150 min./wk	1.00 (ref.)	1.00 (ref.)
150-299 min./wk	0.76 (0.62, 0.92)	0.84 (0.72, 0.98)
>300 min./wk	0.59 (0.50, 0.70)	0.73 (0.63, 0.84)
Family history of diabetes	3.32 (2.75, 4.00)	1.20 (1.07, 1.35)
CHF	6.30 (4.41, 8.99)	2.23 (1.59, 3.11)
CHD	5.62 (4.42, 7.14)	2.24 (1.76, 2.87)
Heart Attack	5.36 (4.14, 6.95)	2.12 (1.73, 2.59)
Hypertension	6.05 (5.18, 7.08)	2.42 (2.17, 2.69)

Abbreviations: BMI, body mass index; CHF, congestive heart failure; CHD, coronary heart disease

Table 7a. Summary of logistic regression output from candidate models for individuals with diabetes.

	OR (95% CI)					
	Model 0	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
	DM	**DM**	**DM**	**DM**	**DM**	**DM**
WHtR						
<0.65	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
≥0.65	4.91 (4.23, 5.71)	4.33 (3.57, 5.26)	4.13 (3.36, 5.06)	4.08 (3.24, 5.15)	3.55 (2.80, 4.50)	3.42 (2.73, 4.29)
OSS						
Q1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2	1.19 (0.92, 1.54)	1.20 (0.91, 1.59)	1.18 (0.88, 1.58)	1.23 (0.89, 1.71)	1.21 (0.87, 1.68)	1.20 (0.81, 1.77)
Q3	1.40 (1.11, 1.76)	1.65 (1.29, 2.11)	1.65 (1.28, 2.12)	1.79 (1.34, 2.40)	1.65 (1.24, 2.21)	1.69 (1.22, 2.34)
Q4	2.31 (1.85, 2.68)	2.52 (1.97, 3.22)	2.32 (1.80, 2.99)	2.28 (1.69, 3.07)	2.07 (1.55, 2.75)	2.15 (1.53, 3.03)
Gender						
Female		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Male		1.77 (1.52, 2.06)	1.95 (1.67, 2.28)	1.88 (1.58, 2.23)	1.94 (1.62, 2.32)	2.11 (1.65, 2.69)
Age						
20-39		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
40-59		7.49 (5.69, 9.86)	7.71 (5.76, 10.34)	7.60 (5.38, 10.73)	6.23 (4.37, 8.87)	5.28 (3.62, 7.72)
60+		24.54 (18.75, 32.12)	29.63 (22.70, 38.68)	31.63 (22.51, 44.44)	20.63 (14.33, 29.70)	17.54 (12.17, 25.27)
Race/Ethnicity						
NH White		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
MA		1.84 (1.47, 2.30)	1.85 (1.48, 2.30)	1.76 (1.39, 2.22)	1.94 (1.52, 2.48)	2.00 (1.50, 2.66)
Other Hispanic		1.78 (1.21, 2.64)	1.77 (1.25, 2.52)	1.70 (1.14, 2.52)	1.90 (1.27, 2.84)	1.83 (1.24, 2.72)
NH Black		1.66 (1.40, 1.97)	1.64 (1.37, 1.97)	1.48 (1.18, 1.85)	1.43 (1.15, 1.77)	1.44 (1.18, 1.76)
Other		3.45 (2.35, 5.05)	3.31 (2.22, 4.95)	2.64 (1.64, 4.23)	2.78 (1.70, 4.53)	2.69 (1.50, 4.83)
Education						
HS grad or less		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Some college		0.81 (0.66, 1.00)	0.77 (0.63, 0.94)	0.79 (0.65, 0.97)	0.80 (0.64, 0.99)	0.87 (0.68, 1.12)
College grad		0.62 (0.47, 0.81)	0.63 (0.48, 0.84)	0.72 (0.52, 0.98)	0.77 (0.57, 1.04)	0.85 (0.59, 1.22)
Marital Status						
Not Married		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)

Married		1.07 (0.87, 1.32)	1.09 (0.89, 1.34)	1.08 (0.83, 1.41)	1.08 (0.82, 1.41)	1.09 (0.81, 1.47)
PIR						
>3.48		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
1.50-3.48		1.23 (0.98, 1.54)	1.17 (0.93, 1.47)	1.14 (0.91, 1.43)	1.18 (0.93, 1.48)	1.17 (0.90, 1.52)
<1.50		1.45 (1.17, 1.79)	1.49 (1.19, 1.86)	1.47 (1.16, 1.86)	1.47 (1.16, 1.86)	1.54 (1.16, 2.04)
Family History						
No			1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes			3.96 (3.23, 4.84)	4.17 (3.39, 5.12)	4.17 (3.36, 5.17)	4.00 (3.20, 4.99)
PA (min/week)						
<150				1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
150-299				0.78 (0.62, 0.97)	0.78 (0.62, 0.98)	0.79 (0.59, 1.06)
≥300				0.74 (0.59, 0.95)	0.76 (0.60, 0.96)	0.86 (0.65, 1.13)
CHD					1.60 (1.04, 2.46)	1.53 (0.89, 2.63)
Hypertension					2.41 (1.91, 3.04)	2.47 (1.89, 3.24)
CHF						1.25 (0.64, 2.47)
MI						0.90 (0.53, 1.52)
Smoking						
Never						1.00 (ref.)
Ever						1.11 (0.87, 1.42)
Current						1.02 (0.80, 1.25)
Binge drinking						
No						1.00 (ref.)
Yes						0.91 (0.69, 1.20)

Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; NH White, non-Hispanic white; MA, Mexican-American; NH Black, non-Hispanic Black; Other race includes multi-racial; HS grad, high school grad; PIR, poverty income ratio; PA, physical activity; CHD, coronary heart disease; CHF, congestive heart failure; MI, myocardial infarction

Table 7b. Summary of logistic regression output from candidate models for individuals with pre-diabetes.

	OR (95% CI)					
	Model 0	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
	Pre-DM	**Pre-DM**	**Pre-DM**	**Pre-DM**	**Pre-DM**	**Pre-DM**
WHtR						
<0.65	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
≥0.65	2.30 (2.01, 2.63)	2.07 (1.78, 2.40)	2.01 (1.71, 2.36)	1.95 (1.66, 2.29)	1.85 (1.56, 2.20)	1.79 (1.52, 2.12)
OSS						
Q1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2	1.30 (1.09, 1.56)	1.30 (1.06, 1.61)	1.29 (1.05, 1.59)	1.27 (1.02, 1.60)	1.26 (1.01, 1.58)	1.24 (0.96, 1.60)
Q3	1.63 (1.31, 2.02)	1.73 (1.33, 2.26)	1.73 (1.31, 2.28)	1.77 (1.33, 2.37)	1.70 (1.28, 2.27)	1.64 (1.19, 2.27)
Q4	2.23 (1.85, 2.68)	2.37 (1.89, 2.98)	2.33 (1.85, 2.94)	2.40 (1.89, 3.05)	2.30 (1.82, 2.91)	2.34 (1.78, 3.07)
Gender						
Female		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Male		1.53 (1.36, 1.74)	1.58 (1.39, 1.80)	1.63 (1.41, 1.88)	1.66 (1.43, 1.92)	1.63 (1.37, 1.93)
Age						
20-39		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
40-59		3.08 (2.65, 3.57)	3.10 (2.68, 3.58)	3.10 (2.61, 3.68)	2.95 (2.48, 3.51)	2.77 (2.31, 3.34)
60+		6.74 (5.75, 7.91)	7.06 (6.05, 8.25)	7.52 (6.18, 9.15)	6.70 (5.43, 8.27)	6.08 (4.82, 7.68)
Race/Ethnicity						
NH White		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
MA		1.29 (1.10, 1.51)	1.30 (1.11, 1.52)	1.11 (0.94, 1.31)	1.13 (0.96, 1.33)	1.12 (0.94, 1.35)
Other Hispanic		1.51 (1.18, 1.92)	1.51 (1.18, 1.92)	1.26 (0.88, 1.80)	1.29 (0.90, 1.85)	1.28 (0.84, 1.95)
NH Black		1.20 (1.02, 1.43)	1.20 (1.00, 1.44)	1.21 (1.00, 1.46)	1.19 (0.98, 1.45)	1.05 (0.84, 1.30)
Other		1.72 (1.27, 2.32)	1.82 (1.35, 2.45)	1.55 (1.03, 2.33)	1.59 (1.05, 2.40)	1.49 (0.86, 2.56)
Education						
HS grad or less		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Some College		0.80 (0.70, 0.93)	0.80 (0.69, 0.93)	0.82 (0.70, 0.97)	0.83 (0.70, 0.98)	0.80 (0.67, 0.96)
College grad/above		0.74 (0.60, 0.92)	0.76 (0.61, 0.95)	0.80 (0.64, 1.00)	0.81 (0.65, 1.01)	0.74 (0.58, 0.96)
Marital Status						
Not Married		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Married		1.15 (0.99, 1.33)	1.16 (0.99, 1.34)	1.12 (0.93, 1.36)	1.11 (0.91, 1.36)	1.05 (0.86, 1.28)

PIR						
>3.48		1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
1.50-3.48		1.01 (0.85, 1.20)	1.02 (0.86, 1.21)	1.06 (0.87, 1.28)	1.08 (0.89, 1.31)	1.10 (0.90, 1.36)
<1.50		1.09 (0.90, 1.32)	1.11 (0.92, 1.35)	1.06 (0.84, 1.34)	1.08 (0.86, 1.36)	1.08 (0.84, 1.38)
Family History of DM						
No			1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes			1.36 (1.20, 1.53)	1.42 (1.24, 1.63)	1.42 (1.24, 1.63)	1.42 (1.24, 1.63)
PA (min/week)						
<150				1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
150-299				0.80 (0.68, 0.93)	0.80 (0.68, 0.94)	0.78 (0.67, 0.92)
300				0.79 (0.66, 0.94)	0.79 (0.67, 0.94)	0.76 (0.63, 1.13)
CHD					0.99 (0.74, 1.33)	1.23 (0.84, 1.81)
Hypertension					1.38 (1.19, 1.60)	1.36 (1.15, 1.60)
CHF						0.70 (0.36, 1.36)
MI						0.82 (0.54, 1.24)
Smoking						
Never						1.00 (ref.)
Ever						1.03 (0.85, 1.26)
Current						0.84 (0.71, 0.99)
Binge Drinking						
No						1.00 (ref.)
Yes						1.00 (0.80, 1.25)

Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; NH White, non-Hispanic white; MA, Mexican-American; NH Black, non-Hispanic Black; Other race includes multi-racial; HS grad, high school grad; PIR, poverty income ratio; PA, physical activity; CHD, coronary heart disease; CHF, congestive heart failure; MI, myocardial infarction

Table 8. Summary of variables included in each multivariate model (Models 0-5).

	MODEL 0	**MODEL 1**	**MODEL 2**	**MODEL 3**	**MODEL 4**	**MODEL 5**
	n=12,486	n=11,062	n=10,848	n=8,345	n=8,259	n=6,884
WHTR	X	X	X	X	X	X
OSS	X	X	X	X	X	X
AGE		X	X	X	X	X
SEX		X	X	X	X	X
RACE/ETHNICITY		X	X	X	X	X
EDUCATION		X	X	X	X	X
MARITAL STATUS		X (n.s.)	X (n.s.)	X (n.s.)	X (n.s.)	X (n.s.)
PIR		X	X	X	X	X
FAMILY HX OF DM			X	X	X	X
PHYSICAL ACTIVITY				X	X	X
SMOKING				n.s.	--	X
BINGE DRINKING				n.s.	--	X
CHD					X	X
HTN					X	X
CHF					n.s.	X
MI					n.s.	X

Note: X denotes variable was included in the specified model; n.s. denotes tested in model, but not significant so therefore was not included in model; X (n.s.) denotes not significant but kept in model; -- indicates not included in model due to previous exclusion from prior model
Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; PIR, poverty income ratio; family hx, family history; CHD, coronary heart disease; HTN, hypertension; CHF, congestive heart failure; MI, myocardial infarction

Table 9a. Sub-analysis of associations between oxidative stress and diabetes among those with cardiovascular fitness data (n=3,141).

	OR (95% CI)					
	Model 0	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
	DM	**DM**	**DM**	**DM**	**DM**	**DM**
WHtR						
<0.65	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
≥0.65	4.10 (3.52, 4.77)	4.13 (3.42, 4.99)	3.93 (3.21, 4.81)	3.85 (3.05, 4.85)	3.40 (2.69, 4.31)	3.25 (2.58, 4.09)
OSS*						
Q1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2	1.23 (0.95, 1.60)	1.21 (0.91, 1.59)	1.19 (0.89, 1.58)	1.23 (0.89, 1.70)	1.21 (0.87, 1.67)	1.20 (0.81, 1.78)
Q3	1.50 (1.19, 1.90)	1.64 (1.29, 2.10)	1.64 (1.28, 2.10)	1.77 (1.32, 2.36)	1.66 (1.24, 2.20)	1.70 (1.23, 2.37)
Q4	2.46 (1.92, 3.15)	2.46 (1.93, 3.14)	2.28 (1.78, 2.93)	2.25 (1.67, 3.04)	2.09 (1.56, 2.79)	2.16 (1.53, 3.04)
CV Fitness§						
Normal (≥32.06)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Low (<32.06)	7.71 (5.63, 10.57)	3.23 (2.25, 4.64)	3.02 (2.08, 4.39)	3.23 (2.09, 5.00)	2.89 (1.86, 4.47)	2.86 (1.73, 4.70)

Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; CV fitness, cardiovascular fitness

* Quartiles of oxidative stress score were determined based on the distribution of the score in this analysis. Q1: <-2.13; Q2: -2.13 to -0.005; Q3: -0.005 to 2.10; Q4: ≥2.10

§ CV fitness was divided into quintiles; the lowest 20th percentile were categorized as having the lowest fitness levels; greater than the 20th percentile was categorized as having normal fitness levels

Table 9b. Sub-analysis of associations between oxidative stress and pre-diabetes among those with cardiovascular fitness data (n=3,141).

	OR (95% CI)					
	Model 0	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
	DM	**DM**	**DM**	**DM**	**DM**	**DM**
WHtR						
<0.65	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
≥0.65	2.08 (1.81, 2.38)	2.03 (1.75, 2.36)	1.97 (1.68, 2.31)	1.91 (1.62, 2.25)	1.83 (1.54, 2.17)	1.75 (1.49, 2.08)
OSS						
Q1	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Q2	1.33 (1.11, 1.59)	1.30 (1.06, 1.60)	1.29 (1.05, 1.59)	1.27 (1.02, 1.60)	1.26 (1.01, 1.58)	1.24 (0.96, 1.60)
Q3	1.69 (1.35, 2.11)	1.73 (1.32, 2.26)	1.73 (1.31, 2.28)	1.77 (1.32, 2.36)	1.71 (1.28, 2.28)	1.65 (1.19, 2.28)
Q4	2.29 (1.90, 2.77)	2.35 (1.87, 2.95)	2.30 (1.83, 2.91)	2.38 (1.87, 3.01)	2.28 (1.80, 2.90)	2.32 (1.77, 3.05)
CV Fitness						
Normal (≥32.06)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Low (<32.06)	2.17 (1.90, 2.47)	1.24 (1.07, 1.45)	1.26 (1.08, 1.48)	1.31 (1.09, 1.58)	1.28 (1.07, 1.54)	1.27 (1.07, 1.51)

Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; CV fitness, cardiovascular fitness

* Quartiles of oxidative stress score were determined based on the distribution of the score in this analysis. Q1: <-2.13; Q2: -2.13 to -0.005; Q3: -0.005 to 2.10; Q4: ≥2.10

§ CV fitness was divided into quintiles; the lowest 20th percentile were categorized as having the lowest fitness levels; greater than the 20th percentile was categorized as having normal fitness levels

Table 10. Summary of variables included in each multivariate model (Models 0-5).

	MODEL 0	MODEL 1	MODEL 2	MODEL 3	MODEL 4	MODEL 5
	n=3,121	n=2,819	n=2,775	N=2,370	n=2,350	n=2,027
WHTR	X	X	X	X	X	X
OSS	X	X	X	X	X	X
CV FITNESS	X	X	X	X	X	X
AGE		X	X	X	X	X
SEX		X	X	X	X	X
RACE/ETHNICITY		X	X	X	X	X
EDUCATION		X	X	X	X	X (n.s.)
MARITAL STATUS		X (n.s.)	X (n.s.)	X (n.s.)	X (n.s.)	X (n.s.)
PIR		X (borderline)	X	X (n.s.)	X (n.s.)	X (borderline)
FAMILY HX OF DM			X	X	X	X
PHYSICAL ACTIVITY				X	X	X
SMOKING				n.s.	--	X (n.s.)
ALCOHOL				n.s.	--	X (n.s.)
CHD					--	--
HTN					X	X
CHF					n.s.	--
MI					n.s.	--

Note: X denotes variable was included in the specified model; n.s. denotes tested in model, but not significant so therefore was not included in model; X (n.s.) denotes not significant but kept in model; -- indicates not included in model due to previous exclusion from prior model
Abbreviations: WHtR, waist-to-height ratio; OSS, oxidative stress score; PIR, poverty income ratio; family hx, family history; CHD, coronary heart disease; HTN, hypertension; CHF, congestive heart failure; MI, myocardial infarction

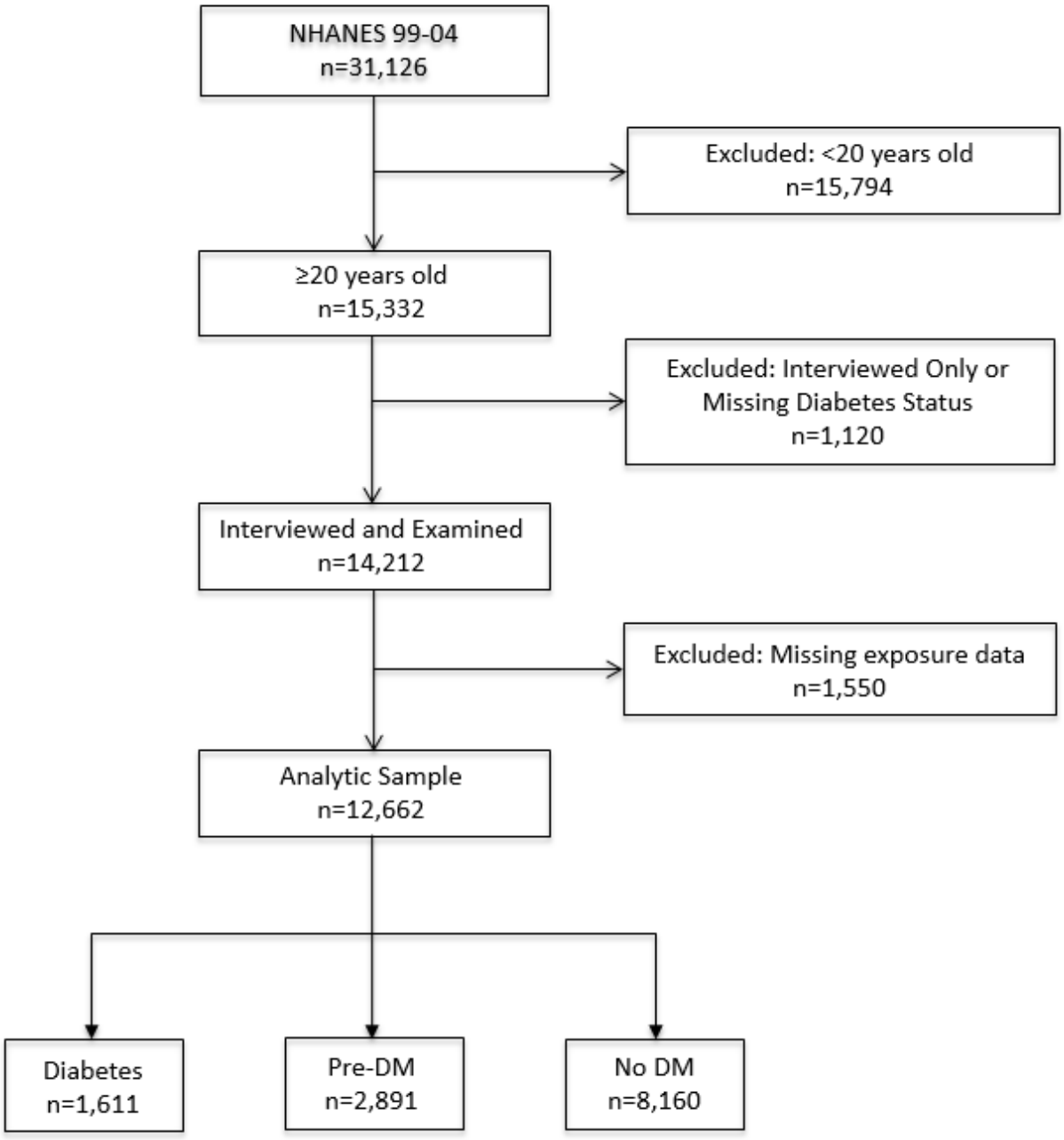


Figure 4. Selection of included survey participants (SP) from NHANES 1999-2004.

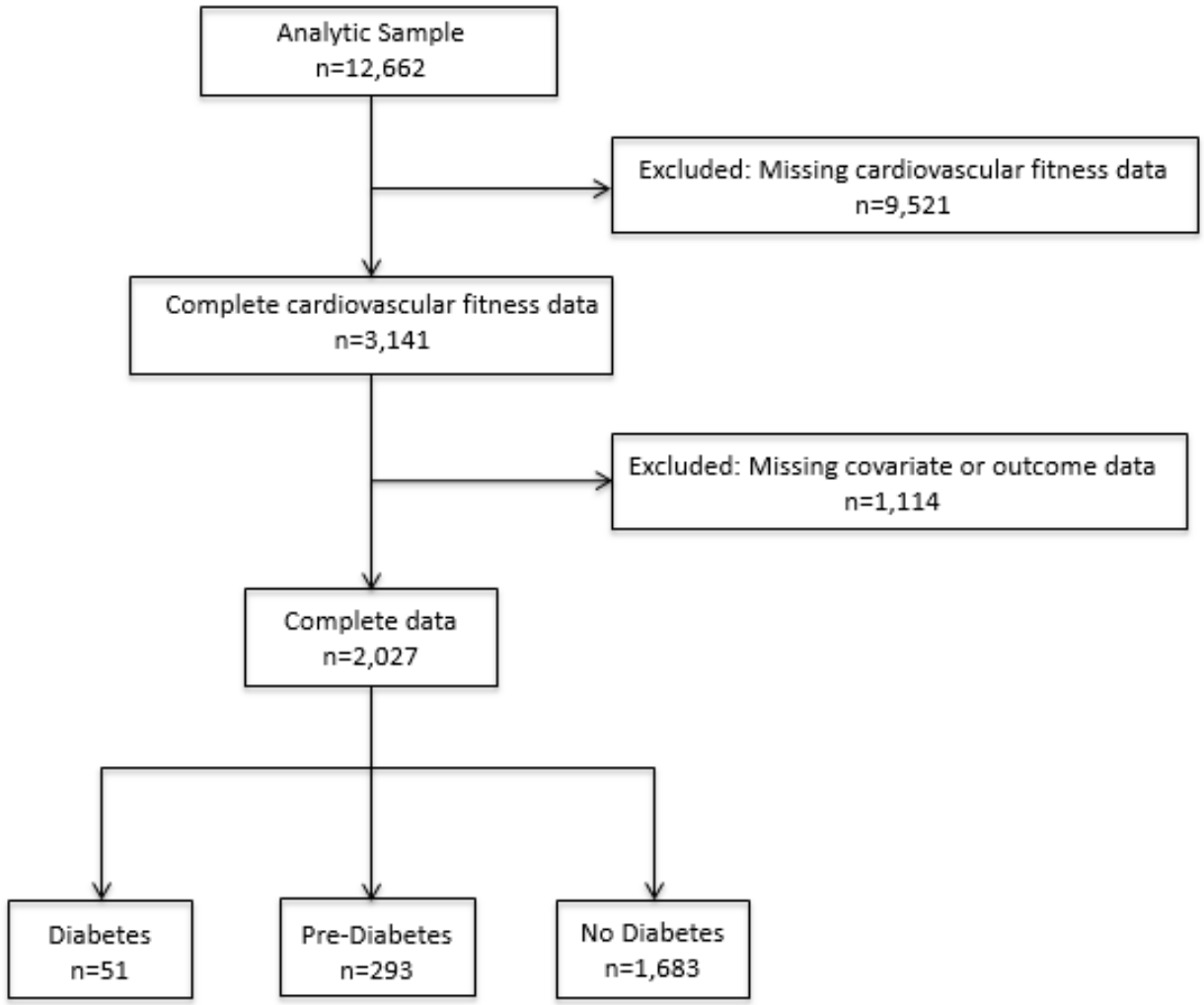
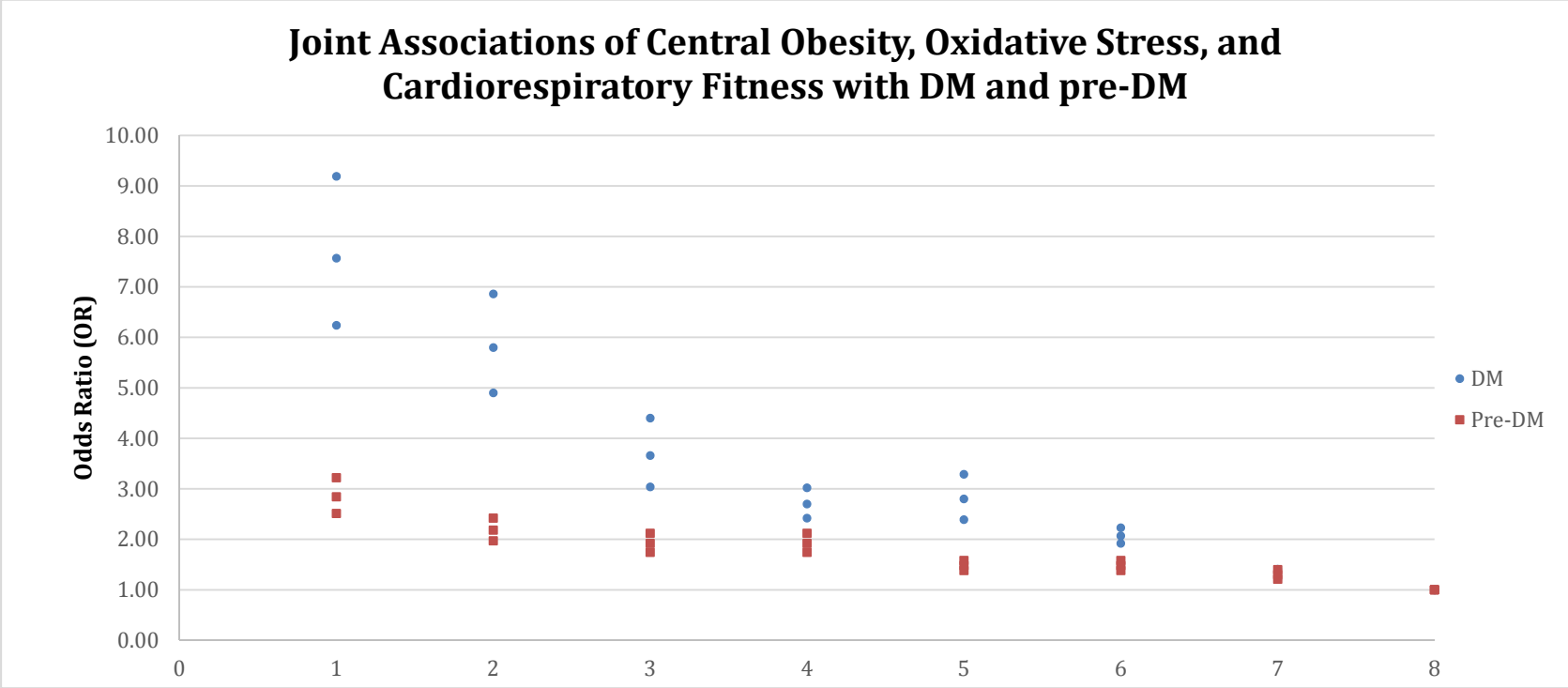


Figure 5. Selection of included survey participants (SP) for sub-analysis with cardiovascular fitness data from NHANES 1999-2004.



	Central Obesity	1	1	0	1	0	1	0	0
	Oxidative Stress	1	0	1	1	0	0	1	0
	Low CRF	1	1	1	0	1	0	0	0
DM	OR (95% CI)	7.57 (6.24, 9.19)	5.80 (4.90, 6.86)	3.66 (3.04, 4.40)	2.70 (2.42, 3.02)	2.80 (2.39, 3.29)	2.07 (1.92, 2.23)	1.31 (1.21, 1.41)	1.00 (ref.)
Pre-DM	OR (95% CI)	2.84 (2.51, 3.22)	2.18 (1.97, 2.42)	1.92 (1.74, 2.12)	1.92 (1.74, 2.12)	1.48 (1.38, 1.58)	1.48 (1.38, 1.58)	1.30 (1.21, 1.40)	1.00 (ref.)

Figure 6. Joint associations of central obesity (WHtR≥0.65), oxidative stress (OSS≥-0.005), and cardiovascular fitness (low fitness if est. VO2max <32.06 mL/min/kg).
Combinations 1-8 refer to 8 different combinations of WHtR, OSS, and CRF (1: WHtR ≥ 0.65, OSS≥-0.005, est. VO2max <32.06 mL/min/kg; 0: WHtR<0.65, OSS<0.005, est. VO2max≥32.06 mL/min/kg)

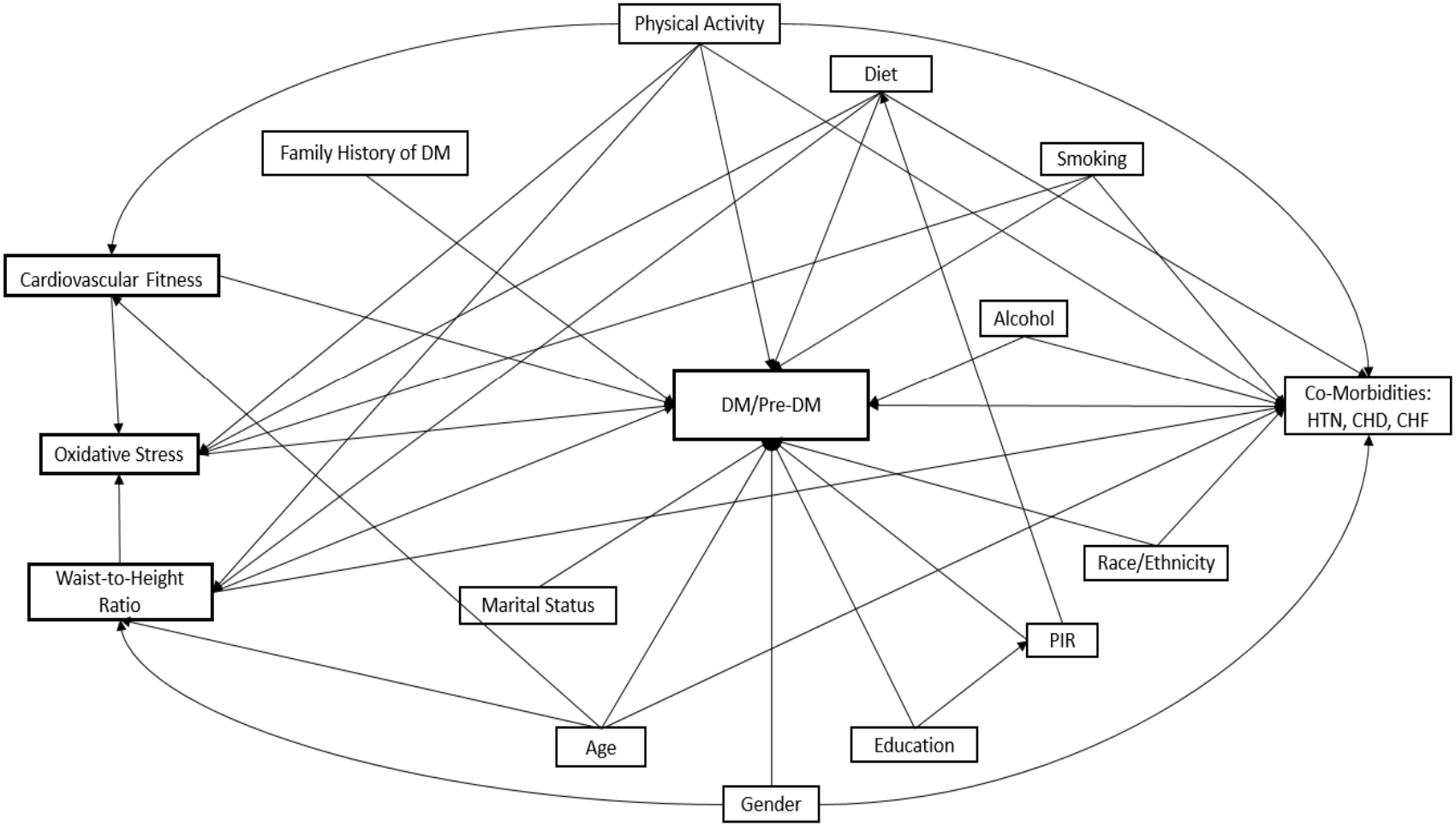


Figure 7. Directed acyclic graph (DAG) showing the associations between predictors and DM/pre-DM in this analysis (NHANES 1999-2004).