UNIVERSITY OF CALIFORNIA SAN DIEGO

Assessing the Mediating Effect of Obesity-Linked SNPs in Nuclear Mitochondrial Genes on Exercise-Enhanced Insulin Sensitivity

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

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2018

The Thesis of Kaitlin E. Bessinger is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

2018

DEDICATION

I dedicate this thesis to my amazing and wonderful parents for all of their support, love, and encouragement throughout my academic career.

EPIGRAPH

The mitochondria is the powerhouse of the cell.

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ABSTRACT OF THE THESIS

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Research shows that mitochondrial function and insulin resistance go hand in hand. As mitochondrial oxidative capacity is decreased, individuals are more likely to become insulin resistant and to develop type 2 diabetes. Exercise is known to increase mitochondrial oxidative capacity, helping these individuals enhance their insulin sensitivity. There are many genetic risk factors that are known to predispose individuals to type 2 diabetes and other metabolic disorders,

some of which may limit the beneficial effects of exercise. I hypothesized that Single Nucleotide Polymorphisms (SNPs) associated with high BMI and type 2 diabetes and located in or near genes that regulate mitochondrial function will mediate the insulin sensitizing effects of exercise.

Introduction

Type 2 diabetes is becoming more prevalent in the United States with more than three million people being diagnosed each year. While the exact cause is unknown, insulin resistance in insulin target tissues (muscle, adipose and liver) is the primary defect leading to type 2 diabetes[1]. Insulin resistance in muscle involves the impaired ability of myocytes to take up glucose in response to the hormone insulin[1]. Since glucose is not readily available in muscles, individuals with type 2 diabetes often experience fatigue. Many other symptoms can result from this increase in glucose concentration, including neuropathy and vision loss. While the side effects of type 2 diabetes are severe, the disease is manageable and if caught early enough, preventable[1].

Many Americans each year are diagnosed with prediabetes, a condition characterized by higher than normal blood glucose levels (100-125mg/dl) or elevated glycated hemoglobin (HbA1c ≥5.7%). These individuals do not tend to display many signs or symptoms. However, diagnosis is important during this time as the disease can progress into type 2 diabetes if interventions are not made[2]. Interventions include lifestyle changes, such as weight loss, a change to a reduced carbohydrate diet, and increasing physical activity. Prediabetes and type 2 diabetes are typically associated with poor lifestyle choices including excess calorie consumption, high BMI, and physical inactivity. Other factors, including age, race, and family history, can also increase a person's likelihood of developing type 2 diabetes[3].

Insulin resistance is a systemic disorder in which tissues, including liver, fat, and muscle, have reduced ability to respond to insulin[3]. While insulin resistance is not a diagnostic tool for diabetes and prediabetes, it is a characteristic of these disorders. Insulin resistance can be gauged by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index. HOMA

-IR factors the relationship between fasting glucose and insulin levels to determine beta cell function and insulin sensitivity[4]. While there is no set HOMA-IR value that defines insulin resistance, a HOMA-IR of approximately 2.7 units or higher is where insulin resistance is thought to occur. At this cut-point, skeletal muscle and liver cells in an individual are significantly limited in their ability to respond to insulin, resulting in a decrease of glucose regulation and increased blood glucose levels following meal consumption. Muscle cells then shift from using glycolysis as a primary energy source to fatty acid oxidation. This switch results in a build-up of fatty acid oxidation by-products in cells, resulting in mitochondrial damage. Lipolysis in adipocytes is also increased, resulting in hyperlipidemia, increasing risk of heart attack and stroke[5]. Insulin resistant individuals can regain sensitivity by losing weight and increasing physical activity levels[3]. The molecular mechanisms surrounding these pathways are well-known.

In healthy individuals, insulin binds to the insulin receptor (IR) and induces autophosphorylation of the IR and tyrosine phosphorylation of the insulin receptor substrate (IRS). This phosphorylation allows for the downstream activation of PI3K, AKT, and their corresponding signaling pathways. Activation of these pathways also results in GLUT4 transporters being translocated to the plasma membrane allowing for uptake of glucose into the cell[6]. Insulin resistant individuals display serine phosphorylation of IRS, limiting tyrosine phosphorylation, and therefore activation of downstream pathways. There are many causes of this serine phosphorylation, including, but not limited to, increased reactive oxygen species (ROS), intracellular stress, inflammation, and circulation of free fatty acids (FFA). Obese individuals have increased FFA levels (known as lipotoxicity) and tumor necrosis factor (TNF-alpha). TNF alpha is a proinflammatory cytokine found in white adipose tissue. TNF-alpha

activates JNK, a serine kinase that results in serine phosphorylation of IRS, preventing tyrosine phosphorylation, downstream signaling, and translocation of GLUT4 receptors to the cell membrane. As a result, insulin resistant individuals display decreased glucose uptake in skeletal muscle[7].

Exercise is an important health-promoting behavior for insulin resistant individuals, as is activates GLUT4 translocation to the plasma membrane and increased glucose uptake despite defects in the IR pathway. During exercise AMP Kinase (AMPK) is activated when the AMP:ATP ratio is elevated. AMPK phosphorylates AS160, a Rab-GTPase, that allows GLUT4 to be translocated to the cellular membrane, increasing glucose uptake. In insulin resistant individuals, this allows for an alternative mechanism for glucose uptake[8]. AMPK also works to improve insulin sensitivity by activating the protein SIRT3, which activates SOD2. SOD2 eliminates superoxide free radicals, reducing the insulin-desensitizing effects of ROS[9]. AMPK is involved in many other cellular pathways, including the activation of PGC-1α, a transcriptional co-activator involved in mitochondrial biogenesis[8].

Mitochondria serve an important role in cellular function, generating ATP as an energy source for cellular activities. This occurs through the Krebs cycle, beta oxidation, and the electron transport chain. Beta oxidation is the process of converting long chain fatty acids (acyl Co-A) to acetyl-CoA, generating co-enzymes that can enter the electron transport chain. The electron transport chain then converts these co-enzymes to ATP. During exercise, the AMP:ATP ratio increases as ATP is converted to AMP during muscle contraction. In order to accommodate increasing energy demands during exercise, mitochondria grow and divide, allowing for increased ATP production[8]. Exercise increases the activity of AMPK, increasing the activity of PGC1alpha. PGC1alpha binds to transcription factor NRF-1, resulting in the transcription of

mitochondrial transcriptional factor TFAM. TFAM enters the mitochondria increasing transcription of mitochondrial genes, allowing for increased biogenesis and oxidative capacity. This increase allows for increased rates of fatty acid oxidation without increase ROS levels[10].

In many insulin resistant individuals, exercise allows for increased glucose uptake while increasing mitochondrial capacity and reducing ROS. Exercise often allows insulin resistant individuals to improve their insulin sensitivity, reducing their risk of diabetes. However, there are some individuals that don't appear to benefit from exercise resulting in impaired sensitivity. One speculation is that these individuals suffer from exercise-resistant mitochondrial dysfunction. These individuals most likely display decreased mitochondrial oxidative capacity and biogenesis that is not improved by exercise. As a result, they display the same lipid and ROS accumulation as sedentary insulin resistant individuals and are not able to increase their mitochondrial capacity from exercise alone. This increase in ROS results in an increase of serine kinase activity, preventing phosphorylation of IRS at the appropriate residues. This allows the cycle of insulin resistance to continue[11].

There are several possible causes for this mitochondrial dysfunction including age, environment, and genetics. Studies have shown that mutations found in genes that either regulate or are directly involved with mitochondrial function result in impaired mitochondrial oxidative capacity and biogenesis[12]. Individuals with these mutations may be more likely to develop metabolic disorders such as type 2 diabetes, meaning they are more likely to be insulin resistant. It is possible that they will have a more difficult time minimizing the effects of these disorders via lifestyle changes than individuals without the mutations[12]. I propose that physically active individuals with mutations in nuclear-encoded mitochondrial genes are more likely to be insulin resistant than physically active counterparts that do not have these mutations.

Single Nucleotide Polymorphisms (SNPs) are single base pair changes that occur throughout the genome. SNPs occur frequently, about every 300 nucleotides, and often serve as biological markers for certain diseases. Researchers have been trying to determine associations between SNPs and clinical outcomes via Genome Wide Association Studies (GWAS). As a result, numerous SNPs in regulatory and coding regions of genes have been identified and correlated with particular phenotypes. For this study, SNPs associated with high Body Mass Index (BMI) and Waist to Hip Ratio (WHR) were selected from various GWAS reports. These criteria were used because insulin resistance is known to be associated with high BMI and WHR. I then selected from these a subset of SNPs located in or near nuclear mitochondrial genes. SNPs in the mitochondrial genome were not considered as the mitochondrial genome tends to be highly conserved, making the frequency of these SNPs lower. I propose that these nuclear mitochondrial SNPs mediate the effect of physical activity on insulin resistance in physically active insulin resistant individuals (**Figure 1**).

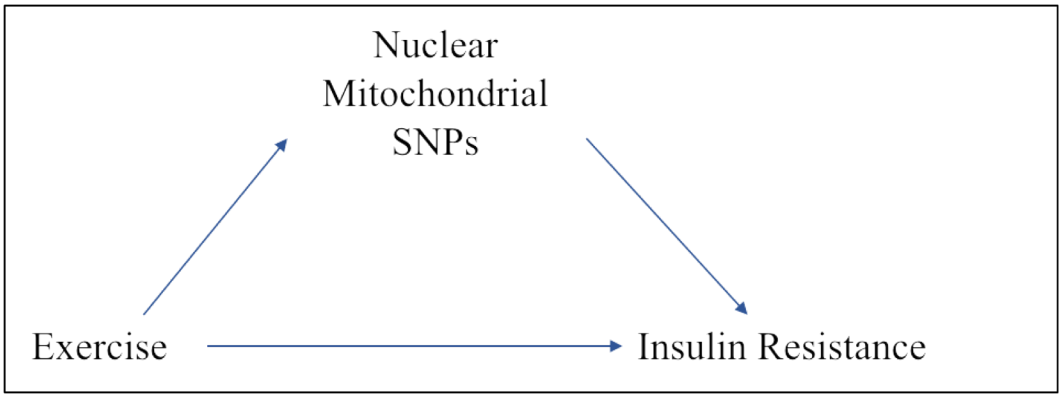

Figure 1: Mediating Effect of SNPs between Exercise and Insulin Resistance
Figure showing the hypothesized mediating effect of SNPs in nuclear mitochondrial genes between exercise and insulin resistance.

Methods

Study Population

Participants were selected from the Community of Mine study, grant number R01CA179977. Participants were randomly selected from various neighborhoods throughout San Diego county, with special effort made to obtain participants from various urban areas. Urban areas were broken up by consensus blocks, and no more than 10 participants were selected from each block. Participants were given a medical questionnaire asking about personal and familial history of diabetes, cancer, heart disease, as well as other metabolic disorders. Demographic characteristics were also collected via survey. Activity data was collected from participants via hip accelerometer for a minimum of 14 days. The accelerometer was worn for a minimum of 10 hours per day. Participants were required to attend one clinic visit where blood and urine samples were collected. Blood pressure, height, weight, BMI, and waist and hip circumference were also recorded during this visit. Two 24-hour food recall surveys were collected, one for a weekend and one for a week day. Clinical characteristics for our study population can be seen in **Table 2**.

Sample Collection

Participants were required to fast for a minimum of 12 hours prior to their clinic visit allowing for a fasting glucose measurement. A 30ml draw of whole blood was collected in an EDTA tube. An aliquot of whole blood was reserved and the remainder of the sample was centrifuged and stored as buffy coat, plasma, and serum. All samples were then stored in a -80°C freezer.

Activity

Physical Activity was measured using a GT3X+ ActiGraph. The device is worn on the hip during waking hours. Moderate to Vigorous Physical Activity (MVPA) was determined to be 1,952 activity counts or higher. This is equivalent to either going for a brisk walk, swim, or bike ride. All Physical Activity (PA) was determined to be 700 counts/minute or higher. This would include other physical activities not likely to result in an increase of heart rate or energy expenditure. Examples could include drinking a glass of water, typing, or washing your hands[13]. Participants will be sorted into two categories, physically active and physically inactive. Physically active individuals will be characterized as those meeting the requirement of 150 minutes of MVPA per week, as per recommendation of The Office of Disease Prevention and Health Promotion[14].

DNA Isolation

DNA was isolated from whole blood or buffy coat samples of participants using a Qiagen DNeasy blood and tissue kit. Following isolation, the concentration of DNA samples was determined using an Invitrogen Qubit dsDNA HS Assay Kit. Samples were then concentrated if needed and stored in a -20°C freezer until sequencing.

Genotyping

Genotyping was done using an Illumina Infinium CoreExome-24 BeadChip. Following genotyping, samples were compared to self-report surveys to ensure that samples matched participants for gender and ethnicity as described below.

Glucose and Insulin Assays

Fasting glucose for participants was measured using a standard glucose oxidase method via a YSI 2900 Bioanalyzer glucose analyzer. Participant plasma samples were thawed and 100ul were plated for each measurement. Insulin was measured using a Meso Scale Discovery Multi-Array Human Insulin Kit (catalog no. K15164C) at the NIH-Funded UC San Diego Clinical and Translational Research Institute Biomarker Laboratory. A standard curve generated using calibrator allows for measurement. Both assays were run in 96 well plates. Each 96 well plate contained multiple replicates of a standardized sample allowing us to ensure uniformity in the assay quality between plates.

HOMA-IR Calculation

Insulin Resistance was determined via HOMA-IR. Insulin resistance for this study was defined as a HOMA-IR of 2.7 or higher.

$$HOMA - IR = \frac{\frac{mg}{dl}\, glucose * \; insulin \frac{mU}{L}}{405}$$

SNP Selection

SNPs were selected from GWAS reports. All SNPs were associated with either high BMI, waist to hip ratio (WHR), or type 2 diabetes. All SNPs had to be located in nuclear genes related to mitochondrial function and must have confirmed significance in GWAS reports. The index of SNPs used for this study will be referred to as the Mitochondrial and Oxidative Capacity Index (MOCI). The SNPs selected were used in a scoring system to determine an individual's genetic predisposition to becoming insulin resistant. Selected SNPs are shown in

Table 1. The Illumina chip used for this project only contained 7 of these particular SNPs. As a result, only 7 will be included in our current analyses.

Table 1: MOCI SNP Selection
Table includes the 25 selected SNPs that contribute to the MOCI Score. Information for each individual SNP including RS number, chromosome/position, effect and non-effect alleles, gene, and mutation location can be seen below. The 7 SNPs included in this analysis are shown in bold.

Name	*Effect allele*	*Non-Effect allele*	*Chr.*	*Position*	*Gene*	*Location of Mutation*	*Phenotype*
rs4994	C	T	8	37,966,820	ABRB3 / W64R	Coding - Missense	BMI[16]
rs470117	A	G	22	50,571,524	CPT1B	Coding - Missense	BMI[16]
rs9939609	T	A	16	53,786,615	FTO	Intron	PCr build up[17]
rs17094222	C	T	10	100,635,683	HIF1AN	3' UTR	BMI[18]
rs1167827	**A**	**G**	**7**	**75,533,848**	**HIP1**	**3'UTR**	**BMI[18]**
rs758747	T	C	16	3,577,357	NLRC3	Intron	BMI[18]
rs13191362	G	A	6	162,612,318	PARK2	Intron	BMI[18]
rs9400239	T	C	6	108,656,460	FOXO3	Intron - 5'UTR	BMI[18]
rs3849570	T	G	3	81,742,961	GBE1	Intron	BMI[18]
rs17724992	G	A	19	18,344,015	PGPEP1	Intron	BMI[18]
rs4787491	**G**	**A**	**16**	**3,004,016**	**MAP3K**	**3' UTR**	**BMI[18]**
rs17203016	**G**	**A**	**2**	**207,390,794**	**CREB1**	**Unknown**	**BMI[18]**
rs2176040	G	A	2	226,228,086	IRS1	Intergenic	BMI[18]
rs1385167	G	T	2	65,973,514	MEIS1	Intron	WHR[18]
rs9991328	**T**	**C**	**4**	**88,791,970**	**FAM13A**	**Intron**	**WHR[18]**
rs1776897	A	C	6	34,227,234	HMGA1	Intergenic	WHR[18]
rs17819328	G	T	3	12,447,843	PPARG	Intron	WHR[18]
rs2645294	**T**	**C**	**1**	**119,031,964**	**WARS2**	**3' UTR**	**WHR[18]**
rs714515	G	A	1	172,383,850	PIGC	Intron	WHR[18]
rs1800592	G	A	4	140,572,807	UCP1	Promoter	BMI[19]
rs659366	**T**	**C**	**11**	**73,983,709**	**UCP2**	**Promoter**	**BMI[19]**
rs660339	T	C	11	73,978,059	UCP2	Coding - Missense	BMI[19]
rs1800849	T	C	11	74,009,120	UCP3	5' UTR	BMI[19]
rs13107325	T	C	4	102,267,552	SLC39A8	Coding - Missense	BMI[20]
rs11868112	**T**	**C**	**17**	**80,517,889**	**RPTOR**	**Unknown**	**Obesity [21]**

Formulation of MOCI Score

Non-effect alleles are those that are not associated with the listed phenotype, while effect alleles are associated with the listed phenotype. Non-effect alleles receive a score of zero. Effect alleles as well as other alleles that vary from the non-effect allele will receive a score of one. This means that the lowest score a person can have for a particular SNP is zero if they are homozygous for the non-effect allele, and the highest would be two if they are homozygous for the effect allele. The scores for individual SNPs were totaled, meaning that each SNP shares the same weight in the determination on the persons MOCI score. As a result, an individual's MOCI score can range from 0-14.

Quality Control of Genetic Data

Seven quality control checks were performed prior to genetic association analyses. These analyses were conducted at UCSD's Center for Computational Biology. All analyses were conducted using PLINK and following *A tutorial on conducting genome-wide association studies: Quality control and statistical analysis*[15].

Participants and SNPs with missingness levels greater than 0.02 (2%) were excluded. Data was checked for sex discrepancy, reported vs. genotypic sex. Sequencing samples that didn't match were excluded. X-chromosome heterozygosity/homozygosity rates for gender determination were examined. Males with a homozygosity estimate of 0.8 or lower and females with an estimate of 0.2 or greater were excluded. Minor Allele Frequency (MAF) was determined for each SNP and SNPs below the MAF threshold of 0.05 were excluded. Hardy Weinberg Equilibrium (HWE) was determined for our population to control for genotyping error. SNPs that violated HWE were excluded from future analysis. Binary traits with a HWE p-

value less that 1e-10 and quantitative traits with a HWE p-value less that 1e-6 were excluded. Heterozygosity in each individual was determined in order to control for potential sample mixing and inbreeding. Individuals with heterozygosity rates that were ±3 standard deviation from the heterozygosity mean rate were excluded. Relatedness was determined by calculating Identity by Descent (IBD) of sample pairs. A pi_hat threshold of 0.2 was set and participants above the threshold were removed. This allowed all second-degree relatives to be removed from the study accounting for heritability of particular SNPs. Population Stratification was conducted using the 10 suggested MDS covariates. All individuals who were identified as outliers in our population were removed. Individual MDS covariant scores were generated for each participant, one for each parameter (C1-C10). These scores will be included in future regressions to further control for heritability.

Linear Regression

Two linear regressions were conducted using the statistical software SPSS to examine the effect of each parameter on HOMA-IR. These parameters included age, BMI, reported type 2 diabetes (either self-reported or reported taking diabetes medications), sex, and the ten MDS covariates. Age, BMI, reported diabetes, sex, and MDS covariates (C1-C10) served as predictors in both regression analyses. The first linear regression was conducted using HOMA-IR as the dependent variable and PA700 as the independent variable. The second linear regression will be conducted using HOMA-IR as the dependent variable and MVPA as the independent variable. These regressions will determine the significance of these parameters on HOMA-IR alone. Significance was defined as p-value ≤ 0.05.

Mediation Analysis

Six mediation analyses were conducted to determine if MOCI mediates the effect of physical activity on HOMA-IR. Mediation analyses were conducted using SPSS statistical software. Age, BMI, sex, reported diabetes, and the 10 MDS covariates were used as predictors. Two mediation analyses were conducted using the entire cohort of 221 individuals. The first analysis was to determine if MOCI mediates the effect of PA700 on HOMA-IR. The second was to determine if MOCI mediates the effect of MVPA on HOMA-IR. Our next two analyses used only the physically inactive subset of participants. We conducted two mediation analyses on this population to determine whether MOCI mediates the effect of PA700/MVPA on HOMA-IR. Our final two analyses used only the physically active subset of participants to determine if MOCI mediates the effect of PA700/MVPA on HOMA-IR. Mediation analyses were considered null if confidence intervals included zero. Statistical significance for each model was defined as $p \leq 0.05$.

Results

Genotyping results were obtained for 417 individuals. The clinical characteristics for our cohort can be seen in **Table 2**. Following data quality control, our population dropped from 417 to 221 individuals. The bulk of individuals dropped were removed in the population stratification step. To account for heritability of particular SNPs and prevent false positives, individuals that displayed high levels of genetic diversity were removed. An example of our MDS population stratification graph can be seen in **Figure 2**. Participants that fell outside of the set parameters were removed from further analysis. 10 MDS covariates were also generated for each participant from the population stratification step and were included in our regression and mediation analyses. Clinical characteristics for our updated population can be seen in **Table 3**.

Table 2: Clinical Characteristics for Original Study Cohort
Table showing average clinical characteristics for our study population. Sample size and standard deviation are also displayed.

CLINICAL CHARACTERISTICS

	n=	Mean	STD
AGE (YRS)	417	48.8	10.9
BMI (kg/m^2)	417	28.4	6
WHR	417	0.92	0.094
HOMA-IR	416	3.06	3.18
REPORTED DIABETES	50	-	-
SEX			
FEMALE	231	-	-
MALE	186	-	-
RACE			
ASIAN/ASIAN PACIFIC ISLANDER	12	-	-
BLACK/AFRICAN AMERICAN	9	-	-
MIXED/UNKNOWN	87	-	-
NATIVE AMERICAN	8	-	-
WHITE	301	-	-
MVPA (MIN/DAY)	417	29.3	23.7

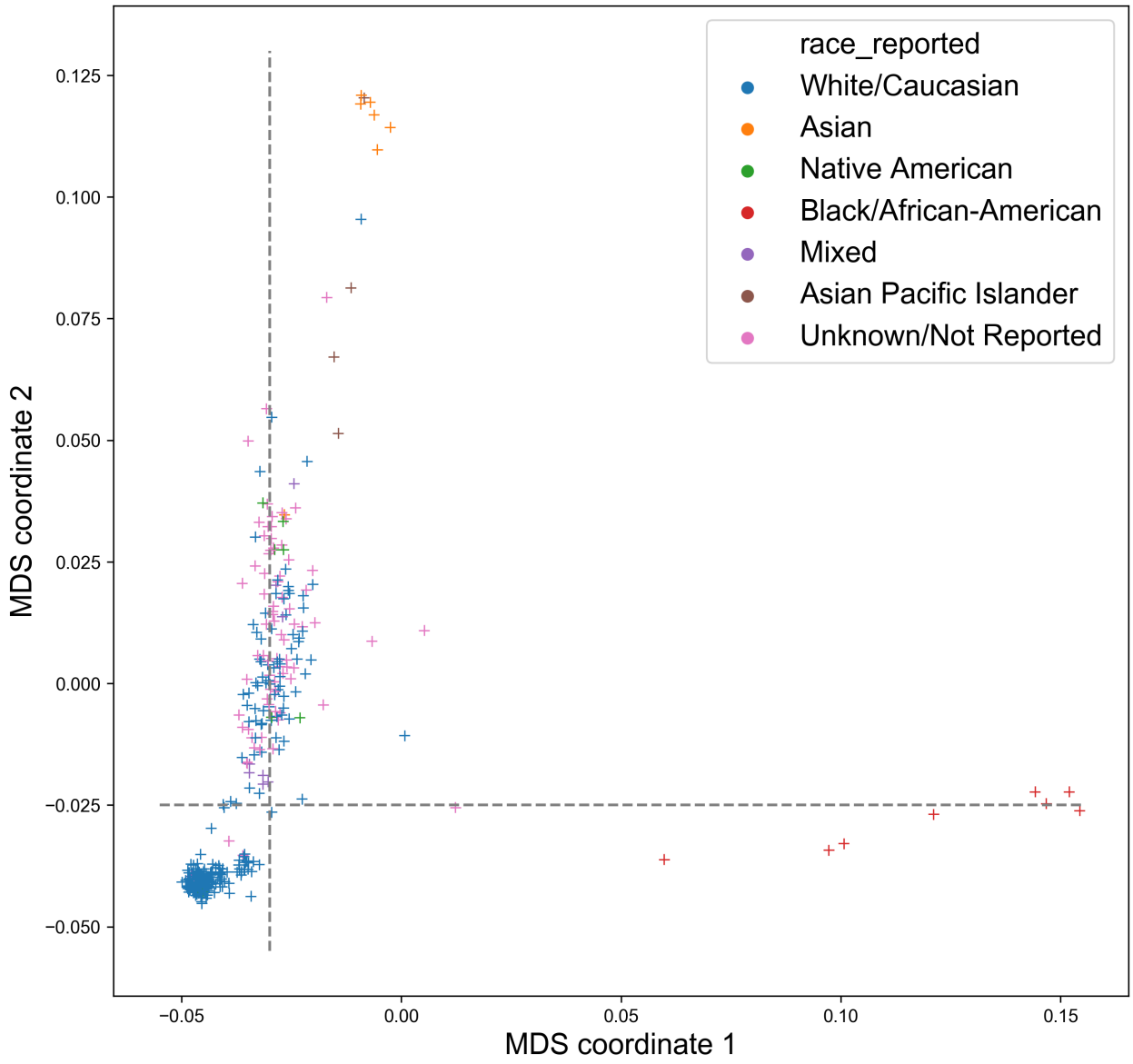

Figure 2: Sample MDS Population Stratification Plot
Shows step 1 of our population stratification. Axis consist of MDS coordinates comprised of known alleles associated with ethnic origins. Dashed lines represent cut-offs generated in this particular step. Legend shows self-reported race/ethnicity for participants.

Table 3: Clinical Characteristics for Updated Study Cohort
Table showing average clinical characteristics for individuals that were included in our regression analyses. Sample size and standard deviation for each parameter are displayed below.

CLINICAL CHARACTERISTICS FOR UPDATED STUDY COHORT

	n=	Mean	STD
AGE (YRS)	221	61.5	10.6
BMI (kg/m^2)	221	27.4	5.9
WHR	221	0.91	0.10
HOMA-IR	221	2.3	2.1
REPORTED DIABETES	15	-	-
SEX			
FEMALE	116	-	-
MALE	105	-	-
RACE			
ASIAN/ASIAN PACIFIC ISLANDER	0	-	-
BLACK/AFRICAN AMERICAN	0	-	-
MIXED/UNKNOWN	5	-	-
NATIVE AMERICAN	1	-	-
WHITE	215	-	-
MVPA (MIN/DAY)	221	22.5	19.9
PA 700 (MIN/DAY)	221	94.3	45.0

Genotyping was performed, and a MOCI score between 0 and 14 was determined for each participant. The MOCI score distribution for our population can be seen in **Figure 3**.

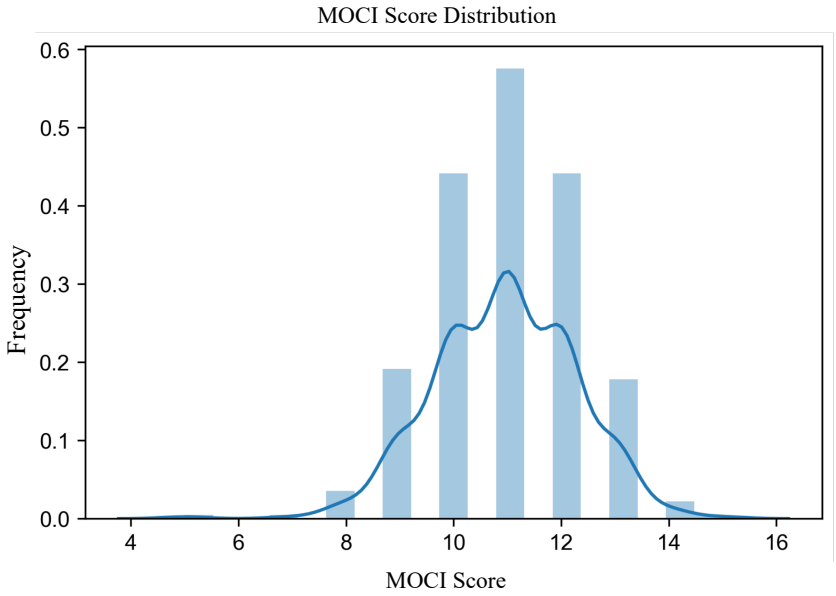

Figure 3: MOCI Score Distribution
The normalized distribution of MOCI score for our sample population.

Model 1

Two linear regression analyses were performed, one for PA700 (model 1A) and one for MVPA (model 1B). All PA700 included minutes of activity for all accelerometer counts above 700 and MVPA included minutes of activity for all accelerometer counts above 1,952. These analyses included our entire sample population of 221 individuals. The summary for the parameters of these regressions can be found in **Table 4**. Sex, age, BMI, and reported diabetes were used as predictors in this model.

Table 4: Model Summary for Models 1A and 1B
Model Summaries showing adjusted R^2 values, sample number (n=), independent and dependents variables for Models 2A and 2B.

Model	Adj. R^2	n=	Independent Variable	Dependent Variable
1A	0.344	221	PA700	HOMA-IR
1B	0.347	221	MVPA	HOMA-IR

From the PA700 model (model 1A) we were able to conclude that BMI, sex (female), and reported diabetes were significantly associated with increased HOMA-IR (**Table 5**). We also saw that PA700 had a negative correlation with HOMA-IR, meaning that as physical activity increased HOMA-IR decreased. However, the R^2 value **(Table 4)** is far from one (0.344) meaning that the predictability of this model is low.

Table 5: Regression Summary for Models 2A and 2B
Regression summary for Models 2A and 2B showing β coefficients, standard error, and p-values for each variable. Asterisks denote significance.

Variables	Unstandardized β	Std. Error	β coefficient	p-value
Model 1A: Linear Regression Using PA700 as Independent Variable				
Sex*	-0.645	0.247	-0.152	0.010
BMI*	0.163	0.031	0.455	0.000
Reported Diabetes*	1.217	0.457	0.157	0.008
PA 700*	-0.001	0.000	-0.136	0.027
Model 1B: Linear Regression Using MVPA as Independent Variable				
Sex*	-0.757	0.251	-0.179	0.003
BMI*	0.158	0.022	0.441	0.000
Reported Diabetes*	1.247	0.455	0.161	0.007
MVPA*	-0.002	0.001	-0.146	0.018

From the MVPA model (model 1B) we were able to conclude that sex (female), BMI, and diabetes were significantly associated with HOMA-IR (**Table 5**). MVPA had an inverse correlation with HOMA-IR. This is similar to what we see in the above model. However, the R^2

value for this summary is significantly less than one (0.347), meaning its accuracy is relatively low. The model summary can be seen in **Table 4**.

Mediation analyses were conducted to determine whether MOCI score mediates the effect of physical activity on insulin resistance. As above, these analyses were conducted using all physical activity (PA700) and then only using MVPA as the dependent variable. The model summaries for the mediation analyses are shown in **Table 6**.

Table 6: Model Summary for All Mediation Analyses
Model Summaries showing adjusted R^2 values, sample number (n), independent and dependents variables, and mediator for all mediation analyses.

Model	R^2	n	Independent Variable	Dependent/Outcome Variable	Mediator
Mediation of MOCI Between PA and HOMA-IR in Entire Study Population					
2A	0.4028	221	PA700	HOMA-IR	MOCI Score
2B	0.4036	221	MVPA	HOMA-IR	MOCI Score
Mediation of MOCI Between PA and HOMA-IR in Physically Inactive Subset					
3A	0.4369	94	PA700	HOMA-IR	MOCI Score
3B	0.4444	94	MVPA	HOMA-IR	MOCI Score
Mediation of MOCI Between PA and HOMA-IR in Physically Active Subset					
4A	0.5025	127	PA700	HOMA-IR	MOCI Score
4B	0.4686	127	MVPA	HOMA-IR	MOCI Score

Model 2

The indirect effect of PA700 on HOMA-IR displayed a lower limit confidence interval (LLCI) of 0.0000 and an upper limit confidence interval (ULCI) of 0.0003. This tells us that the MOCI did not have a mediating effect between PA700 and HOMA-IR. While we did not observe that MOCI score mediated the effect of PA on HOMA-IR, it did have a significant effect on HOMA-IR. From the continuous regression we observed that MOCI was inversely correlated with HOMA-IR. We also saw that PA was inversely correlated with HOMA-IR, while BMI, sex, and reported diabetes positively correlated with HOMA-IR. This data can be found in **Table 7**. While the R^2 is higher than in models 1A and 1B, it is still quite low. This tells us that this model

is not very accurate, but more accurate than our standard linear regressions. The R^2 value for Model 2A can be found in **Table 6**.

Table 7: Regression Summaries for All Mediation Analyses

Regression summary for all mediation analyses showing β coefficients, standard error, and p-values for each variable. Asterisks denote significance.

Independent Variables	β coefficient	Std. Error	p-value
Model 2A – Mediation of MOCI between PA700 and HOMA-IR in All Participants			
PA 700*	-0.0010	0.0004	0.0141
MOCI Score*	-0.2071	0.0948	0.0301
BMI*	0.1636	0.0212	0.0000
Sex*	-0.6220	0.2452	0.0120
Diabetes*	1.2596	0.4534	0.0060
Model 2B – Mediation of MOCI between MVPA and HOMA-IR in All Participants			
MVPA*	-0.0022	0.0009	0.0122
MOCI Score*	-0.1950	0.0943	0.0400
BMI*	0.1588	0.0215	0.0000
Sex*	-0.7418	0.2489	0.0032
Diabetes*	1.2933	0.4524	0.0047
Model 3A – Mediation of MOCI between PA700 and HOMA-IR in Inactive Participants			
PA 700	-0.0003	0.0013	0.8333
MOCI Score*	-0.5571	0.2197	0.0000
BMI*	0.1922	0.0436	0.0000
Sex*	-1.5677	0.5124	0.0030
Diabetes	0.2215	0.8656	0.7987
Model 3B - Mediation of MOCI between MVPA and HOMA-IR in Inactive Participants			
MVPA	-0.0068	0.0065	0.3004
MOCI Score*	-0.5788	0.2177	0.0096
BMI*	0.1855	0.0436	0.0001
Sex*	-1.6771	0.5156	0.0017
Diabetes	0.1717	0.8556	0.8415
Model 4A - Mediation of MOCI between PA700 and HOMA-IR in Active Participants			
PA 700*	-0.0012	0.0004	0.0014
MOCI Score	-0.0624	0.0753	0.4092
BMI*	0.1287	0.0195	0.0000
Sex	-0.1023	0.2113	0.6293
Diabetes*	1.9895	0.4051	0.0000
Model 4B - Mediation of MOCI between MVPA and HOMA-IR in Active Participants			
MVPA	-0.0014	0.0008	0.0828
MOCI Score	-0.0314	0.0770	0.6837
BMI*	0.1260	0.0201	0.0000
Sex	-0.2417	0.2142	0.2617
Diabetes*	2.0637	0.4169	0.0000

The mediation analysis using MVPA (model 2B) showed that MOCI does not mediate the effect between MVPA and HOMA-IR (LLCI of -0.0002 and ULCI of 0.0004). However, like the above model we were able to conclude that the MOCI score inversely correlates with HOMA-IR. This linear regression also showed that MVPA inversely correlates with HOMA-IR. The MOCI score displays a larger β-coefficient than does MVPA, suggesting that MOCI score has a larger effect on HOMA-IR than MVPA. We also observed that BMI, age, and reported diabetes are also significant factors positively associated with HOMA-IR. This data can be found in **Table 7**. The R^2 value is low, meaning that the accuracy of this model isn't very high. The R^2 value for model 2B can be found in **Table 6**.

For the analysis conducted in models 4 and 5, we divided our population into two groups, physically inactive (model 3) and physically active (model 4). Our physically active group reached 150 min or more MVPA per week while our physically inactive group did not. We then re-ran the mediation analysis separately for each group.

Model 3

Our physically inactive group included 94 participants. We performed two mediation analyses for this group - one testing the mediating the effect of MOCI between PA700 (model 3A) and HOMA-IR (model 3B) and one testing the mediating the effect of MOCI between MVPA and HOMA-IR. The summary for these mediation analyses can be found in **Table 6**.

The mediation analysis of MOCI score between PA700 and HOMA-IR showed a LLCI of -0.0004 and a ULCI of 0.0014. This tells us that the MOCI score did not have a significant mediating effect between PA700 and HOMA-IR. However, MOCI score was inversely associated with HOMA-IR. Interestingly, neither PA700 nor reported diabetes were significantly

associated with HOMA-IR in this inactive subpopulation. BMI, reported diabetes, and sex still had significant effects on predicting HOMA-IR. These results can be found in **Table 7**. The R^2 for both models 3A and 3B are higher than those in the previous models. The R^2 values can be found in **Table 6**.

Our second mediation analysis (model 3B) examined whether MOCI score mediated the effect between MVPA and HOMA-IR in the physically inactive subpopulation. The indirect effect of MVPA on HOMA-IR displayed a LLCI of -0.0024 and an ULCI of 0.0075. Thus, MOCI score did not display a mediating affect between MVPA and HOMA-IR. Similar to the analysis done using PA700, MOCI score showed an inverse association with HOMA-IR using a standard continuous regression model. Neither MVPA nor reported diabetes significantly correlated with HOMA-IR in this model. BMI and sex are still significantly associated with HOMA-IR. This data can be found in **Table 7**.

Model 4

Our final set of mediation analyses was done using the physically active subpopulation (met the requirement of 150 min or more of MVPA per week) . This subset included 127 individuals. The same two mediation analyses that were performed on the physically inactive population were performed in this population. Model 4A used PA700 as the independent variable and model 4B used MVPA as the independent variable. A summary for the two analyses can be found in **Table 6**.

We did not observe that MOCI mediated any effect between PA700 and HOMA-IR in this population (LLCI of -0.0001 and an ULCI of 0.0003). Unlike in our physically inactive population, MOCI score was not associated with HOMA-IR using our continuous linear regression with PA700. This suggests that MOCI does not affect HOMA-IR in our physically

active participants. PA700 was inversely correlated with HOMA-IR. BMI and reported diabetes were positively correlated with HOMA-IR. In this regression, sex no longer correlated with HOMA-IR. This data can be found in **Table 7**.

MOCI score also did not mediate the effect between MVPA and HOMA-IR in our physically active population. It also did not have a significant effect in determining HOMA-IR in the regression analysis. MVPA showed a negative correlation with HOMA-IR, where as reported diabetes and BMI showed a positive correlation. This data can be found in **Table 7**. Models 4A and 4B both display higher R^2 values than models 2A and 2B, meaning they are more accurate predictors of HOMA-IR.

Conclusion

MOCI score did not act as a mediator between physical activity and HOMA-IR in any of our mediation analyses. Interestingly, in our regression models, we observed that MOCI was inversely correlated with HOMA-IR. This was only seen in our analyses conducted with the entire population and our inactive subpopulation. In the regression analyses conducted using only our physically active population, MOCI was not significantly associated with HOMA-IR. This tells us that MOCI score does not appear to have an effect on insulin resistance in our physically active population. Our regression analyses conducted in the physically inactive population suggests that as MOCI score increases, insulin sensitivity improves. While this is opposite of what was predicted, the findings lead me to believe that the MOCI score does contribute to insulin sensitivity in some way. It is possible that the particular effect alleles used in the analysis may actually have a beneficial effect. This may be why our physically inactive participants displayed improved insulin sensitivity with increasing MOCI score. Individuals that are meeting the recommended 150 min/wk of MVPA would not display improved insulin sensitivity if MOCI is beneficial since they would be benefitting from the effects of exercise.

We can also conclude that MOCI score is a better predictor of insulin resistance in physically inactive individuals. This model has a higher R^2 value than the model using all participants, making it more accurate. The model using physically active individuals also has a higher R^2 value that the model using all participants. Since MOCI was not significantly associated with HOMA-IR in this model, we can conclude that MOCI is not a significant predictor of insulin resistance in physically active individuals.

Another unexpected finding is that light physical activity (PA700) was also inversely correlated with HOMA-IR. While it is known that MVPA is associated with improved insulin

sensitivity, studies have not shown that light physical activity can improve insulin sensitivity. However, in the regression model using only the physically inactive subset, PA700 did not have a significant effect on HOMA-IR. This suggests that PA700 may only have an effect in a physically active population. MVPA still had a significant effect on insulin sensitivity in the physically inactive subset, showing its importance. MVPA did not appear to have a significant effect in the physically active subset, suggesting that once the requirement of 150 min/wk of MVPA is met, the effect plateaus.

There were several limitations to this study. The most significant one being small sample size of not only our population but for our SNP index as well. We plan to conduct further studies using all of the 25 SNPs that had been identified for the MOCI score, following imputation of our genotype data with the 1000 Genomes database. We also plan to conduct more analyses using the entire sample population, including the individuals that were removed as a result of population stratification. Another limitation is that we only looked at the effect of the SNPs collectively instead of individually. It is possible that particular SNPs have a larger effect than others, or possibly having effects in opposite directions. As a result, our additive scoring system may not be very accurate. We will re-run the analysis for each SNP individually to which SNPs have a significant effect and determine the individual effect size of those SNPs.

Another limitation is that we cannot measure mitochondrial function. If we were to see a correlation between MOCI score and insulin resistance, there is no way to definitively conclude that this effect if the result of mitochondrial dysfunction. Our final limitation is that we did not incorporate dietary intake information for participants. Since we were unable to control for diet, we cannot make conclusions about the effects of physical activity or genetics on insulin resistance alone. It is possible that our physically active individuals that are insulin resistance

could have poor diet quality. Since diet is a major contributing factor to insulin resistance, this could be the main cause instead of genetics.

This study has helped set up a larger study that will be utilizing all 25 SNPs seen above. Our lab plans on further investigating the effects of these SNPs as well as conducting further research into the literature to examine the functional roles of these genes, as well as potential effect sizes. While we were not able to determine that MOCI score mediated the effect of exercise on insulin resistance, we were able to conclude that MOCI score significantly contributed to insulin sensitivity in our physically inactive subset.

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