

EXPLORING COMMUNITY-SPECIFIC ASSOCIATIONS AMONG OBESITY, META-  
INFLAMMATION, AND THE GUT MICROBIOME IN THE CONTEXT OF NATIVE  
HAWAIIAN AND OTHER PACIFIC ISLANDER HEALTH DISPARITIES ON O'AHU

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## **Dedication**

For Nalu and my mama.

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## Abstract

Native Hawaiians and other Pacific Islanders (NHPIs) face a higher prevalence of obesity-related diseases and are more likely to be classified as obese according to conventional body mass index (BMI) standards. However, BMI is differentially effective as an indicator of obesity-related health risk, and its generalized application may introduce biases in clinical or comparative research settings. Community-specific characterizations of obesity risk are needed to improve the accuracy and relevance of health disparities research for NHPIs.

Community clinic events were held for study recruitment, health-related survey administration, clinical health measure collection, blood sample collection, and at-home stool collection kit distribution. Point-of-care glycosylated hemoglobin (A1c; %) tests were used to designate type 2 diabetes mellitus (T2DM) categories/risk status according to thresholds recommended by the American Diabetes Association. Blood pressure (BP) was also measured, and hypertension (HT) status was determined according to 2017 guidelines recommended by the American College of Cardiology. Community-specific definitions for metabolically healthy (MH; non- or pre-diabetic and non-hypertensive) and metabolically unhealthy (MU; diabetic and/or hypertensive) obesity were obtained for each racial-ethnic group in an NHPi-enriched cohort. To achieve this, receiver operating characteristic (ROC) areas under the curve (AUC) values were used to compare the predictive performance of various anthropometric indices for MU status. These included WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio), WHT.5R (waist-to-height<sup>0.5</sup> ratio), ABSI (A body shape index), BAI (body adiposity index), BRI (body roundness index), and WWI (weight-adjusted waist index). Ideal thresholds were then back-calculated for each anthropometric index to maximize accurate classifications for T2DM risk and HT. Youden's J statistic was then calculated for each threshold to determine their predictive performance for MU status.

Plasma concentrations of GLP-1, IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-10, IL-12, IL-13, IL-18, IL-3, IL-6, IL-8 (CXCL8), Insulin, MCP-1 (CCL2), PYY, TNF- $\alpha$ , TNFR1, and VEGF-A (pg/ml) were measured using the 17-Plex Human ProcartaPlex Panel (ThermoFisher Scientific, Warrington, England). Linear discriminant analysis (LDA) was performed to determine the contribution of each biomarker to the separation between MH and MU risk status. Plasma concentrations between ethnic groups were compared using Kruskal and

Wilcoxon rank-sum tests. Ethnic-specific associations between significant meta-inflammatory factors and MH/OB risk were then compared by logistic regression.

Home stool sample self-collection kits were distributed to participants upon biometric data collection. Each kit included one sample tube containing RNAlater (5 ml; a sample preservative supplied by ThermoFisher Scientific, Waltham, MA). DNA (40 ng) isolated from each stool sample was subjected to polymerase chain reaction (PCR) amplification targeting 16S rDNA hypervariable regions V2-4-8 and V3-6,7-9 (Ion Torrent 16S Metagenomics Kit; ThermoFisher Scientific, Warrington, England). 16S rDNA libraries were prepared from 150 ng of pooled amplicons (Ion Plus Fragment Library Kit; ThermoFisher Scientific, Austin, TX, USA) and barcoded using Ion Xpress Barcode Adapters (Life Technologies, Carlsbad, CA, USA). DNA libraries were pooled (80 pmol from up to 60 libraries) and loaded onto Ion 530™ chips (Ion S5 Next-Generation Sequencing System) in preparation for sequencing.

16S Metagenomics Kit analysis was performed using Ion Reporter™ Software v5.18.4.0 (ThermoFisher Scientific). Chimeric sequences were automatically identified and removed. Reads were mapped to reference databases Greengenes v13.5 and MicroSEQ ID v3.0. Gut microbiome profiles were compiled using metagenome taxonomic data via the Curated MicroSEQ(R) 16S Reference Library v2013.1. Differential abundance analyses were performed using Analysis of Compositions of Microbiomes with Bias Correction; the 'ANCOM-BC2' in R.

Anthropometric thresholds associated with MU risk were generally highest among Part/NHPIs compared to other groups. Part/NHPI-specific BMI thresholds resembled the conventional cutoff, ranging from 27.81 to 30.25 kg/m<sup>2</sup>, while those for Asian, White, and Mixed groups were relatively lower, ranging from 23.62 to 25.36 kg/m<sup>2</sup>. However, anthropometric indices were less effective as health risk predictors for NHPIs than for White ( $P=2.3E-5$ ) and Mixed ( $P=0.030$ ) groups. BRI emerged as the most consistent and effective predictor of T2DM and HT risk across the total cohort, suggesting that its use in clinical and research settings may better capture obesity-related health disparities in NHPI-inclusive communities than BMI. However, the effectiveness of anthropometric indices varies among diverse populations and is least effective for NHPIs compared to other racial-ethnic groups.

The features that strongly contributed to the distinction between MH and MU risk categories were age, gender, BRI, IFN- $\alpha$ , IFN- $\gamma$ , IL-10, IL-12, IL-13, IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$ . Among significant meta-inflammatory biomarkers, IFN- $\alpha$ , IL-10, IL-13, IL-1 $\beta$ , and TNF- $\alpha$  were the most important features in the distinction between MH and MU risk status for NHPs. After adjusting for age and gender, IL-1 $\beta$  levels were significantly higher in NHPs compared to the White group ( $P < 0.01$ ). GLP-1 levels were lower in the NHP group than in the White or Mixed groups ( $P < 0.001$ ). PYY was also lower in NHPs compared to Part NHPs and the Mixed group ( $P < 0.01$ ). MCP-1 was lower in NHPs compared to Asian and Mixed groups ( $P < 0.001$ ), and IL-3 was lower in NHPs compared to White ( $P < 0.001$ ) and Mixed groups ( $P < 0.01$ ).

At the genus level, NHPs had the highest prevalence of *Eggerthella*, *Blautia*, *Megamonas*, *Veillonella*, *Lachnoclostridium*, *Lactobacillus*, *Fusobacterium*, *Haemophilus*, *Klebsiella*, and *Mannheimia* and the lowest prevalence of *Ruminiclostridium*, *Barnesiella*, *Prevotella*, *Herbaspirillum*, *Desulfovibrio*, and *Akkermansia* compared to other racial-ethnic groups. Gut bacterial taxa that were more prevalent in NHPs than other groups have been previously linked to inflammation, T2DM, HT, or gastrointestinal complications. Those less prevalent in NHPs than other groups were variably associated with beneficial metabolic effects. However, these associations were primarily observed in NHP-exclusive study populations. In NHPs, A1c levels were positively associated with unclassified members of Clostridiales, *Flavonifractor*, *Megasphaera*, and *Turicibacter* but negatively associated with *Mannheimia* and *Megamonas*. There were no significant associations between gut bacterial genera and A1c levels in the Asian, White, or Mixed groups.

Racial-ethnic differences in obesity, meta-inflammation, and the gut microbiome may contribute to racial-ethnic differences in metabolic health risk. BRI was the most effective predictor for metabolic outcomes in an ethnically diverse population. Still, anthropometric indices may be less effective as health risk indicators for NHPs than other groups. Meta-inflammation may differentially associate with metabolic outcomes between racial-ethnic groups, and the association may be stronger in NHPs than in other groups. Gut bacterial associations with metabolic outcomes also differed by race-ethnicity. Further community-specific research is necessary to characterize these differences effectively.

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## List of Abbreviations

16S: Prokaryotic ribosomal subunit (small)	HFD: High-Fat Diet
A1c: Glycosylated hemoglobin	HT: Hypertension
ABSI: A Body Shape Index	IBS: Irritable/Inflammatory Bowel Syndrome
ACME: Average Causal Mediation Effect	IFN-: Interferon-
AGE: Advanced Glycation End Products	IL-: Interleukin-
AGP: American Gut Project	IR: Insulin Receptor
AIC: Akaike's Information Criterion	IRS: Insulin Receptor Substrate
AMPK: Adenosine monophosphate (AMP)- Activated Protein Kinase	LDA: Linear Discriminant Analysis
ANCOM-BC: Analysis of Compositions of Microbiomes with Bias Correction	LFC: Log-Fold Change
API: Asian and Pacific Islanders	LPS: Lipopolysaccharide (endotoxin)
ATM: Adipose Tissue Macrophages	MCP-1: Monocyte Chemoattractant Protein-1 (CCL2)
AUC: Area Under the Curve	MEC: Multiethnic Cohort (University of Hawai'i Cancer Center)
BAI: Body Adiposity Index	MetS: Metabolic Syndrome
BIA: Bioelectrical Impedance Analysis	MH: Metabolically Healthy (no T2DM or HT)
BMI: Body Mass Index	MH/OB: Obesity-related metabolic health status
BP: Blood Pressure	MHNO: Metabolically Healthy; Non-Obese
BRI: Body Roundness Index	MHOB: Metabolically Healthy; Obese
BUK: Butyrate Kinase	MPC-1: Monocyte Chemoattractant Protein-1
CRP: C-Reactive Protein	MRI: Magnetic Resonance Imaging
CVD: Cardiovascular Disease	MU: Metabolically Unhealthy (T2DM or HT)
DNA: Deoxyribonucleic Acid	MUNO: Metabolically Unhealthy; Non-Obese
DXA: Dual-Energy X-ray Absorptiometry	MUOB: Metabolically Unhealthy; Obese
FMT: Fecal microbiota transplantation	NAFLD: Non-Alcoholic Fatty Liver Disease
GLM: Generalized Linear Modeling	NCBI: National Center for Biotechnology Information
GLP-1: Glucagon-Like Peptide-1	NF-kB: Nuclear Factor Kappa B
GLUT4: Glucose Transporter Type 4	

NGS: Next-Generation Sequencing

NH: Native Hawaiian

NHPI: Native Hawaiian and Other Pacific Islander

NO: Non-Obese

OB: Obese

OTU: Operational Taxonomic Unit

Part/NHPI: NHPI or Part NHPI

PBMCs: Peripheral Blood Mononuclear Cells

PCR: Polymerase Chain Reaction

PI: Pacific Islander

PYY: Peptide tyrosine tyrosine (YY)

RAM: Risk-Adjusted Mean

RNA: Ribonucleic Acid

ROC: Receiver Operating Characteristic

SAT: Subcutaneous Adipose Tissue

SCFA: Short-Chain Fatty Acid

SE: Self-Esteem

SEM: Standard Error of the Mean

SES: Socioeconomic Status

T2DM: Type 2 Diabetes Mellitus

TAb: Total Abdominal Fat

TNF-: Tumor Necrosis Factor-

TNFR1: Tumor Necrosis Factor Receptor I

Treg: Regulatory T cells

VAT: Visceral Adipose Tissue

VEGF-A: Vascular Endothelial Growth Factor A

WC: Waist Circumference

WGS: Whole Genome Sequencing

WHR: Waist-to-Hip Ratio

WHT.5R: Waist-to-Height<sup>0.5</sup> Ratio

WHTR: Waist-to-Height Ratio

WWI: Weight-Adjusted Waist Index

# Chapter 1. Introduction

## 1.1 Background

### 1.2.1 Meta-Inflammation as an Obesity-Related Metabolic Risk Factor

In the United States, Native Hawaiians and other Pacific Islanders (NHPI) are more likely to be classified as “obese” compared to other racial-ethnic groups[1]. In 2022, an estimated 45.6% of NHPIs in the state of Hawai‘i had a BMI over 30 kg/m<sup>2</sup>, classifying them as “obese” by conventional standards[1]. Obesity is a multifaceted risk factor for several serious health complications, including cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), and cancer[2–4], which are especially prevalent in NHPI communities and are among the leading causes of death in Hawai‘i[5]. This disparity is exacerbated by the underrepresentation of NHPIs in biomedical research, a gap contributing to a lack of targeted health interventions for this population[6,7].

The link between excess fat accumulation and cardiovascular health is mediated by chronic low-grade inflammation and its associated disruption of metabolic processes (termed “meta-inflammation”)[8]. Although adipocytes play an intricate role in normal homeostatic functioning[9], sustained excess adiposity may lead to cytokine and adipokine dysfunction, increased recruitment and infiltration of pro-inflammatory immune cells, and further pro-inflammatory polarization of tissue-resident immune cells[10]. The persistence of such inflammation interferes with critical pathways involved in glucose and lipid homeostasis[11].

Proinflammatory cytokine signaling tends to interfere with insulin signaling by various mechanisms. Examples of direct mechanisms include downregulated IRS expression[12], inhibited tyrosine phosphorylation of IR and IRS-1[13], and inhibitory serine phosphorylation of IRS[14]. More indirectly, proinflammatory cytokine signaling can inhibit Akt phosphorylation or downregulate GLUT4 expression/translocation in adipocytes[12,15,16]. Inversely, anti-inflammatory cytokines, such as IL-10, are characteristically suppressed under diabetic conditions[17,18], suggesting a compromised anti-inflammatory defense mechanism in diabetic patients. IL-10 exhibits a significantly reduced capacity to suppress pro-inflammatory TNF- $\alpha$  signaling in T2DM macrophage models[19].

Persistent hyperglycemia may also induce oxidative stress and cellular damage by the accumulation of advanced glycation end products (AGEs) - irreversibly glycated proteins, lipids, or nucleic acids[20]. Elevated AGE accumulation can directly interfere with insulin-related transcription factors, increasing oxidative stress and inducing apoptosis and necrosis in pancreatic islets[21], ultimately resulting in insufficient insulin production. Such pancreatic dysfunction may extend to  $\alpha$ -cells and dysregulated glucagon secretion. Insulin resistance is associated with higher fasting glucagon levels and delayed

postprandial glucagon suppression[22]. In either state, diabetic individuals exhibit hyperglucagonemia compared to nondiabetic controls, in turn stimulating hepatic glucose production and increasing their risk for hyperglycemia[23]. Oxidative and metabolic stressors can exert cellular damage and induce inflammatory responses, enabling a “vicious cycle” of meta-inflammation and metabolic dysfunction. Hyperglycemia can induce an “inflammatory program” in innate immune cells to sustain the cycle[24]. While the mechanistic underpinnings of these interactions are complex and multifaceted, the unresolved hyperglycemia and dyslipidemia that accompany chronic meta-inflammation can lead to several serious health complications, including those aforementioned: CVD, T2DM, and cancer[3,25–27].

### **1.2.2 Obesity-Related Gut Microbiome Research**

From the pursuit of effective strategies to alleviate such complications, the gut microbiome has become a target of increasing translational research interest for its capacity to modulate host physiology[28]. Several modes of fecal microbiota transplantation (FMT) are safe, practical strategies for modulating meta-inflammation, restoring glucose tolerance and lipid homeostasis, and even reducing visceral adiposity[29], demonstrating the functional relevance of a “healthy” or “unhealthy” (or “dysbiotic”) gut microbiome[29]. Dietary and behavioral factors, including prebiotic and probiotic supplements, are also associated with distinct changes among gut bacterial populations. This presents a non-invasive intervention opportunity leveraging the bidirectional nature of host-microbe interactions[29,30].

One way gut bacteria can influence host health is by directly interfacing with inflammatory processes by lipopolysaccharide (LPS)-mediated pathways[31]. Increased plasma levels of LPS are associated with obesity, metabolic inflammation (involving adipose tissues), and  $\beta$ -cell dysfunction[32]. These observations are consistent with the hypothesis that LPS originating from Gram-negative bacteria can be transported into intestinal capillaries and then to target tissues (adipose, liver, and muscle), where it would induce proinflammatory responses[32], thereby disrupting insulin signaling[33]. LPS can also cause local inflammation in the intestines, a hallmark of irritable bowel syndrome (IBS), among other metabolic complications[34]. These responses are attributed to LPS-induced TNF- $\alpha$  expression and subsequent NF- $\kappa$ B signaling in adipocytes, macrophages (including ATMs), and endothelial cells[35]. In this sense, an imbalanced gut microbiome, termed dysbiosis, can mediate or even trigger metabolic inflammation associated with obesity, T2DM, and other metabolic disorders.

Regional and racial-ethnic differences influence the composition of the gut microbiome and, in turn, the conclusions we draw from related research. For example, a microbiota-based predictive model for metabolic disease risk effective in one district in China was unreliable in another district within the same province[36]. Even in a group of individuals from the same region, gut microbiome composition still varies across racial-ethnic groups after controlling for age, sex, dietary habits, and metabolic health status[37,38]. The current body of literature reporting obesity-related trends in specific gut bacteria

presents regional and racial-ethnic diversity that complicates its generalizability to understudied populations. Gut bacterial trends in obesity reported between 2019 and 2024 by 16S-based metataxonomic studies are summarized in **Table 1.1**.

**Table 1.1** Previously reported gut bacterial associations with obesity from recent 16S-based metataxonomic studies. Taxa are listed at phylum and genus levels. Genera are only listed here if they are significantly associated with obesity in at least three studies in the literature review.

	Association with Obesity		Metabolic relevance
	Negative	Positive	
<b>Actinomycetota<sup>a</sup></b>	[39,40]	[41]	Produces fungicidal and antibiotic metabolites [42,43]
<i>Bifidobacterium</i>	[39,44–46]		Attenuates LPS-induced meta-inflammation, insulin resistance [47,48], visceral fat, plasma triglycerides[49]; SCFA producer [50], variable impact on fecal SCFA [51]
<b>Bacteroidota<sup>b</sup></b>	[41,52,53]	[39,40,46,54]	Can produce H <sub>2</sub> S from host mucosal glycans, which causes epithelial damage [55,56]
<i>Bacteroides</i>	[44,57]	[58]	Inhibits lipid accumulation in preadipocytes; alleviates HFD-induced obesity. Restores glucose/lipid homeostasis[59]. SCFA producer [50]; increased fecal acetate [51]
<i>Parabacteroides</i>	[39,41,53]	[54,57] <sup>h</sup>	Facilitates the production of secondary bile acids (lipid homeostasis/insulin signaling), activates intestinal gluconeogenesis, and alleviates obesity[60].
<i>Alistipes</i>	[39,61]	[54] <sup>h</sup>	Alleviates inflammation, atherosclerotic CVD[62]
<i>Prevotella</i>	[41,52,63]	[39,44,54,57,64]	Stimulates proinflammatory cytokine production[57]; Negatively correlated with intestinal permeability[51]; glycemic control in high fiber diet but not HFD[65]
<i>Barnesiella</i>	[39,61,66]		Produces SCFAs involved in glucose/energy homeostasis; mediates inflammation[67]
<b>Bacillota<sup>c</sup></b>	[39,40,46]	[41,52–54]	Enables higher calorie absorption from dietary nutrients, promoting weight gain [68]
<i>Phascolarcto...<sup>d</sup></i>	[66]	[54,64] <sup>h</sup>	Propionate/acetate producer; expanded in HFD-induced obesity[69]; negatively correlated with stool SCFA concentrations [51]
<i>Catenibacterium</i>		[54,61,64] <sup>h</sup>	Enriched in Western diet; more prevalent in MUOB than in MHOB[70]
<i>Megasphaera</i>		[53,57,64,71]	It can produce butyrate from glutamate and lysine. Produces ammonia as a byproduct [72]
<i>Allisonella</i>		[52,58,63,71]	Produces histamine, meta-inflammatory[73]
<i>Oscillibacter</i>	[39,61]	[54]	Enriched in HFD[67]; facilitates diet-induced obesity and inflammation[74].
<i>Faecalibacterium</i>	[39,40,45,58]	[41]	Butyrate-producing; bile-hydrolyzing, which regulates fat absorption and glucose/lipid homeostasis [40]; Inhibits IL-8 and IL-6 production; induces IL-10 production in PBMCs [75]
<i>Ruminococcus</i>	[39,44,52,64,76]	[54,77]	Attenuated MetS symptoms [29]; positively associated with Crohn's[78]; induces TNF production [79]; SCFA producer [50]
<i>Sporobacter</i>	[61]	[54,71] <sup>i</sup>	Negatively correlated with fecal concentrations of butyrate and acetate[51]

**Table 1.1** Previously reported gut bacterial associations with obesity from recent 16S-based metataxonomic studies. Taxa are listed at phylum and genus levels. Genera are only listed here if they are significantly associated with obesity in at least three studies in the literature review.

	Association with Obesity		Metabolic relevance
	Negative	Positive	
<i>Clostridium</i>	[44]	[63,80]	Contains the highest number of bacterial strains with butyrate production capacity [81] but is not significantly correlated to fecal SCFA concentrations [51]
<i>Clostridium XIVa</i>	[39,66]	[54,61] <sup>i</sup>	
<i>Clostridium IV</i>	[39,66]	[54,66] <sup>h</sup>	
<i>Megamonas</i>	[41]	[39,53,57,58,82]	Positively correlated with proinflammatory biomarkers and increased risk for MetS [83]
<i>Romboutsia</i>	[61]	[63,82]	positively associated with fecal SCFA concentrations [51]
<i>Anaerostipes</i>	[63,66,77]		Propionate, acetate production from inositol alleviated insulin resistance in HFD mice[84]
<i>Blautia</i>	[63]	[39,41]	Induces pro and anti-inflammatory responses; varies at the species level [85]
<i>Coprococcus</i>	[39,66,76,77]		May attenuate intestinal permeability [51]
<i>Roseburia</i>	[76]	[41,63]	SCFA producer [50]; positively correlated with fecal SCFA concentrations [51]. Primary degrader of $\beta$ -mannans[86]
<i>Streptococcus</i>		[53,54,57,64] <sup>i</sup>	Correlated with intestinal inflammation [51]
<b>Pseudomonadota<sup>e</sup></b>	[52]	[40,41,46]	May induce meta-inflammation via LPS [87]
<i>Haemophilus</i>	[58]	[44,54,57] <sup>i</sup>	Associated with intestinal inflammation[88]
<b>Fusobacteriota<sup>f</sup></b>	[41,61]	[39,45]	Inhibits inflammation in colorectal cancer [89]
<b>Verrucomicrobiota<sup>g</sup></b>	[46,52,61]	[41]	Major mucin-degrading bacteria; associated with upregulated expression of anti-inflammatory cytokines and Tregs[90]; promotes intestinal barrier integrity [91]; <i>Akkermansia</i> negatively correlated with fecal acetate and butyrate concentrations [51]

<sup>a</sup>Actinobacteria. <sup>b</sup>Bacteroidetes. <sup>c</sup>Firmicutes. <sup>d</sup>Phascolarctobacterium. <sup>e</sup>Proteobacteria. <sup>f</sup>Fusobacteria. <sup>g</sup>Verrucomicrobia. <sup>h</sup>Significant results in Bushbuckridge and not Soweto[54]. <sup>i</sup>Significant results in Soweto and not Bushbuckridge[54]. Phylum names are bolded. For each gut bacterial taxon, studies that reported a significant positive or negative association with obesity are listed in the “Trends in obesity” columns. Positive trends were designated for specific taxa if they were more prevalent in obese (OB) than non-obese (NO) groups or positively associated with obesity metrics in a regression analysis. Negative trends were designated for specific taxa if they were less prevalent in OB than NO groups or negatively associated with obesity metrics in a regression analysis. Baseline trends were prioritized for qualifying intervention studies.

Although each of the studies listed in **Table 1.1** employed 16S-based metataxonomic techniques, their definitions of obesity tended to vary. Conventional or adjusted anthropometric cutoffs were sometimes used for data stratification. It was similarly common for some studies to treat various obesity measures as covariates in regression or other multivariate analyses. Even after adjusting for the metabolic relevance of obesity measures within respective study cohorts, the heterogeneity among study findings suggests that the gut microbiome is differentially associated with health on a community-specific basis.

## 1.2 Gaps in Knowledge

NHPIs are more likely to be classified as obese by conventional standards. However, these standards are variably practical indicators of metabolic health status among diverse populations. Applying conventional BMI cutoffs as thresholds for data stratification may introduce a misclassification bias[92]. In NHPI health research, this bias can also be introduced using generalized thresholds for any obesity index, whether anthropometric or directly measured, especially if obtained in NHPI-exclusive populations. As continuous variables, unadjusted indices may present similarly skewed representations with other biological factors. Additionally, the literature on obesity-related disease etiology in NHPIs is limited. As such, meta-inflammatory associations with metabolic disease risk are understudied in these health-disparate populations, necessitating community-specific research.

Gut bacterial associations with obesity-related metabolic risk may be conditional and community-specific. Racial-ethnic differences in gut microbiota profiles may even be distinct regardless of metabolic health status[37]. However, NHPI-inclusive data tends to be aggregated, and NHPIs remain underrepresented in health disparities research[93]. Community-specific research is required for an accurate characterization of metabolically relevant metataxonomic trends. The obesity-related metabolic disparity among NHPI populations is paralleled by data disparity due to underrepresentation in biomedical research and public health data aggregation. The persistence of such disparities urgently necessitates more accurate assessments of community-specific health needs, which conventional measures may compromise or mischaracterize in clinical and research settings. The purpose of this project is to explore anthropometric indices, meta-inflammatory biomarkers, and gut bacteria as indicators of disparate metabolic risk in NHPI communities. Augmenting foundational knowledge about community-specific patterns in obesity-related disease etiology may better inform future development of targeted treatment options, laying solid groundwork for advancing NHPI health equity in Hawai'i.

## 1.3 Aims and Hypotheses

This dissertation aims to characterize racial-ethnic differences in obesity, meta-inflammation, and the gut microbiome as metabolic health risk factors. These differences may be involved in the persistence of disparate obesity-related metabolic health outcomes, such as type 2 diabetes mellitus (T2DM) and hypertension (HT), in Native Hawaiian and other Pacific Islander (NHPI) communities.

**Aim 1. Obtain ethnic-specific anthropometric thresholds to designate obesity status as a predictor for metabolic health risk.**

**Rationale:** NHPIs face disproportionately high prevalence of and mortality to metabolic diseases, including T2DM, compared to other racial-ethnic groups. In clinical, research, and public health settings,

disparate health outcomes are conventionally described using body mass index (BMI) as a proxy for obesity-related metabolic risk. However, the utility of BMI as a predictor of metabolic outcomes varies across gender and racial-ethnic groups, and its generalized use may obscure real trends in health-disparate populations, especially in those as understudied as NHPs. Therefore, a more uniformly effective indicator for obesity-related metabolic risk is necessary to augment the reliability of study findings in an NHP-enriched community, especially for uncovering racial-ethnic differences in obesity-related outcomes.

**Hypothesis 1:** As predictors of metabolic health risk, anthropometric thresholds for designating obesity status are lower for Native Hawaiian and Pacific Islander (NHP) populations compared to those established for other racial-ethnic groups. This trend may correspond with an elevated risk of obesity-related type 2 diabetes mellitus (T2DM) and hypertension (HT) in NHPs.

**Aim 2. Identify meta-inflammatory risk factors for heightened metabolic health risk in the context of obesity-related disparities in NHPs.**

**Rationale:** Meta-inflammation is increasingly recognized as a causal link between obesity and metabolic diseases. However, meta-inflammatory processes are understudied in NHPs, especially in health disparity.

**Hypothesis 2:** Meta-inflammatory biomarkers are associated with metabolic health risk. Native Hawaiian and Pacific Islander (NHP) populations may exhibit higher relative blood concentrations of pro-inflammatory cytokines than other racial-ethnic groups. Heightened levels of inflammation could partly account for racial-ethnic disparities in metabolic health risk, including the increased susceptibility observed in NHP populations.

**Aim 3. Characterize gut bacterial associations with metabolic health risk in the context of obesity-related disparities in NHPs.**

**Rationale:** Gut bacterial associations with obesity-related disease etiology are influenced by regional, racial-ethnic, and community-specific factors. Due to the limited scope of community-specific research, the gut microbiome and its relationship to metabolic health risk is relatively uncharacterized in NHPs.

**Hypothesis 3:** Specific gut bacteria are associated with type 2 diabetes mellitus (T2DM) and hypertension (HT) risk, and Native Hawaiian and Pacific Islander (NHP) populations will have a higher relative abundance of taxa that have been previously linked to cardiometabolic disorders compared to other racial-ethnic groups. This dysbiosis may contribute to a predisposition or heightened risk for adverse metabolic health outcomes.

## Chapter 2. Racial-Ethnic Differences in the Utility of Anthropometric Indices as Predictors of Obesity-Related Metabolic Outcomes

### 2.1 Abstract

**Background:** In the State of Hawai'i, Native Hawaiians and other Pacific Islanders (NHPIs) face a higher prevalence of obesity-related diseases and are more likely to be classified as obese (BMI=30 kg/m<sup>2</sup>) according to conventional standards. However, BMI's utility as an indicator of obesity-related health risk varies, which may introduce biases in clinical and comparative research settings. It is differentially effective as an indicator of obesity-related health risk, and its generalized application may skew or confound comparative study findings. Community-specific criteria for obesity risk are needed to improve the accuracy and relevance of health disparities research for NHPIs.

**Methods:** Various alternative anthropometric indices were calculated to explore community-specific measures of obesity-related health risk, as BMI may not accurately characterize racial-ethnic differences. These included WC (waist circumference), WHR (waist-to-hip ratio), WHtR (waist-to-height ratio), WHT.5R (waist-to-height0.5 ratio), ABSI (A body shape index), BAI (body adiposity index), BRI (body roundness index), and WWI (weight-adjusted waist index), for men and women from NHPI (N=348), Asian (N=257), White (N=171), Part NHPI (N=638), and multiethnic ("Mixed") groups (N=154). Group-specific thresholds were obtained as dichotomous predictors for type 2 diabetes (T2DM) or hypertension (HT) using receiver operator characteristic (ROC) curves. The risk-adjusted mean (RAM) score and Youden's J Statistic were used to compare the relative efficiency of each index. The most uniformly effective predictor was then used to stratify participants into non-obese (NO) and obese (OB) groups and additionally classified as metabolically healthy (MH) and metabolically unhealthy (MU) based on blood pressure and A1c test results.

**Results:** Anthropometric thresholds associated with MU risk were generally highest among Part/NHPIs compared to other groups. Part/NHPI-specific BMI thresholds resembled the conventional cutoff, ranging from 27.81 to 30.25 kg/m<sup>2</sup>, while those for Asian, White, and Mixed groups were relatively lower, ranging from 23.62 to 25.36 kg/m<sup>2</sup>. However, anthropometric indices were less effective as health risk predictors

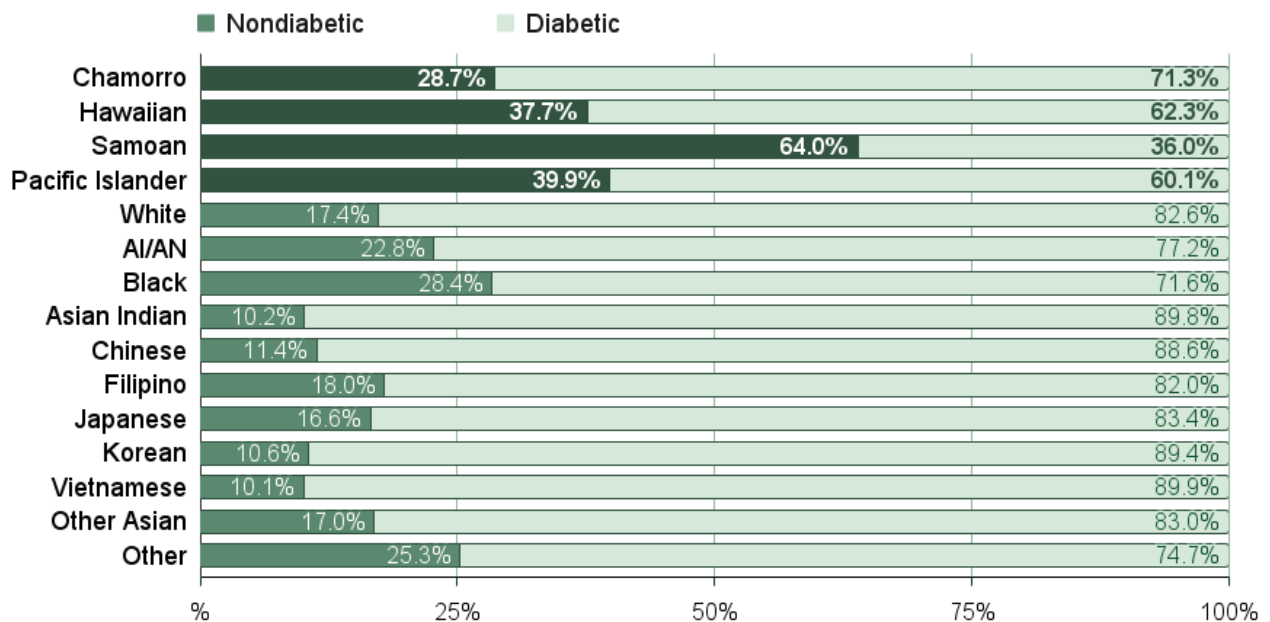
for NHPs than for White ( $P=2.3E-5$ ) and Mixed ( $P=0.030$ ) groups. They were more effective for Part NHPs than for NHPs ( $P=0.048$ ) or Asians ( $P=0.001$ ) but less effective than for Whites ( $P=0.012$ ). BRI was the most uniformly effective predictor of metabolic risk and was significantly more effective than BMI ( $P=0.01$ ). BMI and BRI differentially stratified obesity risk groups ( $P<2.2E-16$ ), and BMI tended to underestimate risk across racial-ethnic groups.

**Conclusions:** BRI emerged as the most consistent and effective predictor of T2DM and HT risk across all gender and racial-ethnic groups, suggesting that its use in clinical and research settings may better capture obesity-related health disparities in NHP-inclusive communities than BMI. However, the utility of anthropometric indices varies among diverse populations and is least effective for NHPs compared to other racial-ethnic groups. Further research is needed to refine obesity-related risk estimates for metabolic disease in understudied, health-disparate populations such as NHPs.

## 2.2 Introduction

According to the World Health Organization (WHO), obesity is abnormal or excessive fat accumulation that presents a risk to health[94]. More specifically, excess adiposity may be causally linked to CVD, heart disease, stroke, T2DM, and osteoarthritis[95]. The standard metric for designating obesity status in adults is body-mass-index (BMI), calculated as weight (kg) divided by square height (m<sup>2</sup>), using the following categorical cutoffs: Underweight (BMI < 18.5 kg/m<sup>2</sup>), Normal weight (18.5 kg/m<sup>2</sup> ≤ BMI < 25 kg/m<sup>2</sup>), Pre-obesity (25.0 kg/m<sup>2</sup> ≤ BMI < 30 kg/m<sup>2</sup>), Obesity Class I (30 kg/m<sup>2</sup> ≤ BMI < 35 kg/m<sup>2</sup>), Obesity Class II (30 kg/m<sup>2</sup> ≤ BMI < 35 kg/m<sup>2</sup>), and Obesity Class III (BMI > 40 kg/m<sup>2</sup>)[96]. According to these standards, an individual with a BMI over 25 kg/m<sup>2</sup> or 30 kg/m<sup>2</sup> would face a heightened risk for chronic metabolic and cardiovascular diseases compared to an individual with a BMI between 18.5 and 25 kg/m<sup>2</sup>, which is estimated to increase with increasing BMI[96].

Although BMI-related health risk assessments are commonplace in clinical and research settings[97], the utility of BMI as a universal tool for health risk assessment is inherently limited. The original formulation for BMI was determined using observational studies in a sample of healthy men of European ancestry[97,98]. A universal definition of obesity is based solely on BMI; therefore, it may not be a universally effective indicator for adiposity-related metabolic disease risk.



**Figure 2.1** T2DM status in individuals with BMI ≥ 30 kg/m<sup>2</sup> in Hawai'i by racial-ethnic group (2022). AI/AN=American Indian and Alaska Natives.

This discrepancy can be observed among diverse populations in the State of Hawai'i. In 2022, NHPs had the highest rate of obesity compared to all other census race groups[1]. However, among Hawai'i state residents who were classified as "obese" (BMI ≥ 30 kg/m<sup>2</sup>), NHPs had the highest proportion of

nondiabetic individuals (**Figure 2.1**)[1]. This demonstrates that the conventional definition of obesity (based on BMI) is limited as a proxy measure for metabolic risk across racial-ethnic groups in this community, and it is particularly ineffective in NHPI communities in Hawai'i. More importantly, this discrepancy may suggest that metabolic health risk is commonly mischaracterized in NHPI and minority populations.

To circumvent such discrepancies, independent institutions have recommended modified, ethnic-specific BMI cutoffs[99]. In 2000, the International Diabetes Institute (IDI) proposed modified BMI cutoffs to designate obesity status for communities in the Asia-Pacific region. Pacific-Islander (PI)-specific BMI cutoffs proposed by the IDI for overweight and obesity were 26 kg/m<sup>2</sup> and 32 kg/m<sup>2</sup>, respectively. These thresholds were developed in a 1999 study that measured body fat (BF) mass at fixed BMI values in European individuals. Those BF measurements were then used as fixed values to back-calculate equivalent BMI cutoffs for a Polynesian (Māori and Sāmoan) cohort[100].

A 2010 study revisited these standards and found that the increased threshold for obesity at 32 kg/m<sup>2</sup> did not significantly improve the utility of BMI as a predictor for insulin resistance or metabolic syndrome (MetS) in Māori populations[101]. This demonstrates that the correlation between BF mass and cardiometabolic risk is inequivalent between communities - an assumption that served as a basis for proposing a 32 kg/m<sup>2</sup> obesity threshold for Polynesian populations.

Differences in the relationship between BF and metabolic health are sometimes attributed to individual-specific patterns in body composition, which can be measured directly[102]. Bioelectrical Impedance Analysis (BIA) can be used to estimate total body fat, fat mass, lean mass, and bone mineral density, among other parameters[103]. Imaging-based techniques like Dual-Energy X-ray Absorptiometry (DXA) can estimate total and regional body fat distribution, and magnetic resonance imaging (MRI) is effectively able to differentiate between subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT)[104]. Applying such techniques has led to the establishment of a causal link between abdominal fat and metabolic dysfunction in some populations[104,105].

However, even direct measures of adiposity lack universality as a tool for health risk assessment due to racial-ethnic differences in obesity-related disease etiology[105,106]. In a multiethnic cohort (MEC) study, the association between MetS risk and %BF was weaker, and VAT volume was stronger among Native Hawaiian individuals than in White individuals. More specifically, different regions of the VAT compartment were differentially associated with MetS risk[107].

Alternative anthropometric indices have been proposed for improved predictive capacity for abdominal adiposity. Such indices include waist circumference (WC), hip circumference (HC), waist-to-hip ratio

(WHR), waist-to-height ratio (WHtR), waist-to-height<sup>0.5</sup> ratio (WHT.5R)[108], a body shape index (ABSI), body adiposity index (BAI), and body roundness index (BRI)[109].

These anthropometric indices are variably effective as indicators for adiposity. At a fixed BMI, Māori and other Pacific Islanders (PI) tend to have less total percentage body fat but significantly more accumulated fat in the VAT compartment[100,110]. More specifically, a New Zealand study found that BMI was differentially associated with BF (kg; DXA) by gender and race-ethnicity[100]. The association was highest for Māori men ( $R^2=0.85$ ) compared to European ( $R^2=0.82$ ) and Sāmoan men ( $R^2=0.81$ ). Similar trends were observed between Māori ( $R^2=0.92$ ), European ( $R^2=0.89$ ), and Sāmoan women ( $R^2=0.88$ ).

Another New Zealand study compared anthropometric indices to SAT, VAT, and total abdominal fat (TAb) volumes estimated by MRI[110]. Age- and sex-adjusted models revealed that WC explained 56.9% of the variance in VAT, 61.2% of the variance in SAT, and 74.6% of the variance in TAb volume in the Māori/PI group. WC was also a better predictor of SAT, VAT, and TAb in the Māori/PI group than in the Caucasian group. BMI demonstrated slightly better predictive capacity, explaining 57.9% of VAT variance, 86.9% of SAT variance, and 87.5% of TAb variance in the Māori/PI group, with lower predictive capacities in the Caucasian group. In contrast, a separate New Zealand study reported that age- and sex-adjusted regression models between BMI and VAT were significant for all VAT compartments in the European group, significant for specific regions in Māori/PI, and nonsignificant for all regions in the Asian cohort[111]. WC and WHtR were not significantly associated with all VAT regions for European, Māori/PI, and Asian groups.

These indices are also variably effective as predictors of metabolic health outcomes. WC cutoffs are integrated into the diagnostic criteria for MetS, although its predictive utility for different health risks may vary depending on the method of measurement[112]. Although WHR was proposed as a controlled way to estimate abdominal obesity, it is a worse predictor of metabolic health risk than BMI and WC in some populations[113]. Compared to WHR, WHtR may be a better predictor for metabolic health risks in some communities[114], but it may overestimate health risks in shorter individuals[108]. WHT.5R was formulated to mitigate this bias, demonstrating improved predictive capacity in some populations[108] but reduced utility in others[115]. ABSI is an effective predictor of mortality risk in European cohorts[109], though its practical value may be limited in other contexts[116]. BAI is a generally consistent proxy measure for %BF based on DXA validation, but the relationship between %BF and metabolic risk is not universal[117]. BRI may be a more effective predictor for MetS than other anthropometric indices[118]. Still, neither BAI nor BRI were uniformly effective indicators of obesity-related insulin resistance[119].

To illustrate racial-ethnic differences in the utility of anthropometric indices, their predictive power for various metabolic outcomes in different Caucasian, Asian, and NHPI cohorts are presented in **Table 2.1** as reported ROC-AUC values. In this context, an ROC (Receiver Operating Characteristic) curve

evaluates the performance of a predictive model by plotting the true positive detection rate (sensitivity) against the true negative detection rate (1 - specificity) for a binary classifier. Its Area Under the Curve (AUC) summarizes the model's capacity to distinguish between the two classes[120]. An AUC of 1 would indicate perfect discriminative ability, while an AUC of 0.5 would indicate random discrimination or no predictive power. AUC values may not necessarily be comparable between studies due to modeling differences and covariate considerations. Still, AUC values reported by the same study may be used to compare the predictive capabilities of independent variables.

**Table 2.1** Racial-ethnic differences in the utility of anthropometric indices as predictors of cardiometabolic outcomes in some Caucasian, Asian, and NHPI communities.

Ethnicity	Men							Women						
	BMI	WC	WHR	WHtR	%BF	ABSI	BRI	BMI	WC	WHR	WHtR	%BF	ABSI	BRI
<b>Metabolic syndrome (MetS)</b>														
[121] Caucasian	0.75			<b>0.76</b>	0.74	0.60	0.73	0.73			<b>0.76</b>	0.72	0.64	0.75
[101] Caucasian	0.78	<b>0.85</b>		0.81				0.77	<b>0.84</b>		<b>0.84</b>			
Māori	0.81	<b>0.84</b>		0.82				0.77	<b>0.81</b>		<b>0.81</b>			
[122] Chinese	<b>0.72</b>	0.71	0.67	0.71		0.57	0.71	0.69	<b>0.70</b>	0.66	<b>0.70</b>		0.59	<b>0.70</b>
<b>Hypertension (HT)</b>														
[123] Caucasian	<b>0.66</b>	<b>0.66</b>		<b>0.66</b>	0.65	0.54	0.64	0.68	0.68		<b>0.69</b>	0.68	0.58	0.68
[124] Tongan	0.67	<b>0.68</b>	0.64	<b>0.68</b>	0.61			0.71	<b>0.72</b>	0.62	<b>0.72</b>	0.64		
[122] Chinese	0.64	0.66	0.64	<b>0.67</b>			0.60	<b>0.67</b>	0.67	0.70	0.66	<b>0.71</b>		0.63
<b>Dyslipidemia</b>														
[123] Caucasian	0.64	0.64		0.64	0.64	0.54	0.62	0.64	0.66		0.66	0.64	0.61	0.66
[125] Tongan	0.70	0.72	0.69	0.71	<b>0.75</b>			0.60	0.66	0.65	0.66	0.55		
[122] Chinese	<b>0.69</b>	0.68	0.65	0.67		0.55	0.67	0.66	0.67	0.65	<b>0.68</b>		0.58	<b>0.68</b>
<b>Type 2 diabetes mellitus (T2DM)</b>														
[126] Caucasian	<b>0.75</b>	0.74	0.71	<b>0.75</b>				0.78	0.78	0.75	<b>0.79</b>			
Hawaiian	<b>0.70</b>	0.69	0.67	0.69				<b>0.72</b>	<b>0.72</b>	0.69	<b>0.72</b>			
Japanese	<b>0.68</b>	0.66	0.64	0.66				<b>0.72</b>	0.71	0.69	0.71			
[125] Tongan	0.67	0.72	<b>0.75</b>	0.73	0.71			0.63	0.69	<b>0.72</b>	0.70	0.54		
[122] Chinese	0.66	0.70	0.69	<b>0.71</b>		0.60	0.67	0.67	0.70	0.66	<b>0.71</b>		0.63	<b>0.71</b>

The highest AUC values for men and women from each study are bolded. Covariate adjustments may vary between studies. %BF was measured by DXA.

As an indicator of MetS in Caucasians, WHtR was the most effective index in the Polish cohort. In a New Zealand cohort, WC was an equally effective indicator in women and a more effective indicator for men. For Chinese men, BMI was a slightly better predictor for MetS compared to other indices, but WC, WHtR, and BRI demonstrated equally moderate predictive capacity for MetS in Chinese women. WHtR was a moderately effective predictor for hypertension in Polish, Tongan, and Chinese cohorts, but this predictive capacity was matched by other indices (except in Polish women). WHtR was also a moderate indicator for dyslipidemia in Polish, Tongan, and Chinese women but not superior to WC (in Caucasian and Tongan cohorts) or BRI (in Polish and Chinese cohorts). In men, however, %BF was a better predictor of dyslipidemia in the Tongan cohort, and BMI was a slightly better predictor in the Chinese cohort. In Polish men, BMI, WC, WHtR, and %BF exhibited comparable, moderate predictive capacity for dyslipidemia.

In a Hawai'i cohort, WHtR was a slightly better predictor of T2DM in Caucasians, though BMI matched this predictive capacity in Caucasian men. WHtR was also better than other indices as a predictor of T2DM in a cohort in China, though BRI was equally effective in Chinese women. In Hawai'i, BMI was better than other indices as a moderate predictor of T2DM in Japanese-Americans and in Native Hawaiian men. In Native Hawaiian women, BMI, WC, and WHtR had similar predictive capacities for T2DM. In a Tongan cohort, WHR was consistently more effective than BMI, WC, WHtR, and %BF as a predictor of T2DM. Overall, ABSI had the worst predictive performance for MetS, hypertension, dyslipidemia, and T2DM in Polish and Chinese cohorts.

NHPIs are more likely to be classified as obese by conventional standards. However, these standards are variably effective as an indicator of metabolic health status among diverse populations. Applying conventional BMI cutoffs as thresholds for data stratification may introduce a misclassification bias[92]. Misrepresentation in biomedical data is particularly dangerous in understudied populations that face disparate health outcomes, as misinformation will hinder efforts to understand community-specific needs.

To mitigate the risk of such biases, the community-specific relationship between accessible obesity measure data and the outcome of interest should be considered in scientific communication. Additionally, clarifying anthropometric associations with adiposity may recontextualize their relevance in obesity-related outcomes. Further research is necessary to elucidate the role of fat accumulation in metabolic disease etiology in understudied populations. Until then, the variable metabolic relevance of obesity-related measures should be acknowledged in health disparities research, and such transparency in scientific communication may mitigate the risk of misrepresentations of Indigenous health.

The purpose of this study is to characterize community-specific associations between anthropometric indices and obesity-related health risks and then to obtain ethnic-specific thresholds for designating obesity status. This approach may augment the metabolic relevance of future study findings, especially for the members of a health-disparate NHPI community.

## **2.3 Methods**

### **2.3.1 Human Subjects Data Collection**

Approval was obtained from the University of Hawai'i Institutional Review Board (UH IRB) under protocol 2019-00376. All laboratory procedures were evaluated and approved by the Hawaii Institutional Biosafety Committee under protocol number B22-100652. Individuals who had been previously diagnosed with T2DM were excluded from the study. Participants enrolled in an NHPI-enriched cohort (N=2137; aged 16 to 88 years) mainly resided in one of two NHPI-enriched communities on O'ahu, Hawai'i, where community clinic events were held for study recruitment and sample collection.

Informed consent, sociodemographic information, medical history, and behavioral risk factor data were collected using self-reported survey responses. Personal information was de-identified for each participant by assigning a unique numerical ID. Due to missingness of survey responses (regarding age, gender, or racial-ethnic group) or variable quality/missingness of clinical data (A1c level, height, weight, anthropometric measures), N=1568 participants (aged 15 to 88 years) were included for downstream analyses in the present study. Racial-ethnic data was self-reported and categorized as follows: NHPI (N=348), Asian (N=257), White (N=171), Part NHPI (N=638), and Mixed groups (N=154). Participants in the NHPI, Asian, or White groups exclusively identified with racial-ethnic groups from those regions. Participants in the Part NHPI and Mixed groups were multiethnic and identified with more than one regional group. Individuals in the Mixed group did not identify with any NHPI racial-ethnic groups. Participants with a previous diagnosis of T2DM were excluded from the present analysis. Informed consent, sociodemographic information, medical history, and behavioral risk factor data were collected using self-reported survey responses. Personal information was de-identified for each participant by assigning a unique numerical ID. Self-esteem (SE) was measured using the Rosenberg SE Assessment[127]. Proportional dietary vegetable consumption (VEG) was calculated as previously described[45].

Blood pressure and A1c levels (PTS Diagnostics, Whitestown, IN) were collected upon survey completion. T2DM status was determined according to A1c test result thresholds recommended by the American Diabetes Association (ADA): Nondiabetic (ND; A1c < 5.7%), Prediabetic (PD; 5.7% ≤ A1c < 6.5%), and Diabetic (DM; A1c ≥ 6.5%)[128]. Blood pressure (BP) categories were determined using the 2017 Guideline for High Blood Pressure in Adults from the American College of Cardiology: Normal (systolic < 120 mm Hg; diastolic < 80 mm Hg), Elevated (systolic 120-129 mm Hg; diastolic < 80 mm Hg); Hypertension I (systolic 130-139 mm Hg; diastolic 80-89 mm Hg), and Hypertension II (systolic ≥140 mm Hg; diastolic ≥ 90 mm Hg)[129].

**Table 2.2** Anthropometric indicators for obesity-related metabolic health risk.

Symbol	Index name	Formula
BMI	Body mass index	weight · height <sup>-2</sup>
WHR	Waist-to-hip ratio	WC · HC <sup>-1</sup>
WhtR	Waist-to-height ratio	WC · height <sup>-1</sup>
WHT.5R	Waist-to-height <sup>0.5</sup> ratio	WC · height <sup>-1/2</sup>
ABSI	A body shape index	1000 · WC · BMI <sup>-2/3</sup> · height <sup>-1/2</sup>
BRI	Body roundness index	364.2 - 365.5 · ( 1 - ( WC · π <sup>-1</sup> · height <sup>-1</sup> ) <sup>2</sup> ) <sup>1/2</sup>
BAI	Body adiposity index	100 · HC · height <sup>3/2</sup> - 18
WWI	Weight-adjusted waist index	0.1 · weight · WC <sup>-1/2</sup>

Alternative obesity-related indices were explored to identify the most consistently effective anthropometric predictor of metabolic health risk in an ethnically diverse population. Weight was measured in kilograms. Height and hip circumference (HC) were measured in meters. Waist circumference is conventionally reported in centimeters but in meters for the present study to maintain consistency among measures. Anthropometric indices were calculated using the formulas listed in **Table 2.2**.

### **2.3.2 Ethnic-Specific Obesity Thresholds**

The 'mgcv' package in R was used for generalized linear modeling (GLM) and residual-outcome covariate adjustment[130]. Akaike's 'An Information Criterion' (AIC) was used to select the best-fit model for the relationship between outcome variables and covariates (such as age and gender), from which residual values were extracted. To obtain covariate-adjusted outcome values, the unadjusted group mean of each outcome variable was then added to these residuals. The 'pROC' package in R was used to generate Receiver operating characteristic (ROC) curves for type 2 diabetes mellitus (T2DM) and hypertension (HT) with age-adjusted anthropometric indices as predictor variables[131]. The same package obtained AUC (area under the curve), sensitivity, specificity, and estimated cutoff values. Risk-adjusted mean (RAM) scores were used to assist in selecting the index with the most consistent efficiency by gender and race-ethnicity using within-group rank-transformed data (mean + 0.2 · standard deviation)[132]. Youden's J statistic was calculated for the lowest of the two thresholds obtained for each group to assess its predictive capacity for the presentation of either T2DM or HT[133], which designated "metabolically unhealthy" (MU) status. The absence of both conditions qualified for "metabolically healthy" (MH) status. Youden's J Statistic was calculated to estimate the predictive performance of each ethnic-specific cutoff for MU. The index with the lowest cumulative RAM score was used to stratify obese (OB) and non-obese (NO) groups.

### **2.3.3 Data Analysis**

Residual-outcome covariate adjustment was performed using the steps described in Section 2.3.2. Kendall's  $\tau$  was used as an exploratory measure to assess the strength and direction of correlations between continuous and ordinal categorical variables. Wilcoxon rank-sum and signed-rank tests were used for intergroup comparisons. Statistical significance was determined at  $P=0.05$ . Data were visualized via 'ggplot2', 'ggpubr,' and 'ggsignif'[134,135]. McNemar's test was used to assess correlations between binary variables.

## 2.4 Results

### 2.4.1 Preliminary T2DM Risk Assessment

**Table 2.3** Sociodemographic summary and metabolic health risk factors by racial-ethnic group.

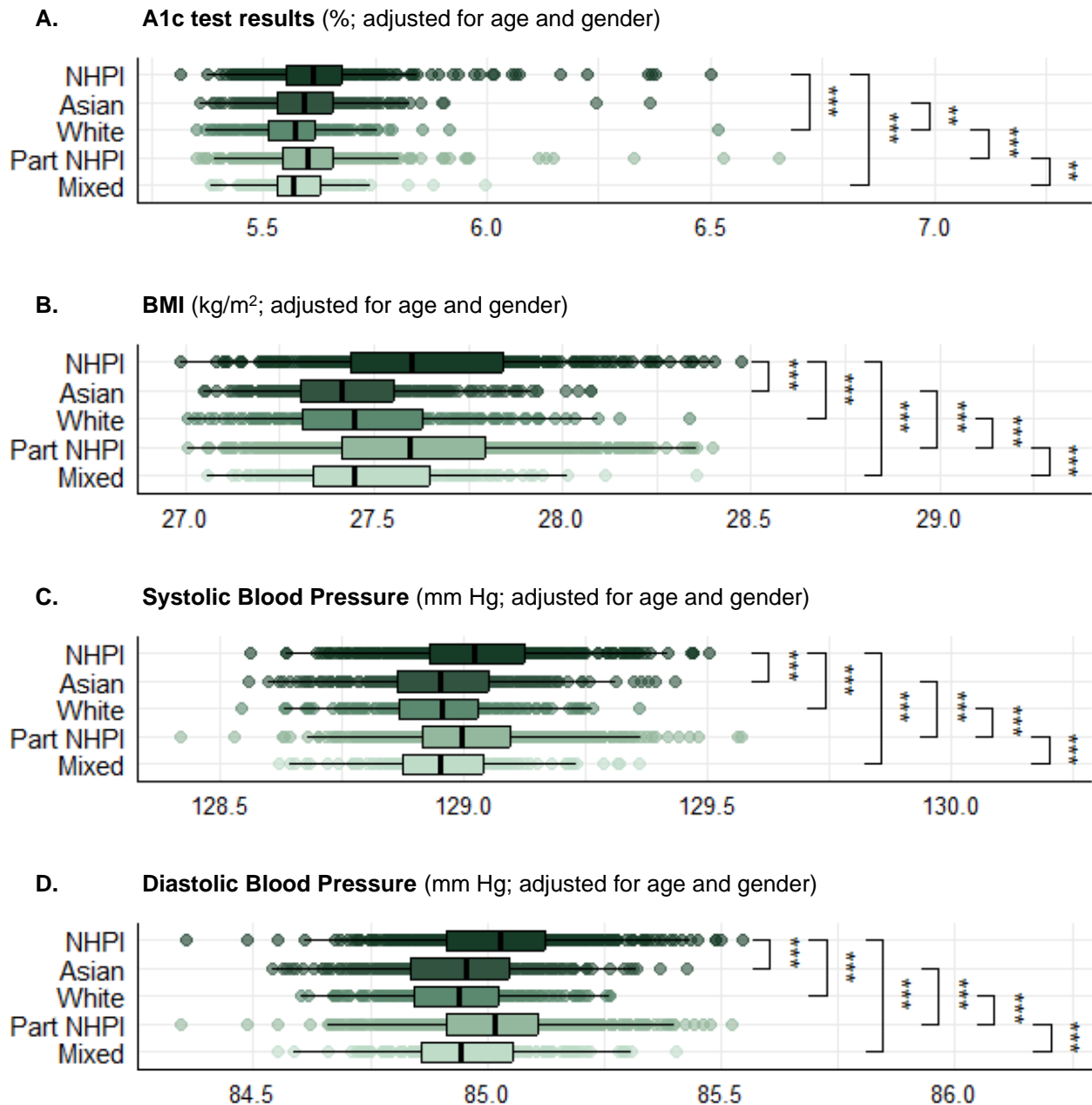
	Total	Racial-Ethnic Groups					P
		NHPI	Asian	White	Part NHPI	Mixed	
<b>Total (N)</b>	1568	348	257	171	638	154	
<b>Gender (N, %)</b>							$\chi^2 = 4.07$ 0.3968 <sup>a</sup>
Men	658 (42.0%)	156 (44.8%)	95 (37.0%)	75 (43.9%)	267 (41.8%)	65 (42.2%)	
Women	910 (58.0%)	192 (55.2%)	162 (63.0%)	96 (56.1%)	371 (58.2%)	89 (57.8%)	
<b>Systolic BP (mm Hg)</b>							$\chi^2 = 114.11$ <0.001 <sup>b</sup>
mean	132.2	140.3	125.1	125.6	134.0	125.2	
SEM	0.57	1.26	1.30	1.49	0.87	1.66	
<b>Diastolic BP (mm Hg)</b>							$\chi^2 = 117.59$ <0.001 <sup>b</sup>
mean	82.3	91.4	81.2	80.8	88.0	82.0	
SEM	0.38	0.83	0.85	0.98	0.58	1.15	
<b>BP Categories (N, %)</b>							$\chi^2 = 118.66$ <0.001 <sup>a</sup>
Low	7 (0.4%)		3 (1.2%)		3 (0.5%)	1 (0.6%)	
Normal	339 (21.6%)	38 (10.9%)	82 (31.9%)	55 (32.2%)	110 (17.2%)	54 (35.1%)	
Elevated	122 (7.8%)	25 (7.2%)	21 (8.2%)	20 (11.7%)	45 (7.1%)	11 (7.1%)	
Hypertension I	398 (25.4%)	77 (22.1%)	72 (28.0%)	48 (28.1%)	163 (25.5%)	38 (24.7%)	
Hypertension II	702 (44.8%)	208 (59.8%)	79 (30.7%)	48 (28.1%)	317 (49.7%)	50 (32.5%)	
<b>A1c level (%)</b>							$\chi^2 = 82.94$ <0.001 <sup>b</sup>
mean	5.70	5.94	5.64	5.49	5.70	5.50	
SEM	0.02	0.05	0.05	0.06	0.03	0.04	
<b>T2DM Status (N, %)</b>							$\chi^2 = 70.648$ <0.001 <sup>a</sup>
Nondiabetic	739 (47.1%)	121 (34.8%)	132 (51.4%)	113 (66.1%)	280 (43.9%)	93 (60.4%)	
Prediabetic	705 (45.0%)	181 (52.0%)	105 (40.9%)	51 (29.8%)	315 (49.4%)	53 (34.4%)	
Diabetic	124 (7.91%)	46 (13.2%)	20 (7.78%)	7 (4.09%)	43 (6.74%)	8 (5.19%)	
<b>BMI (kg/m<sup>2</sup>)</b>							$\chi^2 = 144.75$ <0.001 <sup>b</sup>
mean	29.2	31.6	25.4	26.6	30.6	26.8	
SEM	0.02	0.47	0.34	0.54	0.32	0.52	
<b>BMI Groups (N, %)</b>							$\chi^2 = 139.3$ <0.001 <sup>a</sup>
Underweight	50 (3.19%)	8 (2.30%)	12 (4.67%)	12 (7.02%)	11 (1.72%)	7 (4.55%)	
Normal	520 (33.2%)	75 (21.6%)	134 (52.1%)	75 (43.9%)	170 (26.6%)	66 (42.9%)	
Overweight	393 (25.1%)	90 (25.9%)	65 (25.3%)	42 (24.6%)	161 (25.2%)	35 (22.7%)	
Obese I	281 (17.9%)	66 (19.0%)	31 (12.1%)	21 (12.3%)	130 (20.4%)	33 (21.4%)	
Obese II	159 (10.1%)	53 (15.2%)	11 (4.28%)	12 (7.02%)	75 (11.8%)	8 (5.19%)	
Obese III	165 (10.5%)	56 (16.1%)	4 (1.56%)	9 (5.26%)	91 (14.3%)	5 (3.25%)	
<b>Age (years)</b>							$\chi^2 = 86.74$ <0.001 <sup>b</sup>
mean	38.04	43.7	35.4	36.1	37.5	34.1	
SEM	0.37	0.74	0.92	1.18	0.55	1.15	
<b>Age Groups (N)</b>							$\chi^2 = 120.6$ <0.001 <sup>a</sup>
G1 ( $\leq$ 22 yrs)	298 (19.0%)	27 (7.76%)	52 (20.2%)	50 (29.2%)	124 (19.4%)	45 (29.2%)	
G2 (23-34 yrs)	404 (25.8%)	61 (17.5%)	97 (37.7%)	44 (25.7%)	158 (24.8%)	44 (28.6%)	
G3 (35-42 yrs)	285 (18.2%)	73 (21.0%)	37 (14.4%)	22 (12.9%)	127 (19.9%)	26 (16.9%)	
G4 (43-55 yrs)	369 (23.5%)	114 (32.8%)	41 (16.0%)	29 (17.0%)	161 (25.2%)	24 (15.6%)	
G5 (55+ yrs)	212 (13.5%)	73 (21.0%)	30 (11.7%)	26 (15.2%)	68 (10.7%)	15 (9.74%)	

<sup>a</sup>Chi-squared test for independence. <sup>b</sup>Kruskal-Wallis ANOVA. BP=Blood pressure. yrs=years. Percentages are calculated in columns.

A summary of major T2DM risk factor measures is presented by racial-ethnic group in **Table 2.3**. NHPIs and Part NHPIs (Part/NHPIs) tended to have higher systolic blood pressure (BP) compared to other racial-ethnic groups. NHPIs also had the highest diastolic BP compared to all other groups, among which there were no other significant differences. NHPIs had the highest proportion of individuals with hypertension (HT; 81.9%), followed by Part NHPIs (75.2%). Based on A1c test results, NHPIs also had the highest proportion of individuals that qualify as diabetic (13.2%), while Part NHPIs had a similar rate for diabetic status among other groups (6.74%). NHPIs had the highest proportion of individuals classifying for conventional obesity ( $BMI > 30 \text{ kg/m}^2$ ; 50.3%), and Part NHPIs had the second highest (46.4%). Part/NHPIs represented 69.8% of class I, 80.5% of class II, and 89.1% of class III obesity categories.

A1c test results, BMI, systolic BP, diastolic BP, and age significantly differed between racial-ethnic groups. NHPI and Part NHPI groups had consistently higher BMIs than other racial-ethnic groups. The intergroup differences in A1c and BMI are directionally inconsistent, possibly due to the differential utility of BMI as a predictor of obesity-related health risk between racial-ethnic groups. Members of the NHPI group tended to be older than all other participants, and Part NHPI participants tended to be older than Mixed participants. Thus, it is unclear whether racial-ethnic differences in A1c levels and BP measurements are partially attributed to differences in age, requiring age adjustment for downstream analyses.

After adjusting for age and gender, A1c levels were significantly higher in NHPIs compared to White and Mixed groups (**Figure 2.2A**). There were no significant differences in A1c levels among NHPI, Asian, and Part NHPI groups, suggesting that previously observed differences may have been attributed to intergroup differences in age. BMI still tended to be higher for NHPIs and Part NHPIs compared to all other groups after adjusting for age and gender (**Figure 2.2B**). Interestingly, intergroup differences between NHPI and Part NHPI were nonsignificant for BP only after adjusting for age and gender, revealing that both groups tended to have higher systolic (**Figure 2.2C**) and diastolic (**Figure 2.2D**) BP compared to all other racial-ethnic groups. The interaction effects of age and race-ethnicity on A1c levels are marginally significant ( $P=0.072$ ; two-way ANOVA). Additionally, previous research has demonstrated that age-specific analyses are crucial to health disparities research, including in NHPI communities[136]. To mitigate the confounding effects of age upon intergroup comparisons, age groups were created as an option for downstream analyses: G1 (N=298; aged  $\leq 22$  years), G2 (N=404; aged 23-34 years), G3 (N=285; 35-42 years), G4 (N=369; 43-55 years), and G5 (N=212; aged 55+ years).



**Figure 2.2** Racial-ethnic differences in **(A)** A1c test results, **(B)** BMI, **(C)** systolic BP, and **(D)** diastolic BP after adjusting for age and gender.

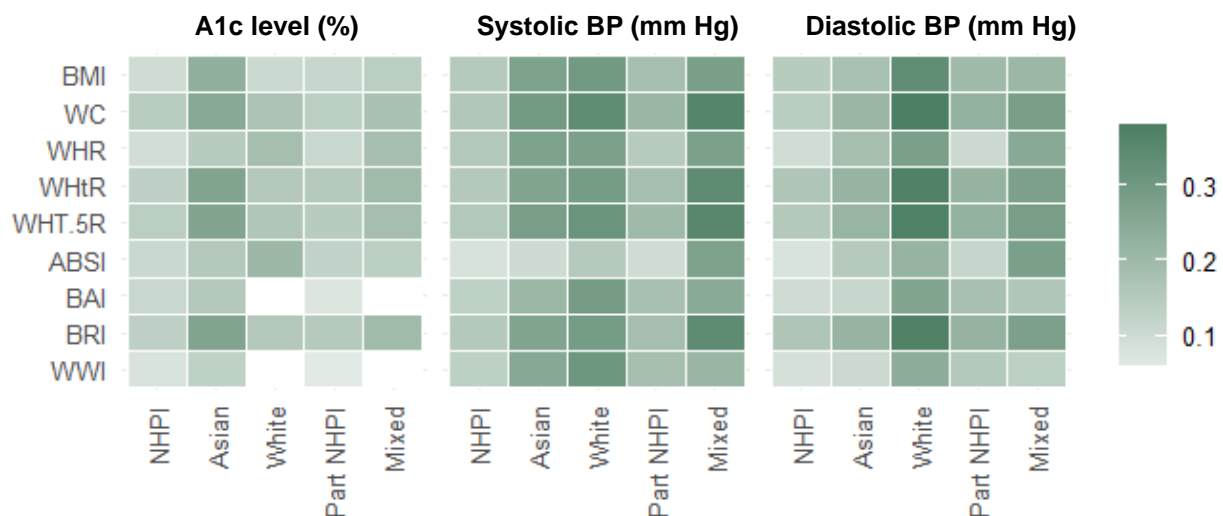
#### 2.4.2 Anthropometric Indices and the Definition of Obesity

BMI may be differentially associated with metabolic health risk between racial-ethnic groups. To assess these associations, correlations between alternative anthropometric indices and clinical health measures were evaluated for each racial-ethnic group using Kendall's  $\tau$  test (summarized in **Table 2.4**; illustrated in **Figure 2.3**). Among significant results, anthropometric indices correlated more strongly with systolic and diastolic BP (median  $\tau=0.248$  and  $0.211$ , respectively) than with A1c levels (median  $\tau=0.149$ ). The

significant correlations between A1c and anthropometric indices tended to be stronger in Asian (median  $\tau=0.232$ ), White (median  $\tau=0.164$ ), and Mixed (median  $\tau=0.187$ ) groups. BRI and WHtR tended to exhibit stronger correlations with A1c than other indices. The same correlations tended to be weaker in the NHPI (median  $\tau=0.113$ ) and Part NHPI (median  $\tau=0.127$ ) groups.

Overall, the strongest (yet moderate) correlations with A1c were observed in the Asian group for BRI ( $\tau=0.265$ ;  $P=5.5E-10$ ), WHtR ( $\tau=0.265$ ;  $P=5.6E-10$ ), WHT.5R ( $\tau=0.262$ ;  $P=9.3E-10$ ), WC ( $\tau=0.251$ ;  $P=7.6E-09$ ), and BMI ( $\tau=0.232$ ;  $P=5.4E-08$ ). Aside from a correlation with ABSI in the White group ( $\tau=0.205$ ;  $P=8.720E-5$ ), all other significant  $\tau$  coefficients were  $< 0.2$ . Among these, the weakest correlations with A1c were observed in Part NHPIs for WWI ( $\tau=0.060$ ;  $P=2.43E-02$ ) and BAI ( $\tau=0.072$ ;  $P=6.98E-03$ ) and NHPIs for WWI ( $\tau=0.079$ ;  $P=2.93E-02$ ) and WHR ( $\tau=0.095$ ;  $P=8.41E-03$ ).

Correlations between systolic BP and anthropometric indices tended to be stronger in Asian (median  $\tau=0.267$ ), White (median  $\tau=0.291$ ), and Mixed (median  $\tau=0.282$ ) groups. Interestingly, WC consistently had the strongest correlation with systolic BP compared to all other indices for all racial-ethnic groups. Like those with A1c, anthropometric correlations with systolic BP tended to be lower in NHPI (median  $\tau=0.155$ ) and Part NHPI (median  $\tau=0.187$ ) groups. The strongest correlations were observed in the Mixed group for WC ( $\tau=0.361$ ;  $P=7.8E-11$ ), WHT.5R ( $\tau=0.354$ ;  $P=1.0E-10$ ), BRI ( $\tau=0.342$ ;  $P=4.2E-10$ ), and WHtR ( $\tau=0.342$ ;  $P=4.2E-10$ ). Correlation coefficients were similarly strong in the White group for WC ( $\tau=0.338$ ;  $P=1.3E-10$ ), WHT.5R ( $\tau=0.316$ ;  $P=1.2E-09$ ), WWI ( $\tau=0.306$ ;  $P=3.5E-09$ ), and BMI ( $\tau=0.297$ ;  $P=1.0E-08$ ). The strongest correlation in the Asian group was for WC ( $\tau=0.296$ ;  $P=5.4E-12$ ). The weakest correlations with systolic BP were observed for ABSI in NHPI ( $\tau=0.082$ ;  $P=2.3E-02$ ), Part NHPI ( $\tau=0.101$ ;  $P=1.5E-04$ ), and Asian groups ( $\tau=0.103$ ;  $P=1.4E-02$ ).



**Figure 2.3** Kendall's  $\tau$  for significant correlations between anthropometric indices and clinical health measures for each racial-ethnic group. Nonsignificant correlations are white.

**Table 2.4** Kendall's  $\tau$  correlation coefficients for anthropometric associations with clinical health measures for each racial-ethnic group.

	NHPI		Asian		White		Part NHPI		Mixed	
	$\tau$	P	$\tau$	P	$\tau$	P	$\tau$	P	$\tau$	P
<b>A1c level</b>										
ABSI	0.110	2.4E-3	0.154	3.0E-4	<b>0.205</b>	<b>8.7E-5</b>	0.127	2.0E-6	0.139	1.2E-2
BAI	0.113	1.8E-3	0.157	2.3E-4	0.092	7.7E-2	0.072	7.0E-3	0.041	4.6E-1
BMI	0.100	5.6E-3	0.232	5.4E-8	0.106	4.2E-2	0.115	1.8E-5	0.140	1.1E-2
BRI	0.135	2.0E-4	<b>0.265</b>	<b>5.5E-10</b>	0.158	2.5E-3	<b>0.153</b>	<b>9.6E-9</b>	<b>0.196</b>	<b>4.1E-4</b>
WC	<b>0.147</b>	<b>5.6E-5</b>	0.251	7.6E-9	0.172	1.2E-3	0.142	1.6E-7	0.177	1.6E-3
WHR	0.095	8.4E-3	0.148	5.2E-4	0.188	3.3E-4	0.113	2.4E-5	0.188	7.2E-4
WHT.5R	0.142	8.5E-5	0.262	9.3E-10	0.164	1.7E-3	0.148	3.0E-8	0.187	7.5E-4
WhtR	0.135	2.0E-4	<b>0.265</b>	<b>5.6E-10</b>	0.158	2.5E-3	<b>0.153</b>	<b>9.6E-9</b>	<b>0.196</b>	<b>4.1E-4</b>
WWI	0.079	2.9E-2	0.133	1.8E-3	0.050	3.4E-1	0.060	2.4E-2	0.049	3.7E-1
<b>Systolic Blood pressure (BP)</b>										
ABSI	0.082	2.3E-2	0.103	1.4E-2	0.153	3.2E-3	0.101	1.5E-4	0.274	5.4E-7
BAI	0.134	2.2E-4	0.209	7.7E-7	0.289	2.7E-8	0.182	9.7E-12	0.248	6.1E-6
BMI	0.153	2.3E-5	0.273	1.0E-10	0.297	1.0E-8	0.187	2.5E-12	0.282	2.5E-7
BRI	0.155	1.8E-5	0.267	2.6E-10	0.291	2.1E-8	0.187	2.5E-12	0.342	4.2E-10
WC	<b>0.162</b>	<b>9.2E-6</b>	<b>0.296</b>	<b>5.4E-12</b>	<b>0.338</b>	<b>1.3E-10</b>	<b>0.212</b>	<b>4.2E-15</b>	<b>0.361</b>	<b>7.8E-11</b>
WHR	0.158	1.2E-5	0.273	1.1E-10	0.277	1.0E-7	0.151	1.5E-8	0.275	5.2E-7
WHT.5R	0.159	1.1E-5	0.283	1.9E-11	0.316	1.2E-9	0.200	5.8E-14	0.354	1.0E-10
WhtR	0.155	1.8E-5	0.267	2.7E-10	0.291	2.1E-8	0.187	2.5E-12	0.342	4.2E-10
WWI	0.136	1.7E-4	0.254	1.7E-9	0.306	3.5E-9	0.188	1.7E-12	0.212	1.1E-4
<b>Diastolic Blood Pressure (BP)</b>										
ABSI	0.079	3.0E-2	0.153	3.0E-4	0.217	3.0E-5	0.118	1.1E-5	0.279	3.7E-7
BAI	0.099	6.1E-3	0.116	6.1E-3	0.262	5.0E-7	0.181	1.4E-11	0.163	2.9E-3
BMI	0.148	4.4E-5	0.180	2.0E-5	0.335	1.2E-10	0.199	1.1E-13	0.209	1.4E-4
BRI	<b>0.166</b>	<b>4.8E-6</b>	<b>0.217</b>	<b>3.1E-7</b>	0.370	1.2E-12	0.220	1.8E-16	0.276	4.8E-7
WC	0.144	8.8E-5	0.211	9.4E-7	<b>0.379</b>	<b>6.9E-13</b>	<b>0.226</b>	<b>6.7E-17</b>	0.281	4.4E-7
WHR	0.096	8.0E-3	0.188	9.3E-6	0.278	9.2E-8	0.108	5.8E-5	0.251	4.9E-6
WHT.5R	0.155	1.8E-5	0.216	3.4E-7	0.374	6.7E-13	0.224	5.0E-17	<b>0.283</b>	<b>2.4E-7</b>
WhtR	<b>0.166</b>	<b>4.8E-6</b>	<b>0.217</b>	<b>3.1E-7</b>	0.370	1.2E-12	0.220	1.8E-16	0.276	4.8E-7
WWI	0.089	1.4E-2	0.108	1.1E-2	0.238	4.9E-6	0.160	2.2E-9	0.137	1.3E-2

Results are sectioned by response variable (A1c level, systolic BP, and diastolic BP). For each outcome, the highest  $\tau$  and its P-value are bolded for each racial-ethnic group.

Diastolic BP correlated more strongly with anthropometric indices for the White (median  $\tau=0.335$ ) and Mixed (median  $\tau=0.276$ ) groups. The same correlations tended to be weaker for Asian (median  $\tau=0.188$ ), NHPI (median  $\tau=0.144$ ), and Part NHPI (median  $\tau=0.199$ ) groups. The strongest correlations were observed in the White group for WC ( $\tau=0.379$ ;  $P=6.9E-13$ ), WHT.5R ( $\tau=0.374$ ;  $P=6.7E-13$ ), BRI ( $\tau=0.370$ ;  $P=1.2E-12$ ), WhtR ( $\tau=0.370$ ;  $P=1.2E-12$ ), and BMI ( $\tau=0.335$ ;  $P=1.2E-10$ ). These coefficients were followed by correlations between diastolic BP and WHT.5R ( $\tau=0.283$ ;  $P=2.4E-07$ ), WC ( $\tau=0.281$ ;  $P=4.4E-07$ ), and ABSI ( $\tau=0.279$ ;  $P=3.7E-07$ ) in the Mixed group.

The weakest correlations were observed in the NHPI group for ABSI ( $\tau=0.079$ ;  $P=3.0E-02$ ), WWI ( $\tau=0.089$ ;  $P=1.4E-02$ ), WHR ( $\tau=0.096$ ;  $P=8.0E-03$ ), and BAI ( $\tau=0.099$ ;  $P=6.1E-03$ ). Intergroup differences suggest that anthropometric indices are differentially effective as health risk indicators by race-ethnicity. Moreover, the strength of significant correlations varies between clinical measures for each group. Racial-

ethnic and community-specific thresholds for obesity may be obtained to be more uniformly informative of obesity-related health risks between groups, improving the metabolic relevance of research outcomes for these communities.

### 2.4.3 Anthropometric Thresholds for Obesity-Related T2DM Risk

To compare the utility of these indices across racial-ethnic groups, the predictive capacity of each anthropometric index was assessed using Receiver Operating Characteristic (ROC) analysis with T2DM status as a binary classifier. Ethnic-specific thresholds, or “cutoffs,” for designating obesity status (comparable to the conventional BMI cutoff at 30 kg/m<sup>2</sup>) were also obtained from these analyses (after adjusting for age and gender; **Table 2.5**). In this context, a ROC curve evaluates the performance of a predictive model by plotting the true positive detection rate (sensitivity) against the true negative detection rate (1 - specificity) for a binary classifier. Its Area Under the Curve (AUC) summarizes the model's capacity to distinguish between the two classes[57]. An AUC of 1 would indicate perfect discriminative ability, while an AUC of 0.5 would indicate random discrimination or no predictive power.

Upon gender stratification, BMI thresholds were higher in NHPI and Part NHPI women (30.46 kg/m<sup>2</sup> and 30.00 kg/m<sup>2</sup>, respectively) than men (29.44 kg/m<sup>2</sup> and 30.00 kg/m<sup>2</sup>, respectively) in the same groups. In contrast, the BMI thresholds were lower in Asian women (23.62 kg/m<sup>2</sup>), White women (24.84 kg/m<sup>2</sup>), and Mixed women (24.81 kg/m<sup>2</sup>) compared to men from the same groups (25.05 kg/m<sup>2</sup>, 25.47 kg/m<sup>2</sup>, and 25.58 kg/m<sup>2</sup>, respectively). Since ROC analyses are inequivalent between racial-ethnic groups due to differential test performance, risk-adjusted means (RAM) were used to compare the relative efficiency of each index within groups[132]. This metric depends on the mean and standard deviations of rank-transformed data within each racial-ethnic subgroup. Lower RAM scores for AUC values indicate more consistency and higher performance of the indices as predictors of T2DM risk.

BRI consistently performed better than other indices for Part NHPI men (AUC=0.703) and women (AUC=0.660). WHR was similarly consistent among men and women from the Mixed group (AUC=0.641 and 0.659, respectively). For NHPIs, BAI was the best T2DM predictor in men (AUC=0.636) but relatively ineffective in women (AUC=0.524). Instead, WHR was the most effective predictor in NHPI women (AUC=0.697) but the least effective in NHPI men (AUC=0.570). For the Asian group, the best T2DM predictor was WC in men (AUC=0.774) and BMI in women (AUC=0.682). For White men, BMI, WHtR, and BRI were equally effective (AUC=0.865), while WHR was the most effective predictor in White women (AUC=0.896). BMI was the most effective predictor of T2DM risk for Asian women (AUC=0.682) but least effective for NHPI women (AUC=0.511). The index with the largest discrepancy in predictive capacity between genders was WHR, a more consistent predictor for T2DM in women (RAM=4.07) than in men (RAM=7.06).

**Table 2.5** ROC-AUC values and corresponding ethnic-specific anthropometric thresholds associated with increased risk of T2DM.

	NHPI		Asian		White		Part NHPI		Mixed		RAM
	AUC	Cutoff	AUC	Cutoff	AUC	Cutoff	AUC	Cutoff	AUC	Cutoff	
<b>Men</b> (age-adjusted; T2DM risk prediction)											
BMI	0.587	29.44	0.701	25.05	<b>0.865</b>	<b>25.47</b>	0.675	28.04	0.547	25.58	5.70
WC	0.624	0.952	<b>0.774</b>	<b>0.917</b>	0.784	1.042	0.683	1.182	0.531	0.896	<b>4.55</b>
WHR	<i>0.570</i>	<i>0.945</i>	0.704	0.956	<i>0.662</i>	<i>0.976</i>	<i>0.593</i>	<i>0.999</i>	<b>0.641</b>	<b>0.936</b>	7.06
WHtR	0.596	0.464	0.749	0.523	<b>0.865</b>	<b>0.683</b>	0.700	0.758	<i>0.516</i>	<i>0.490</i>	5.04
WHT.5R	0.612	7.569	0.759	6.833	0.838	6.958	0.690	7.525	0.563	6.862	<b>3.58</b>
ABSI	0.584	79.56	0.725	78.59	0.649	80.10	0.596	79.11	0.578	78.65	6.74
BAI	<b>0.636</b>	<b>221.7</b>	0.679	187.8	0.649	209.4	0.633	209.0	0.547	206.0	6.37
BRI	0.600	4.756	0.751	3.791	<b>0.865</b>	<b>3.998</b>	<b>0.703</b>	<b>4.929</b>	0.563	3.739	<b>3.16</b>
WWI	0.613	8.763	<i>0.661</i>	<i>7.832</i>	0.757	8.443	0.627	8.672	0.578	8.037	5.98
<b>Women</b> (age-adjusted; T2DM risk prediction)											
BMI	0.511	30.46	<b>0.682</b>	<b>23.62</b>	0.629	24.84	0.629	30.00	0.597	24.81	5.94
WC	0.615	1.106	0.613	1.126	0.748	0.816	0.648	0.972	0.626	0.822	4.32
WHR	<b>0.697</b>	<b>0.938</b>	0.536	0.935	<b>0.896</b>	<b>0.947</b>	0.628	0.938	<b>0.659</b>	<b>0.907</b>	<b>4.07</b>
WHtR	0.605	0.684	0.637	0.849	0.733	0.483	0.658	0.659	0.630	0.537	4.32
WHT.5R	0.608	7.868	0.625	6.743	0.737	6.676	0.653	7.815	0.628	6.596	<b>4.14</b>
ABSI	0.695	79.84	<i>0.453</i>	<i>77.91</i>	0.654	76.85	<i>0.442</i>	<i>78.91</i>	0.580	77.35	7.18
BAI	0.524	208.4	0.607	164.6	<i>0.481</i>	<i>193.1</i>	0.597	202.8	<i>0.557</i>	<i>178.0</i>	8.02
BRI	0.608	5.768	0.638	4.439	0.739	3.668	<b>0.660</b>	<b>5.847</b>	0.637	3.716	<b>2.83</b>
WWI	<i>0.497</i>	<i>7.707</i>	0.656	6.654	0.541	7.500	0.596	7.944	0.562	7.138	7.57

The highest AUC values and their corresponding cutoffs are bolded for each gender and racial-ethnic group. The lowest are italicized.

Anthropometric thresholds for T2DM-related obesity status also differed between genders. WHR, BAI, and WWI cutoffs were consistently higher for men than women for all racial-ethnic groups. In NHPI women, the thresholds for WC (1.106m), WHtR (0.684), WHT.5R (7.868), and BRI (5.768) were notably higher than those in NHPI men (0.952m, 0.464, 7.569, and 4.756, respectively). In Asian women, thresholds were higher for WC (1.126m), WHtR (0.849), and BRI (4.439) compared to those for Asian men (0.917m, 0.523, and 0.3791, respectively). For Part NHPI women, the obesity cutoffs were higher for WHT.5R (7.815) and BRI (5.87) compared to those for Part NHPI men (7.525 and 4.929, respectively). For individuals in the White group, obesity thresholds were consistently higher for all indices in men compared to women. A similar trend was observed in the Mixed group, although the threshold for WHtR was higher for women (0.537) than for men (0.49).

Overall, the indices with the highest ranking in predictive performance and consistency (and the lowest cumulative RAM scores) were BRI (2.98), WHT.5R (3.87), and WC (4.42). The indices with the worst predictive performance (highest cumulative RAM scores) were BAI (7.2), ABSI (6.93), and BMI (5.79). It must be noted that ROC analyses were performed using T2DM as a binary classifier, and the White and Mixed groups had a low N number of diabetic individuals. This small sample size may have compromised the discriminatory capacity of any anthropometric test for T2DM status. Nonetheless, these results indicate that although the utility of anthropometric indices varies between gender and ethnic groups, WC, WHT.5R, and BRI may be more uniformly effective as health risk predictors for this cohort, at least

compared to BMI. This improved consistency may mitigate intergroup biases introduced by differential predictive performance.

#### 2.4.4 Anthropometric Thresholds for Obesity-Related Hypertension (HT)

While hypertension (HT) and T2DM are closely linked to obesity, anthropometric indices may be differentially associated with each outcome. To obtain ethnic-specific anthropometric thresholds associated with increased risk for HT, ROC analyses were performed using HT (which includes HT I and II) as a binary classifier (**Table 2.6**).

Cumulatively, the most consistent and effective predictors of HT (lowest RAM) were BMI (3.30), BRI (3.29), and WHT.5R (4.12). Overall, the least effective predictors (highest cumulative RAM) were ABSI (8.88), WHR (7.20), and BAI (6.86). In men, the most consistently effective predictors of HT risk (lowest RAM) were BMI (3.35), BRI (3.63), and WHT.5R (3.97). In women, the most effective predictors for HT (lowest RAM) were BRI (2.99), BMI (3.30), and WHT.5R (4.28).

**Table 2.6** ROC-AUC values and corresponding ethnic-specific anthropometric thresholds associated with increased risk of hypertension (HT).

	NHPI		Asian		White		Part NHPI		Mixed		RAM
	AUC	Cutoff	AUC	Cutoff	AUC	Cutoff	AUC	Cutoff	AUC	Cutoff	
<b>Men</b> (age-adjusted; HT risk prediction)											
BMI	0.638	29.49	0.608	24.94	0.732	25.26	<b>0.701</b>	<b>27.81</b>	0.595	25.36	<b>3.35</b>
WC	0.616	1.130	0.613	0.788	0.711	0.900	0.696	0.865	0.595	0.797	4.20
WHR	0.568	0.907	<b>0.616</b>	<b>0.888</b>	0.682	0.979	0.622	0.997	<i>0.469</i>	<i>0.967</i>	7.24
WhtR	0.607	0.528	0.610	0.449	<b>0.733</b>	<b>0.446</b>	0.697	0.503	0.579	0.401	5.06
WHT.5R	0.612	7.635	0.614	6.720	0.722	6.783	0.699	7.220	0.586	6.745	<b>3.97</b>
ABSI	<i>0.537</i>	<i>79.60</i>	<i>0.459</i>	<i>78.59</i>	<i>0.522</i>	<i>80.16</i>	<i>0.569</i>	<i>79.07</i>	0.557	78.67	8.89
BAI	0.605	221.8	0.561	187.9	0.640	209.3	0.658	208.7	<b>0.617</b>	<b>205.9</b>	6.79
BRI	0.607	4.864	0.614	3.420	0.732	3.346	0.705	4.271	0.585	3.421	<b>3.63</b>
WWI	<b>0.641</b>	<b>8.807</b>	0.588	7.718	0.701	8.159	0.670	8.532	0.616	7.847	4.94
<b>Women</b> (age-adjusted; HT risk prediction)											
BMI	<b>0.672</b>	<b>30.25</b>	<b>0.654</b>	<b>23.63</b>	0.706	24.73	<b>0.679</b>	<b>30.00</b>	0.668	24.74	<b>3.30</b>
WC	0.663	0.914	0.624	0.892	0.721	0.779	0.656	0.892	0.706	0.800	4.70
WHR	0.619	0.847	0.585	0.901	0.584	0.910	0.548	0.889	<b>0.722</b>	<b>0.919</b>	7.23
WhtR	0.656	0.564	0.642	0.523	0.708	0.551	0.659	0.559	0.701	0.486	4.90
WHT.5R	0.662	7.707	0.633	6.498	0.721	6.648	0.658	7.723	0.706	6.533	<b>4.28</b>
ABSI	<i>0.560</i>	<i>79.80</i>	<i>0.490</i>	<i>77.94</i>	<i>0.483</i>	<i>76.79</i>	<i>0.528</i>	<i>79.02</i>	0.634	77.33	8.89
BAI	0.663	208.4	0.595	164.4	0.701	193.0	0.635	203.0	<i>0.592</i>	<i>178.0</i>	6.96
BRI	0.660	5.469	0.643	3.590	<b>0.740</b>	<b>3.650</b>	0.666	5.576	0.715	3.571	<b>2.99</b>
WWI	0.669	7.991	0.635	6.594	0.681	7.547	0.659	7.898	0.637	7.138	5.06

The highest AUC values and their corresponding cutoffs are bolded for each gender and racial-ethnic group. The lowest are italicized.

Anthropometric indices were better predictors of T2DM than HT for Asian (P=0.004) and White (P=0.017) men. For NHPI, Part NHPI, and Mixed men, these indices were similarly predictive of both outcomes. In men from NHPI (P=0.013), Part NHPI (P=0.009), and Mixed groups (P=0.02), anthropometric thresholds

were significantly higher to associate with HT risk compared to T2DM risk. Index thresholds were significantly lower for HT risk compared to T2DM risk for Asian (P=0.042) and White (P=0.024) men.

Anthropometric indices were slightly more effective as predictors of T2DM than HT in women from the mixed group (P=0.009). For women in other groups, the indices were similarly effective predictors for both outcomes. However, for women from the Asian and Mixed groups, risk-related thresholds were significantly higher for HT than for T2DM (P=0.024 and 0.035, respectively). Ethnic-specific thresholds from ROC analyses corresponded with HT and T2DM risk for Part/NHPI and White women.

#### **2.4.5 Anthropometric Thresholds for Increased Metabolic Health Risk**

Although there were observable differences in anthropometric associations with T2DM and HT as independent outcomes, they are closely linked and tend to present as a comorbidity. However, using the comorbid state of T2DM and HT as a binary classifier would limit the prediction of non-comorbid outcomes. To obtain metabolically relevant thresholds for obesity-related risk without excluding non-comorbid cases, the presentation of either T2DM or HT was considered “metabolically unhealthy” (MU), and the presentation of neither was considered “metabolically healthy” (MH). For each anthropometric index, the smaller of the two previously obtained risk-related thresholds were assessed as predictors for MU. Youden’s J statistic (sensitivity + specificity - 1) for the predictive performance of risk-related thresholds for MU was compared for each anthropometric index across gender and racial-ethnic groups (**Table 2.7**). J statistics were then ranked within each group to obtain a summative RAM score for both genders.

Uniquely in NHPI men, the WWI threshold for obesity performed better than other thresholds (J=0.190; WWI=8.763). However, the highest J Statistic obtained for this group was 0.190, indicating poor overall diagnostic performance of these age-adjusted anthropometric indices as predictors for metabolic health risk. BRI cutoffs were most effective for Asian (J=0.292; BRI=3.420) and Part NHPI (J=0.396; BRI=4.271) men. Uniquely for men in the Mixed group, the ABSI threshold was the most effective dichotomous predictor (J=0.370; ABSI=78.65), although it was the least effective for NHPI, White, and Part NHPI men. Additionally, in men from the Mixed group, the J statistic was negative for WHtR. The most effective and consistent data stratification thresholds were for BRI (RAM=3.29), WHT.5R (RAM=4.33), and WC (RAM=3.90) in men. The least consistent was ABSI (RAM=7.69).

The conventional definition of obesity (30 kg/m<sup>2</sup>) was ineffective as a dichotomous predictor of metabolic risk for any group. For men of all racial-ethnic groups, the conventional BMI threshold had lower J scores than the risk-related cutoffs. However, the conventional cutoff performed almost as well as the group-specific cutoffs for Mixed women (J=0.346 vs. 0.351) and better than the risk-related thresholds obtained for NHPI (J=0.275 vs. 0.269) and White (J=0.468 vs. 0.458). Although, the difference between the

conventional and obtained cutoffs differed by 0.25 kg/m<sup>2</sup> in NHPI women. Additionally, the risk-related threshold for Part NHPI women was equal to 30 kg/m<sup>2</sup>, lending to its accuracy for that group. Notably, BRI performed better than conventional BMI for every group except NHPI men (J=0.157 vs. 0.159) and Part NHPI women (J=0.316 vs. 0.337). WC performed better than conventional BMI for every group except Part NHPI women (J=0.321 vs. 0.337). WHT.5R performed better than conventional BMI for every group except NHPI men (J=0.157 vs. 0.159).

**Table 2.7** Relative performance of the minimum risk-related threshold for each anthropometric index as a predictor of either T2DM or HT (MU) for each gender and racial-ethnic group.

	NHPI		Asian		White		Part NHPI		Mixed		RAM
	J	Cutoffs	J	Cutoffs	J	Cutoffs	J	Cutoffs	J	Cutoffs	
<b>Men</b> (age-adjusted; MU status prediction)											
BMI <sup>a</sup>	0.159	30.00	0.179	30.00	0.255	30.00	0.258	30.00	0.020	30.00	
BMI <sup>b</sup>	0.174	29.44	0.267	24.94	<b>0.403</b>	<b>25.26</b>	0.344	27.81	0.160	25.36	4.26
WC	0.186	0.952	0.246	0.788	0.329	0.900	0.345	0.865	0.275	0.797	<b>3.90</b>
WHR	0.160	0.907	0.271	0.888	0.306	0.976	0.242	0.997	0.090	0.936	6.12
WHT <sub>R</sub>	0.044	0.464	0.141	0.449	0.377	0.446	0.392	0.503	<i>-0.880</i>	<i>0.401</i>	6.50
WHT.5R	0.157	7.569	0.264	6.72	0.350	6.783	0.382	7.220	0.195	6.745	<b>4.33</b>
ABSI	<i>0.024</i>	<i>79.56</i>	0.154	78.59	<i>0.040</i>	<i>80.10</i>	<i>0.128</i>	<i>79.07</i>	<b>0.370</b>	<b>78.65</b>	7.69
BAI	0.127	221.7	0.202	187.8	0.271	209.3	0.266	208.7	0.175	205.9	6.51
BRI	0.157	4.756	<b>0.292</b>	<b>3.420</b>	0.338	3.346	<b>0.396</b>	<b>4.271</b>	0.255	3.421	<b>3.29</b>
WWI	<b>0.190</b>	<b>8.763</b>	<i>0.118</i>	<i>7.718</i>	0.260	8.159	0.248	8.532	0.235	7.847	6.45
<b>Women</b> (age-adjusted; MU status prediction)											
BMI <sup>a</sup>	0.275	30.00	0.232	30.00	0.468	30.00	0.337	30.00	0.346	30.00	
BMI <sup>b</sup>	0.269	30.25	0.288	23.62	0.458	24.73	0.337	30.00	0.351	24.74	4.81
WC	0.344	0.914	0.297	0.892	0.472	0.779	0.321	0.892	<b>0.488</b>	<b>0.800</b>	<b>3.32</b>
WHR	0.263	0.847	0.177	0.901	0.300	0.910	0.171	0.889	0.464	0.907	6.47
WHT <sub>R</sub>	0.256	0.564	<b>0.336</b>	<b>0.523</b>	<b>0.538</b>	<b>0.483</b>	0.322	0.559	0.370	0.486	4.42
WHT.5R	0.362	7.707	0.275	6.498	0.500	6.648	<b>0.340</b>	<b>7.723</b>	0.443	6.533	<b>3.05</b>
ABSI	<i>0.232</i>	<i>79.80</i>	<i>0.087</i>	<i>77.91</i>	0.271	76.79	<i>0.145</i>	<i>78.91</i>	0.378	77.33	8.23
BAI	0.257	208.4	<i>0.087</i>	<i>164.4</i>	0.521	193.0	0.216	202.8	0.240	178.0	6.82
BRI	<b>0.387</b>	4.756	0.274	3.590	0.500	3.650	0.316	5.576	0.443	3.571	<b>3.93</b>
WWI	0.269	7.707	0.188	6.594	<i>0.250</i>	<i>7.500</i>	0.205	7.898	<i>0.206</i>	<i>7.138</i>	7.56

<sup>a</sup>Conventional BMI cutoff (30 kg/m<sup>2</sup>). <sup>b</sup>Gender and ethnic-specific BMI cutoff obtained from risk assessment. J=Youden's J statistic (sensitivity + specificity -1). For each group, the highest J Statistics and their corresponding cutoffs are bolded. The lowest are italicized.

Anthropometric thresholds tended to be more effective as dichotomous predictors of metabolic health status in NHPI women than in NHPI men. For NHPI women, a BRI threshold of 5.469 had the highest predictive capacity compared to thresholds obtained for other indices (J=0.387). Additionally, in NHPI women, risk-related thresholds tended to be higher than those for women in other groups. For Asian and White women, WHT<sub>R</sub> was the best predictor for MU status (J=0.336 and 0.538, respectively), and the threshold was higher for Asian women (WHT<sub>R</sub>=0.523) compared to White women (WHT<sub>R</sub>=0.483). For Part NHPI women, WHT.5R was the best dichotomous predictor (J=0.340; WHT.5R=7.723). For women in the Mixed group, WC was the best predictor (J=0.488; WC=0.8 m). Overall, the most consistent and effective predictors for MU status in women were BRI (RAM=2.83), WHR (RAM=4.07), and WHT.5R (RAM=4.14).

Consistently, BRI had the highest relative predictive performance and the lowest relative variance across gender and racial-ethnic groups. It was the best overall predictor of T2DM for both men and women. It performed similarly well as a predictor for HT, as it was the most consistent predictor across racial-ethnic groups for women. In men, BMI performed slightly better overall as a predictor of HT. BMI was one of the better predictors of metabolic outcomes among the anthropometric indices analyzed.

Nonetheless, BRI was the more favorable predictor. Intergroup differences in the predictive capacity of any anthropometric index may compromise the metabolic relevance of generalized study findings. These gender and ethnic-specific, risk-related thresholds for BRI were used to define obesity related to metabolic health risk within this community.

#### **2.4.6 Community-Specific Trends in Metabolically Healthy Obesity**

Using the risk-related thresholds obtained for BRI, participants were stratified into obesity (OB) and non-obese (NO) groups. Participants were then stratified into metabolically healthy (MH) and metabolically unhealthy (MU) categories depending on the presentation of either T2DM or HT (**Table 2.8**). Accordingly, systolic BP ( $\chi^2=699.6$ ;  $P < 2.2E-16$ ), diastolic BP ( $\chi^2=866.3$ ;  $P < 2.2E-16$ ), and A1c levels ( $\chi^2=299.2$ ;  $P < 2.2E-16$ ) significantly differed across metabolic health risk groups.

Using risk-related, ethnic-specific BRI cutoffs as thresholds for data stratification yielded significantly different results from those obtained using conventional BMI cutoffs (30 kg/m<sup>2</sup>;  $\chi^2=596.38$ ,  $P < 2.2E-16$ ). The conventional BMI threshold differentially characterized obesity-related health risk for each of the NHPI ( $\chi^2=174.7$ ;  $P < 2.2E-16$ ), White ( $\chi^2=44.3$ ;  $P=2.8E-11$ ), Asian ( $\chi^2=41.0$ ;  $P=1.5E-10$ ), Part NHPI ( $\chi^2=343.6$ ;  $P < 2.2E-16$ ), and Mixed groups ( $\chi^2=39.6$ ;  $P=3.1E-10$ ).

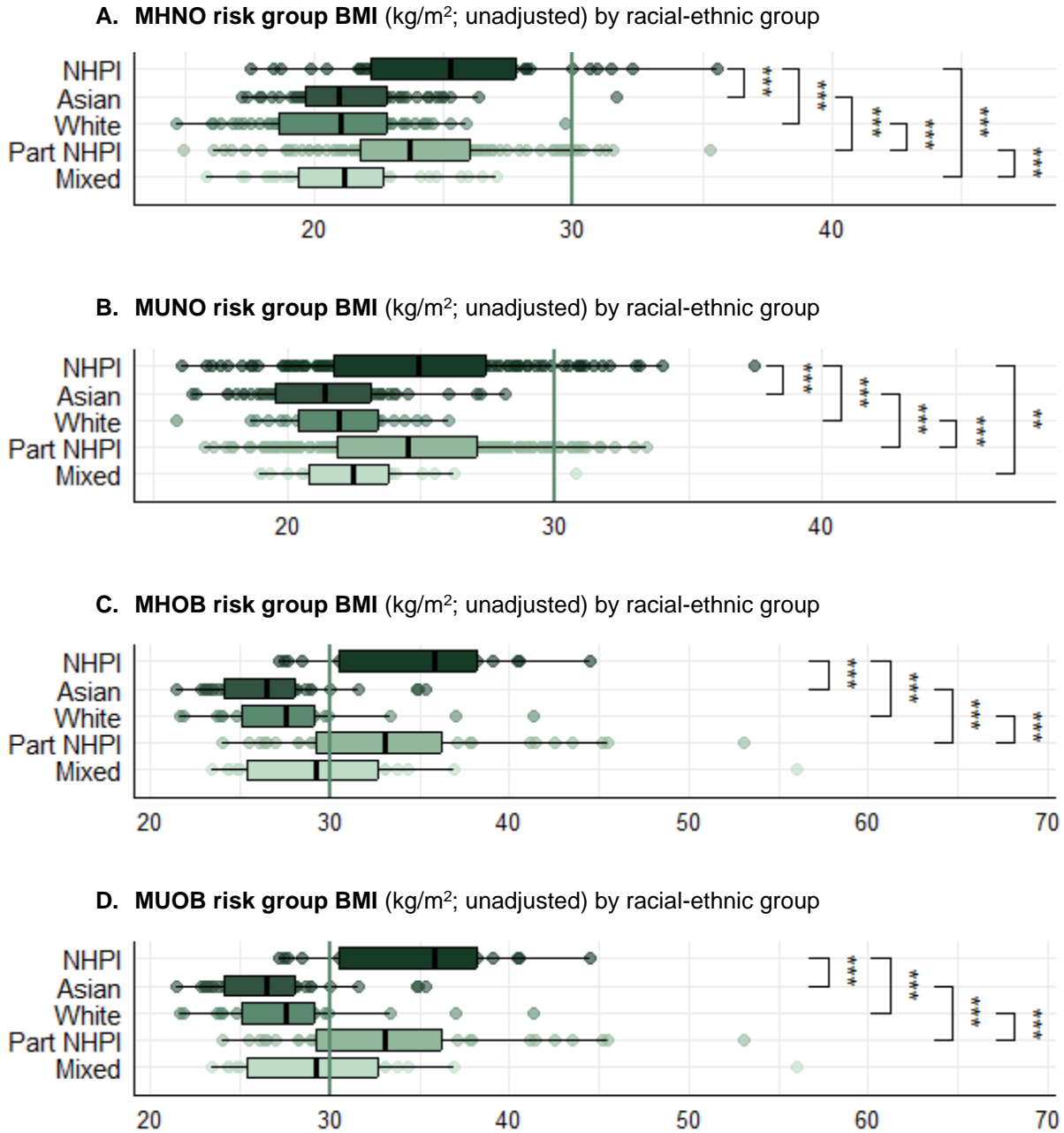
NHPIs and Part NHPIs had the least representation in the MHNO group (11.5% and 17.1%, respectively). They also had the highest representation in the MUNO group (34.8% and 29.5%, respectively) and the lowest relative representation in the MHOB group (4.89% and 6.27%, respectively). Additionally, NHPIs and Part NHPIs had similarly high representation in the MUOB group (48.9% and 47.2%, respectively).

To further illustrate intergroup discrepancies in the definition of obesity, BMI was compared across racial-ethnic groups within metabolic risk categories. In the MHNO group, NHPIs and Part NHPIs tended to have higher BMIs compared to all other groups (**Figure 2.4A**), some of whom would have qualified as obese under conventional standards. This trend was similarly observed in the MUNO group (**Figure 2.4B**), although there was no significant difference in BMI between Part NHPI and Mixed groups. For both the MHOB (**Figure 2.4C**) and MUOB (**Figure 2.4D**) groups, NHPIs and Part NHPIs had significantly higher BMIs than individuals in the Asian and White groups. In contrast, White and Asian individuals qualified for MHOB and MUOB groups at a BMI below 30 kg/m<sup>2</sup>.

**Table 2.8** Obesity and metabolic health (MH/OB) groups stratified by ethnic-specific thresholds.

	Total	Metabolic health risk groups				P
		MHNO	MHOB	MUNO	MUOB	
<b>Total (N; %)</b>	1568	305 (19.5%)	139 (8.86%)	413 (26.3%)	711 (45.3%)	
<b>Racial-Ethnic Group (N; %)</b>					$\chi^2=93.68$	9.5E-15 <sup>a</sup>
NHPI	348 (22.2%)	40 (11.5%)	17 (4.89%)	121 (34.8%)	170 (48.9%)	
Asian	257 (16.4%)	64 (24.9%)	37 (14.4%)	54 (21.0%)	102 (39.7%)	
White	171 (10.9%)	52 (30.4%)	22 (12.9%)	26 (15.2%)	71 (41.5%)	
Part NHPI	638 (40.7%)	109 (17.1%)	40 (6.27%)	188 (29.5%)	301 (47.2%)	
Mixed	154 (9.82%)	40 (26.0%)	23 (14.9%)	24 (15.6%)	67 (43.5%)	
<b>Gender (N; %)</b>					$\chi^2=10.08$	0.01791 <sup>a</sup>
Male	658 (42.0%)	104 (15.8%)	58 (8.81%)	179 (27.2%)	317 (48.1%)	
Female	910 (58.0%)	201 (22.0%)	81 (8.90%)	234 (25.7%)	394 (43.3%)	
<b>Systolic BP (mm Hg)</b>					$\chi^2=699.6$	< 2.2E-16 <sup>b</sup>
mean	132.2	110.1	113.5	137.4	142.2	
SEM	0.567	0.613	0.890	0.971	0.771	
<b>Diastolic BP (mm Hg)</b>					$\chi^2=866.3$	< 2.2E-16 <sup>b</sup>
mean	86.30	69.83	72.04	91.12	93.33	
SEM	0.378	0.383	0.545	0.595	0.478	
<b>BP Categories (N; %)</b>					$\chi^2=1508$	< 2.2E-16 <sup>a</sup>
Low	7 (0.45%)	5 (71.4%)	2 (28.5%)			
Normal	122 (7.78%)	67 (54.9%)	44 (36.0%)	1 (0.82%)	10 (8.20%)	
Elevated	339 (21.6%)	233 (68.7%)	93 (27.4%)	3 (0.88%)	10 (2.95%)	
Hypertension I	398 (25.3%)			186 (46.7%)	212 (53.2%)	
Hypertension II	702 (44.7%)			223 (31.7%)	479 (68.2%)	
<b>A1c level (%)</b>					$\chi^2=299.2$	1.61E-11 <sup>b</sup>
mean	5.70	5.39	5.48	5.67	5.90	
SEM	0.02	0.02	0.04	0.04	0.03	
<b>T2DM Status (N; %)</b>					$\chi^2=125.2$	< 2.2E-16 <sup>a</sup>
Nondiabetic	739 (47.1%)	204 (27.6%)	76 (10.3%)	204 (27.6%)	255 (34.5%)	
Prediabetic	705 (45.0%)	101 (14.3%)	63 (8.94%)	181 (25.7%)	360 (51.1%)	
Diabetic	124 (7.91%)			28 (22.6%)	96 (77.4%)	
<b>BRI</b>					$\chi^2=1170$	3.6E-15 <sup>b</sup>
mean	5.161	2.883	5.676	3.354	7.086	
SEM	0.067	0.051	0.159	0.049	0.091	
<b>BMI (kg/m<sup>2</sup>)</b>					$\chi^2=857.9$	< 2.2E-16 <sup>b</sup>
mean	29.16	22.68	30.50	23.95	34.69	
SEM	0.203	0.206	0.525	0.183	0.281	
<b>Conventional BMI Groups (N; %)</b>					$\chi^2=596.38$	< 2.2E-16 <sup>a</sup>
NO < 30 kg/m <sup>2</sup>	963 (61.4%)	292 (30.3%)	80 (8.31%)	384 (39.8%)	207 (21.5%)	
OB ≥ 30 kg/m <sup>2</sup>	605 (38.6%)	13 (2.15%)	59 (9.75%)	29 (4.79%)	504 (83.3%)	
<b>Age (years)</b>					$\chi^2=273.3$	< 2.2E-16 <sup>b</sup>
mean	38.0	29.0	34.5	38.7	42.2	
SEM	0.37	0.68	1.21	0.69	0.52	
<b>Age Groups (N; %)</b>					245.99	< 2.2E-16 <sup>a</sup>
G1 (≤ 22 yrs)	298 (19.0%)	133 (44.6%)	42 (14.0%)	58 (19.4%)	65 (21.8%)	
G2 (23-34 yrs)	404 (25.7%)	96 (23.7%)	37 (9.16%)	115 (28.4%)	156 (38.6%)	
G3 (35-42 yrs)	285 (18.1%)	29 (10.1%)	21 (7.37%)	73 (25.6%)	162 (56.8%)	
G4 (43-55 yrs)	369 (23.5%)	39 (10.5%)	25 (6.78%)	113 (30.6%)	192 (52.0%)	
G5 (55+ yrs)	212 (13.5%)	8 (3.77%)	14 (6.60%)	54 (25.4%)	136 (64.1%)	

<sup>a</sup>Chi-squared test for independence. <sup>b</sup>Kruskal-Wallis rank sum test. Percentages were calculated by row.



**Figure 2.4** Racial-ethnic differences in BMI in **(A)** metabolically healthy non-obese (MHNO), **(B)** metabolically unhealthy non-obese (MUNO), **(C)** metabolically healthy obese (MHOB), and **(D)** metabolically unhealthy obese (MUOB) risk groups. A solid green line indicates BMI=30 kg/m<sup>2</sup>.

Gender was differentially distributed across MH/OB groups for all race-ethnicities ( $\chi^2=10.08$ ;  $P=0.018$ ), and women tended to represent a larger proportion of MU status than men. Of members in the G1 age group, Part NHPs had the highest representation of MUNO (25.8%), and NHPs had the highest representation of MUOB (40.7%). For G2, NHPs had the highest representation of MUNO (41.0%), and Part NHPs had the highest MUOB (45.6%). Generally, the Part/NHPI groups tended to have a consistently high representation of MU status for all age groups. The interaction between age and race-ethnicity was marginally significant for A1c levels ( $F=1.56$ ,  $P=0.072$ ; Two-way ANOVA) but nonsignificant for systolic and diastolic BP.

MU status was consistently higher in Part/NHPs. In non-NHPI groups, obesity status in the MU risk groups tended to skew toward OB rather than NO. In contrast, obesity status was comparatively more evenly distributed in Part/NHPs with MU status. These trends illustrate that obesity status is more closely linked to metabolic risk in Asian, White, and Mixed groups, as there is a higher tendency for comorbidity in these groups. This observation is consistent with previous results, as correlations between anthropometric indices and clinical measures were significantly weaker in NHPs compared to other racial-ethnic groups (Kendall's  $\tau$ ; **Table 2.4**).

#### **2.4.7 NHPI-Specific Trends in Metabolic Risk Factors**

Metabolic risk factor trends for NHPs and non-NHPs are presented in **Table 2.9**.

For NHPs, gender did not significantly differ across MH/OB risk groups, although about half of the women in the NHPI cohort qualified for MUOB (50.5%). As aforementioned, participants in the NHPI group tended to be older than participants in other racial-ethnic groups ( $\chi^2=95.28$ ;  $P<0.001$ ). The interaction effect between age group and MH/OB risk was significant for diastolic BP ( $F=1.86$ ;  $P=0.039$ ) but nonsignificant for A1c level and systolic BP. Tobacco use was more prevalent in NHPs compared to non-NHPs ( $\chi^2=100.53$ ,  $P<0.001$ ). Interaction effects between racial-ethnic groups and tobacco use were significant for diastolic BP ( $F=0.289$ ;  $P=0.003$ ).

In NHPs, the majority of tobacco users were MU (84.7%), but obesity status did not differ among MU tobacco users. The group of tobacco non-users still had a higher relative representation in the MUOB risk group compared to tobacco users (55.0% and 43.6%, respectively). However, the difference in tobacco use across NHPI MH/OB risk groups was only marginally significant ( $\chi^2=11.98$ ;  $P=0.062$ ).

**Table 2.9** NHPI-specific trends in metabolic health risk factors.

	Non-NHPI	NHPI	P <sup>a</sup>	NHPI Metabolic Risk Groups				P <sup>a</sup>
				MHNO	MHOB	MUNO	MUOB	
<b>Total</b>	582	348		40 (11.5%)	17 (4.9%)	121 (34.8%)	170 (48.9%)	
<b>Gender</b>		$\chi^2=1.59$	0.207				$\chi^2=2.93$	0.402
Male	235 (40.4%)	156 (44.8%)		15 (9.6%)	10 (6.4%)	58 (37.2%)	73 (46.8%)	
Female	347 (59.6%)	192 (55.2%)		25 (13.0%)	7 (3.6%)	63 (32.8%)	97 (50.5%)	
<b>Age group</b>		$\chi^2=95.28$	<0.001				$\chi^2=31.49$	0.002
G1 ( $\leq 22$ )	147 (25.3%)	27 (7.76%)		10 (37.0%)	2 (7.4%)	4 (14.8%)	11 (40.7%)	
G2 (23-34)	185 (31.8%)	61 (17.5%)		8 (13.1%)	4 (6.6%)	25 (41.0%)	24 (39.3%)	
G3 (35-42)	85 (14.6%)	73 (21.0%)		6 (8.2%)	4 (5.5%)	21 (28.8%)	42 (57.5%)	
G4 (43-55)	94 (16.2%)	114 (32.8%)		13 (11.4%)	6 (5.3%)	44 (38.6%)	51 (44.7%)	
G5 (55+)	71 (12.2%)	73 (21.0%)		3 (4.1%)	1 (1.4%)	27 (37.0%)	42 (57.5%)	
<b>Tobacco use</b>		$\chi^2=100.53$	<0.001				$\chi^2=11.98$	0.062
Never	441 (76.0%)	151 (43.4%)		21 (13.9%)	6 (4.0%)	41 (27.2%)	83 (55.0%)	
1-2/month	31 (5.34%)	32 (9.20%)		5 (15.6%)		12 (37.5%)	15 (46.9%)	
3+/month	108 (18.6%)	163 (46.8%)		14 (8.6%)	11 (6.7%)	67 (41.1%)	71 (43.6%)	
NA	2	2				1	1	
<b>Alcohol use</b>		$\chi^2=41.99$	4.0E-9				$\chi^2=11.39$	0.249
Never	257 (44.3%)	214 (62.0%)		24 (11.2%)	11 (5.1%)	68 (31.8%)	111 (51.9%)	
1-2/month	169 (29.1%)	95 (27.5%)		11 (11.6%)	4 (4.2%)	39 (41.1%)	41 (43.2%)	
3-4/month	63 (10.9%)	19 (5.51%)			1 (5.3%)	9 (47.4%)	9 (47.4%)	
4+/month	91 (15.7%)	17 (4.93%)		5 (29.4%)	1 (5.9%)	4 (23.5%)	7 (41.2%)	
NA	2	3				1	2	
<b>Activity (Moderate)<sup>b</sup></b>		$\chi^2=32.31$	1.6E-6				$\chi^2=35.21$	4.3E-4
Rarely	58 (25.1%)	80 (44.7%)		11 (13.8%)	4 (5.0%)	19 (23.8%)	46 (57.5%)	
2-3/month	42 (18.2%)	28 (15.6%)		4 (14.3%)	1 (3.6%)	9 (32.1%)	14 (50.0%)	
1/week	87 (37.7%)	65 (36.3%)		4 (6.2%)	3 (4.6%)	22 (33.8%)	36 (55.4%)	
2-4/week	28 (12.1%)	4 (2.23%)		3 (75.0%)	1 (25.0%)			
4+/week	16 (6.93%)	2 (1.12%)		2 (100.0)				
NA	351	169		16	8	71	74	
<b>Activity (Vigorous)<sup>b</sup></b>		$\chi^2=20.87$	3.4E-4				$\chi^2=18.17$	0.006
Rarely	173 (51.3%)	123 (68.7%)		12 (9.8%)	8 (6.5%)	28 (22.8%)	75 (61.0%)	
2-3/month	4 (1.19%)	2 (1.12%)		1 (50.0%)	1 (50.0%)			
1/week	15 (4.45%)							
2-4/week	140 (41.5%)	54 (30.2%)		9 (16.7%)	4 (7.4%)	21 (38.9%)	20 (37.0%)	
4+/week	5 (1.48%)							
NA	245	169		18	4	72	75	
<b>Diet (Vegetables)<sup>b,c</sup></b>		$\chi^2=46.69$	4.0E-10				$\chi^2=4.03$	0.9096
Never	21 (3.63%)	38 (11.1%)		5 (13.2%)	2 (5.3%)	11 (28.9%)	20 (52.6%)	
Sometimes	221 (38.2%)	176 (51.5%)		23 (13.1%)	8 (4.5%)	63 (35.8%)	82 (46.6%)	
Often	205 (35.5%)	85 (24.9%)		7 (8.2%)	4 (4.7%)	27 (31.8%)	47 (55.3%)	
Always	131 (22.7%)	43 (12.6%)		5 (11.6%)	3 (7.0%)	17 (39.5%)	18 (41.9%)	
NA	4	6				3	3	

<sup>a</sup>Chi-squared test for independence. <sup>b</sup>Frequency-related questions answered on a Likert scale. <sup>c</sup>Question: How often did you eat cooked vegetables (in the past month)? Percentage values were calculated by column for racial-ethnic comparisons and by row for MH/OB risk group comparisons. NA=No survey response provided for N number of participants (italicized).

Alcohol use was less prevalent in NHPIs compared to non-NHPIs ( $\chi^2=41.99$ ;  $P=4.0E-9$ ), as 62% of NHPI participants never drink alcohol. There was no significant difference in alcohol use across NHPI MH/OB risk groups. Obesity status seemed evenly distributed among MU individuals who drink 1-2 (NO=41.1%; OB=43.2%) and 3-4 (NO=47.4%; OB=47.4%) times per month. Of NHPI individuals who drink 4+ times per month, 29.4% were MHNO (N=5), 23.5% were MUNO (N=4), and 41.2% were MUOB (N=7). The behavioral risk factor with the largest discrepancy between racial-ethnic groups was physical activity levels. For moderate exercise, 44.7% of NHPI participants and 25.1% of non-NHPI participants exercise at a moderate level less than twice per month. NHPI individuals who exercise vigorously less than twice per month were largely represented by the MUOB group (61.0%). Individuals who got vigorous exercise 2-4 times per week were largely represented by MU individuals (MUNO=38.9%; MUOB=37.0%). Interestingly, there was a significant difference in the frequency of vegetable consumption between NHPIs and non-NHPIs ( $\chi^2=46.69$ ;  $P=4.0E-10$ ), but no significant differences within the NHPI group by MH/OB risk. For NHPIs, most participants in every metabolic risk group tended to “never” (11.1%) or “sometimes” (51.5%) consume vegetables over a month.

#### **2.4.8 Socioeconomic and Mental Health Risk Factors**

In addition to behavioral factors, socioeconomic and mental health factors may also be associated with metabolic health risks. Interaction effects between racial-ethnic groups and socioeconomic factors, including household income and education, were nonsignificant for A1c, systolic BP, and diastolic BP. The only exception to this pattern was the interaction between household income and race-ethnicity for diastolic BP ( $F=1.870$ ;  $P=0.0114$ ), although 423 observations were missing whenever participants declined to respond to socioeconomic questions.

However, these factors were significantly correlated with metabolic outcomes. Household income ( $\tau=-0.174$ ;  $P=1.2E-10$ ), highest grade completed ( $\tau=-0.145$ ;  $P=4.9E-10$ ), mom’s education ( $\tau=-0.192$ ;  $P=3.3E-12$ ), and dad’s education ( $\tau=-0.159$ ;  $P=4.5E-8$ ) were each negatively correlated with MU status. Although these factors did not significantly vary across MH/OB risk groups in NHPIs, household income ( $\chi^2=96.3$ ;  $P<2.2E-16$ ), highest grade completed ( $\chi^2=241.7$ ,  $P<2.2E-16$ ; excluding participants younger than 18 years), mom’s education ( $\chi^2=195.9$ ;  $P<2.2E-16$ ) and dad’s education ( $\chi^2=214.8$ ;  $P<2.2E-16$ ) were significantly different between NHPI and non-NHPI groups. Nonetheless, these significant results were obtained using bivariate tests, and their associations with health are not so straightforward. Further investigation is necessary to elucidate the complex relationships among socioeconomic factors and MU risk in health-disparate populations. Mental health risk factors were also significantly associated with MU status. Responses to the question, “During the past year, did you ever feel sad or hopeless for more than 2 weeks or felt as if you had nothing to look forward to?” were positively associated with MU ( $\chi^2=308.9$ ;  $P<2.2E-16$ ; McNemar’s test). The same trends were observed for the questions regarding intentional self-harm ( $\chi^2=805.8$ ;  $P<2.2E-16$ ) and previous thoughts of suicide ( $\chi^2=903.9$ ;  $P<2.2E-16$ ).

Self-esteem (SE) and mental health-related survey responses indicate similar associations. After conversion to binary variables (agree vs. disagree), SE and mental health-related survey responses were used as predictor variables in a binomial regression model for MU status (**Table 2.10**). In the total cohort, agreeing to the statement, “I feel like I do not have much to be proud of,” was *negatively* associated with MU risk ( $\beta=-0.400$ ;  $P=0.046$ ). However, identifying with the statement, “Felt sad or hopeless for more than two weeks [within the past year],” was positively associated with MU risk ( $\beta=-0.400$ ;  $P=0.046$ ). While individually, each type of SE/mental health-related survey response significantly correlated with MU status, additional factors may be involved in their relationship with metabolic health outcomes. Moreover, cumulative SE scores were not significantly correlated with MU status and did not vary with MU risk or by racial-ethnic group.

**Table 2.10** Self-esteem (SE) and mental health-related survey responses associated with MU risk.

	Binomial Regression				McNemar	
	Coeff.	SE	Z value	Pr(> z )	$\chi^2$	P
<b>Self-Esteem (SE) Statements</b>						
I'm a person of worth, on an equal plane with others.	0.293	0.279	1.048	0.295	181.1	<0.001
I feel that I have a number of good qualities.	-0.302	0.364	-0.829	0.407	245.3	<0.001
All in all, I am inclined to feel that I am a failure.	-0.333	0.195	-1.707	0.088	21.77	<0.001
I am able to do things as well as most other people.	0.455	0.247	1.842	0.066	164.65	<0.001
I feel I do not have much to be proud of.	-0.400	0.200	-1.996	0.046	19.41	<0.001
I take a positive attitude toward myself.	0.299	0.237	1.261	0.207	84.05	<0.001
On the whole, I am satisfied with myself.	-0.326	0.207	-1.573	0.116	19.55	<0.001
I wish I could have more respect for myself.	0.260	0.152	1.710	0.087	162.4	<0.001
I certainly feel useless at times.	0.257	0.181	1.418	0.156	37.21	<0.001
At times, I think I am not good at all.	0.203	0.192	1.059	0.290	8.522	0.004
<b>Mental Health-Related Statements<sup>a</sup></b>						
Felt sad or hopeless for more than two weeks	0.327	0.162	2.014	0.044	308.88	<0.001
Previous history of intentional self-harm	-0.028	0.217	-0.130	0.897	805.77	<0.001
Seriously contemplated suicide	0.365	0.271	1.349	0.177	903.91	<0.001

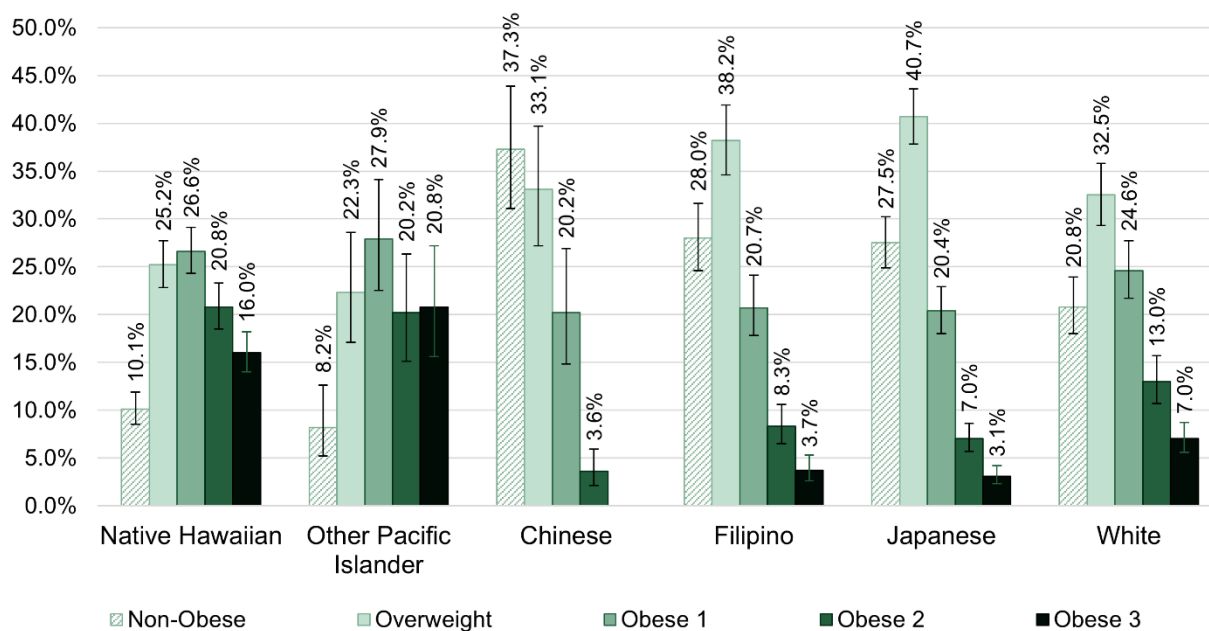
<sup>a</sup>Responses were “Yes/No” regarding the past year. Coeff.=Estimated coefficient. SE=Standard error. Binomial regression model results are presented after adjustment for age and gender.

## 2.5 Discussion

In this cohort, NHPIs tended to be older than individuals from other racial-ethnic groups. To mitigate confounding effects of age, and because age-specific analyses are crucial to health disparities research, including in NHPI communities[136]. After adjusting for age and gender, NHPIs and Part NHPIs (Part/NHPIs) had consistently higher BMI, systolic BP, and diastolic BP than other groups. Despite significantly reduced BMI and BP compared to Part/NHPIs, the Asian group had comparably high A1c test results (**Figure 2.3**). Preliminary correlation tests between anthropometric indices and A1c levels were strongest for the Asian group but comparably weak in Part/NHPI groups (**Figure 2.4; Table 2.3**). Regarding BP, anthropometric indices showed the strongest correlations in Asian, White, and Mixed groups, yielding weak associations for Part/NHPIs.

Ethnic-specific obesity thresholds associated with heightened T2DM risk tended to be *higher* for Part/NHPI men and women (**Table 2.5**). Originally, the expected outcome was for risk-related thresholds to have been lower for Part/NHPIs, limiting the amount of fat accumulation that could occur before facing an increased risk for T2DM. Additionally, ethnic-specific thresholds tended to be lowest for the Asian group, which was associated with heightened MU risk (**Table 2.7**). Notably, risk-related BMI thresholds for obesity were closest to the conventional definition of obesity for Part/NHPIs (~30kg/m<sup>2</sup>), while those for Asian and White groups were closest to the conventional definition of overweight (~25kg/m<sup>2</sup>).

These trends are consistent with those for categorical BMI distribution among diabetic individuals from different racial-ethnic groups in the State of Hawai'i (2012-2022; **Figure 2.5**)[137]. Among Filipino, Japanese, and White groups, the highest proportion of diabetic individuals had a BMI that fell in the “overweight” category (38.2%, 40.7%, and 32.5%, respectively). Although the Chinese group had a similarly high proportion of diabetic individuals with an overweight BMI (33.1%), most of the diabetic individuals in this group were in the “standard” BMI category (37.3%). In contrast, NHPIs had a more even distribution of BMI categories among diabetic individuals. Although the largest percentage of NHPI individuals had a conventionally obese BMI (NH=26.6%, PI=27.9%), these groups also had high representation in the Obese II (NH=20.8%; PI=20.2%) and Obese III (NH=16.0%; PI=20.8%) categories. This discrepancy may be due to the increased prevalence of “obesity” in NHPIs, as defined by BMI.



**Figure 2.5** Body mass index (BMI) category distribution among diabetic individuals in Hawai'i by racial-ethnic group (2012-2022)[137].

Anthropometric indices are differentially predictive of clinical measures across gender and racial-ethnic groups. In NHPI men, the best predictor of T2DM was BAI (AUC=0.636; **Table 2.5**). In the same group, the best predictor of HT was WWI (AUC=0.641; **Table 2.6**), the worst predictor of T2DM in NHPI women (AUC=0.497). Also, in NHPI women, the best predictor of T2DM was WHR (AUC=0.697), the worst predictor for NHPI men (AUC=0.570). At the same time, the best predictor for HT in NHPI women was BMI (AUC=0.642). Overall, the most consistently effective predictor of either T2DM or HT risk across gender and racial-ethnic groups was BRI.

However, anthropometric indices were generally ineffective as predictors of T2DM risk in NHPs compared to other racial-ethnic groups. For NHPI men, the maximum J statistic was 0.190, which was obtained using WWI. For comparison, the maximum J statistic for White men was 0.403 (BMI), for Part NHP men was 0.396 (BRI), and for Mixed men was 0.370 (ABSI). Interestingly, ABSI was the overall worst predictor of MU risk but was the best predictor for MU, specifically in men from the Mixed group (J=0.370). Anthropometric indices performed slightly better as MU predictors in NHPI women but much worse for Asian women.

This variable predictive capacity suggests that the relationship between anthropometric measures may not be as strong in NHPs and Asians as it is in the White group. Perhaps obesity-related disease etiology cannot be sufficiently gleaned from anthropometric data. The relationship between anthropometric indices and adipose tissue volume varies between racial-ethnic groups. As this relationship is relatively uncharacterized in NHPs, so is the relationship between adipose tissue and metabolic risk, which may also vary by race-ethnicity[107]. Thus, community-specific research is required to characterize racial-ethnic differences in obesity-related disease risk effectively.

Ethnic-specific BRI thresholds differentially distributed participants across obesity categories and were more effective at discriminating by MU status than BMI ( $\chi^2=596.38$ ,  $P<0.001$ ; **Table 2.8**). NHPs and Part NHPs had the least representation in the MHNO group (11.5% and 17.1%, respectively). They also had the highest representation in the MUNO group (34.8% and 29.5%, respectively) and the lowest relative representation in the MHOB group (4.89% and 6.27%, respectively). Additionally, NHPs and Part NHPs had similarly high representation in the MUOB group (48.9% and 47.2%, respectively).

Tobacco use was more prevalent in NHPs than in non-NHPs ( $\chi^2=100.53$ ,  $P<0.001$ ; **Table 2.9**), and alcohol use was significantly less prevalent comparatively ( $\chi^2=41.99$ ,  $P=4.0E-9$ ). The NHPI group also tended to get less moderate-level exercise than other racial-ethnic groups ( $\chi^2=32.31$ ;  $P=1.6E-6$ ), with only N=6 participants in this group getting moderate exercise more than once per week. Vigorous exercise was similarly rare, although there was no significant difference in vigorous exercise between NHPI MH/OB groups ( $P=0.9096$ ). However, many study participants declined to answer activity-related questions, which may complicate the interpretation of behavioral risk factor data.

Within the total cohort, general and moderate associations were observed between socioeconomic factors and clinical outcomes. Notably, the interaction between race-ethnicity and these factors did not have a significant effect on A1c or BP. If socioeconomic status (SES) does not differentially affect health outcomes across racial-ethnic groups, then broader racial-ethnic differences in SES may partially contribute to community health status. As for mental health and wellbeing, feeling sad or hopeless for longer than two weeks was positively associated with MU risk (**Table 2.10**). As a symptom associated with depression[138], this trend underscores the necessity for further investigations into the link between mental and metabolic health within this community.

Survey questions regarding socioeconomic status and mental well-being were missing a similarly high proportion of responses, and further investigation is necessary to elucidate associations among them. This issue is of particularly urgent necessity: in the State of Hawai'i, the average age-adjusted death rate due to intentional self-harm jumped from 18.1 to 26 between the years 2021 and 2022 for Native Hawaiians[5]. However, the handling and reporting of such data is especially sensitive in Indigenous and NHPI communities[139], and the persistent effects of colonization shouldered by Indigenous populations should be considered appropriately[140]. Therefore, while these effects may significantly contribute to community health and well-being, it is beyond the scope of this project to draw conclusions about socioeconomic status as a health risk factor in this context.

In summary, NHPIs are more likely to be classified as obese by conventional standards. However, these standards are variably effective as an indicator of metabolic health status among diverse populations. Applying conventional BMI cutoffs as thresholds for data stratification may introduce a misclassification bias[92]. In NHPI health research, this bias can also be introduced using generalized thresholds for any obesity index, whether anthropometric or directly measured, especially if obtained in NHPI-exclusive populations. As continuous variables, unadjusted indices may present similarly skewed representations with other biological factors. It must also be noted that anthropometric indices are not uniformly indicative of adiposity or body composition and should not be communicated as such until those measures are verifiably relevant in a study population. Therefore, community-specific research is required to characterize the relationship between anthropometrics and metabolic health risk in NHPIs.

## Chapter 3. Meta-Inflammatory Associations with Metabolic Risk

### 3.1 Abstract

**Background:** Meta-inflammation is increasingly recognized for its causal role in the etiology of obesity-related diseases. Despite the disproportionate prevalence of obesity-related diseases in Native Hawaiian and Other Pacific Islander (NHPI) populations in the State of Hawai'i, racial-ethnic differences in meta-inflammation are relatively uncharacterized in the context of obesity-related metabolic health (MH/OB) disparities for the community.

**Methods:** To explore community-specific trends in obesity-related metabolic health (MH/OB) risk, clinic events were held for study recruitment, clinical measures, and sample collection. Up to 20mL of blood was collected upon survey completion and processed within 48 hours. Plasma concentrations of GLP-1, IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-10, IL-12, IL-13, IL-18, IL-3, IL-6, IL-8 (CXCL8), Insulin, MCP-1 (CCL2), PYY, TNF- $\alpha$ , TNF-RI, and VEGF-A (pg/ml) were measured using the 17-Plex Human ProcartaPlex Panel (ThermoFisher Scientific, Warrington, England). To determine the contribution of each factor in the distinction between MH and MU status, linear discriminant analysis (LDA) was performed based on metabolic biomarker concentrations. Plasma concentrations of significant biomarkers were compared between NHPI (N=102), Asian (N=99), White (N=65), Part NHPI (N=215), and Mixed (N=68) groups. Ethnic-specific associations between significant meta-inflammatory factors and MH/OB risk were compared by logistic regression.

**Results:** Together with age, body roundness index (BRI), and gender, the LDA model based on meta-inflammatory biomarkers was highly accurate in classifying MU status in non-obese (NO) and obese (OB) groups. Its accuracy was highest for NHPIs (NO AUC=0.961; OB AUC=0.969) but lowest for Part NHPIs (NO AUC=0.782; OB=0.828). The features that strongly contributed to the distinction between MH and MU classes (LDA coefficient  $>|0.1|$ ) were age, gender, BRI, IFN- $\alpha$ , IFN- $\gamma$ , IL-10, IL-12, IL-13, IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$ . After adjusting for age and gender, IL-1 $\beta$  levels were significantly higher in NHPIs compared to the White group ( $P<0.01$ ). All other significant pairwise intergroup differences were observed for biomarkers that did not contribute substantially to MU classification within OB:racial-ethnic groups:

GLP-1 levels were lower in the NHPI group than in the White or Mixed groups ( $P < 0.001$ ). PYY was also lower in NHPIs compared to Part NHPIs and the Mixed group ( $P < 0.01$ ). MCP-1 was lower in NHPIs compared to Asian and Mixed groups ( $P < 0.001$ ), and IL-3 was lower in NHPIs compared to White ( $P < 0.001$ ) and Mixed groups ( $P < 0.01$ ). In logistic regression models with MU status as a response variable, meta-inflammatory biomarkers only had significant coefficients in NHPIs (IL-13  $\beta = -0.241$ ,  $P = 0.017$ ; IL-6  $\beta = 0.048$ ,  $P = 0.024$ ) and Part NHPIs (IL-13  $\beta = -0.199$ ,  $P = 0.022$ ). Regression models based on significant meta-inflammatory biomarkers had higher  $R^2$  values for Part/NHPIs than for other groups.

**Conclusion:** Among significant meta-inflammatory biomarkers, IFN- $\alpha$ , IL-10, IL-13, IL-1 $\beta$ , and TNF- $\alpha$  were the most important features in the distinction between MH and MU risk status for NHPIs. While those factors were important for risk classification *within* racial-ethnic groups, IL-1 $\beta$ , GLP-1, PYY, MCP-1, and IL-3 may be associated with differences in MU risk *between* racial-ethnic groups. Further investigation is necessary to determine the impact of racial-ethnic differences in meta-inflammation and racial-ethnic differences in metabolic health risk.

## 3.2 Introduction

Native Hawaiians and other Pacific Islanders (NHPI) face a disproportionately high prevalence of and mortality to metabolic diseases, including type 2 diabetes mellitus (T2DM). T2DM is clinically characterized by persistent hyperglycemia, a significant risk factor for a wide range of cardiovascular complications[141]. At the cellular level, hyperglycemia contributes to such complications by inducing oxidative stress, which, in turn, can induce intracellular damage and disrupt normal metabolic functioning in various tissues throughout the body[142]. While it has been argued that obesity-related inflammation is causal in T2DM progression, emerging evidence suggests a bidirectional relationship between dysregulated cytokine signaling and insulin resistance[33,143]. Obesity-related metabolic inflammation is classically attributed to the increased accumulation and polarization of immune cells to a pro-inflammatory profile in various tissues, including adipose tissue, skeletal muscle, liver, gut, brain, and pancreas[33].

Upregulated adipocyte production of chemoattractant cytokines, including IL-8 and MCP-1[144], facilitates the recruitment and infiltration of macrophages into adipose tissues, possibly by upregulating the expression of adhesion molecules in endothelial cells[145] or modulating adipocyte physiology[146]. Persistent proinflammatory crosstalk between adipose tissue and ATMs can exacerbate insulin resistance. Thus, interactions between glucose metabolism and inflammatory processes are a focal point for current investigations into T2DM pathophysiology. Compared to healthy controls, plasma levels of pro-inflammatory cytokines such as interleukin-8 (IL-8)[146], monocyte chemoattractant protein-1 (MCP-1)[147], IL-1 $\beta$ [148], tumor necrosis factor-alpha (TNF- $\alpha$ )[149], IL-6[150], and interferon-gamma (IFN- $\gamma$ )[151] are generally elevated or hyperactive in individuals with T2DM. TNF- $\alpha$  and IL-6 stimulate hepatocytic c-reactive protein (CRP) production[152], a clinically relevant marker for chronic low-grade systemic inflammation[153]. Additionally, IL-1 $\beta$  and IL-6 can induce the expression of vascular endothelial growth factor-A (VEGF-A)[154], potentiating chronic inflammation.

Adipocyte cytokine secretion can also significantly affect glucose homeostasis. Adiponectin, an obesity-related adipokine, has been shown to enhance insulin sensitivity by promoting IRS-1 serine phosphorylation via Adenosine monophosphate (AMP)-Activated Protein Kinase (AMPK)-mediated pathways[155] and upregulating GLUT4 translocation and glucose uptake while suppressing glucagon production[156]. Its insulin-sensitizing effects are often attributed to anti-inflammatory activity, as it stimulates IL-10 production while inhibiting macrophage TNF-mediated NF- $\kappa$ B pathways[35]. This regulatory activity is bidirectional: TNF- $\alpha$  and IL-6 can downregulate adiponectin production[157]. Another T2DM-relevant adipokine, leptin, may also enhance insulin sensitivity[158,159] and facilitate GLUT4 translocation and glucose uptake[156]. In contrast to adiponectin, however, leptin is a proinflammatory

adipokine and, in tandem with IL-1, may upregulate IL-6 and IL-8 expression in adipocytes[160]. Adiponectin and leptin are bidirectionally regulated by stress responses.

Cortisol influences glucose homeostasis by inhibiting insulin secretion, disrupting insulin signaling, suppressing GLUT4 translocation, and stimulating glucagon secretion[161]. Cortisol generally exerts anti-inflammatory effects by inhibiting the production of proinflammatory cytokines[162], though chronically high plasma cortisol levels can lead to cortisol resistance and attenuate its anti-inflammatory capacity. In contrast to cortisol, glucagon-like peptide 1 (GLP-1) inhibits glucagon secretion[23] and potentiates glucose-stimulated insulin secretion[163]. GLP-1 also carries anti-inflammatory properties, as it stimulates the expression of IL-10[164] and inhibition of TNF- $\alpha$ [165]. Another metabolically relevant enteroendocrine hormone, peptide tyrosine tyrosine (PYY), has garnered similar attention due to its capacity to regulate appetite and mitigate pancreatic islet dysfunction[166]. Recent literature suggests a bidirectional relationship between cortisol and enteroendocrine hormones GLP-1 and PYY.

Emerging evidence indicates that drugs prescribed for T2DM management modulate the interface between insulin signaling and inflammation. Glycine supplementation has been observed to elevate IFN- $\gamma$  levels in T2DM subjects and diminish serum levels of proinflammatory cytokines[167]. Similarly, metformin has been shown to attenuate systemic pro-inflammatory cytokine concentrations in a dose-responsive manner[168,169]. Metformin's ability to directly inhibit NF- $\kappa$ B, bypassing the AMPK pathway, suggests its insulin-sensitizing effects may partly arise from modulating intracellular signaling pathways where inflammation and insulin signaling intersect[169].

These meta-inflammatory factors are increasingly recognized as a causal link between obesity and metabolic disease. However, the relationship between meta-inflammation and obesity-related health risk is relatively uncharacterized in NHPIs, especially in health disparity. This study aims to address this gap in knowledge by characterizing community-specific associations between meta-inflammatory biomarkers and obesity-related health risks.

### **3.3 Methods**

#### **3.3.1 Human Subjects Data Collection**

*See Section 2.3.1: Human Subjects Data Collection*

Of N=1568 participants in the NHPI-enriched study cohort, a subset of biological samples was included in the present analysis depending on sample availability. N=549 participants (aged 16 to 82 years) were included in the present analysis from the following racial-ethnic groups: Native Hawaiian and other Pacific Islander (NHPI; N=102), Asian (N=99), White (N=65), Part NHPI (N=215), and Mixed (N=68).

### 3.3.2 Quantifying Plasma Biomarkers of Metabolic Inflammation

Blood was collected from participants (up to 20 mL) at our community sites by venipuncture upon biometric data collection. Patients receiving medical treatment for either type 1 or type 2 diabetes were not included in the study cohort. Plasma and peripheral blood mononuclear cells (PBMCs) were stratified within 24 h of sample collection using density-gradient centrifugation in SepMate tubes (Stemcell Technologies, Canada). Five plasma aliquots (1 mL) from each sample were stored at  $-80^{\circ}\text{C}$  until further application. PBMCs were stored at  $-150^{\circ}\text{C}$  until the moment of assay performance.

Plasma concentrations of GLP-1, IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-10, IL-12, IL-13, IL-18, IL-3, IL-6, IL-8 (CXCL8), Insulin, MCP-1 (CCL2), PYY, TNF- $\alpha$ , TNF-RI, and VEGF-A (pg/ml) were assessed using the 17-Plex Human ProcartaPlex Panel (ThermoFisher Scientific, Warrington, England) under manufacturer provided protocols. Samples were centrifuged at  $13,000 \times g$  for 2 min to pellet aggregates. A standard curve was generated using antigen standards provided by the manufacturer. Bead counts below 35 were excluded from further analyses. Fluorescent signals were analyzed using the Luminex 200™ instrument (R&D Systems, Inc., Minneapolis, MN, United States). Bio-Plex Manager™ software (Bio-Rad Laboratories, Inc., Hercules, CA, United States) was used for data processing.

### 3.3.3 Data Analysis

To characterize community-specific trends in obesity-related risk, we stratified metabolic health risk groups (MH/OB) using ethnic-specific body roundness index (BRI) cutoffs, which were obtained relative to group-specific risk for hypertension (HT) or type 2 diabetes mellitus (T2DM; *See Section 2.3.2*). After stratifying racial-ethnic groups by non-obese (NO) and obese (OB) status, linear discriminant analysis (LDA) was performed to evaluate the contribution of each cytokine target analyte to the distinction between metabolically healthy (MH) and metabolically unhealthy (MU) status using the 'MASS' package in R[170]. The 'pROC' package was used to obtain receiver operating characteristic area under the curve (ROC-AUC) values for LDA analyses[131]. The 'MASS' package was also used for logistic regression through generalized linear modeling (GLM) to determine the relationship between meta-inflammatory biomarkers and MU status. Age, BRI, and gender were included in regression analyses for covariate adjustment. Data was visualized via 'ggplot2', 'ggpubr,' and 'ggsignif' packages[134,135].

### 3.4 Results

#### 3.4.1 Statistical Summary of the Study Cohort

A statistical cohort summary is presented in **Table 3.1**. The representation of type 2 diabetes (T2DM; 12.7%) and hypertension (HT; 82.3%) were disproportionately high for the NHPI group. As such, metabolically unhealthy (MU) groups were highly represented in the NHPI group for the non-obese (NO; 23.5%) and obese (OB; 58.8%) groups. NHPI had the lowest percentage of metabolically healthy obesity (MHOB; 3.92%) compared to other groups.

**Table 3.1** Statistical cohort summary of sociodemographic and metabolic health factors.

	Total	Racial-Ethnic Groups					P <sup>a</sup>
		NHPI	Asian	White	Part NHPI	Mixed	
<b>Total</b>	549	102	99	65	215	68	
<b>Gender</b>							$\chi^2=2.259$ 0.6882
Male	235 (42.8%)	44 (43.1%)	43 (43.4%)	33 (50.8%)	87 (40.5%)	28 (41.2%)	
Female	314 (57.2%)	58 (56.9%)	56 (56.6%)	32 (49.2%)	128 (59.5%)	40 (58.8%)	
<b>T2DM Status</b>							$\chi^2=23.09$ 0.0034
Nondiabetic	310 (56.5%)	47 (46.1%)	58 (58.6%)	47 (72.3%)	111 (51.6%)	47 (69.1%)	
Prediabetic	203 (37.0%)	42 (41.2%)	36 (36.4%)	15 (23.1%)	92 (42.8%)	18 (26.5%)	
Diabetic	36 (6.56%)	13 (12.7%)	5 (5.05%)	3 (4.62%)	12 (5.58%)	3 (4.4%)	
<b>BP Categories</b>							$\chi^2=41.90$ 0.0004
Low	3 (0.55%)		1 (1.01%)		2 (0.93%)		
Normal	132 (24.0%)	14 (13.7%)	29 (29.3%)	20 (30.8%)	46 (21.4%)	23 (33.8%)	
Elevated	51 (9.29%)	4 (3.92%)	8 (8.08%)	10 (15.4%)	23 (10.7%)	6 (8.82%)	
HT I	146 (26.6%)	24 (23.5%)	36 (36.4%)	15 (23.1%)	52 (24.2%)	19 (27.9%)	
HT II	217 (39.5%)	60 (58.8%)	25 (25.3%)	20 (30.8%)	92 (42.8%)	20 (29.4%)	
<b>Obesity-Related Metabolic Health Risk (MH/OB) Status</b>							$\chi^2=25.56$ 0.0124
MHNO	113 (20.6%)	14 (13.7%)	22 (22.2%)	17 (26.2%)	44 (20.5%)	16 (23.5%)	
MHOB	64 (11.7%)	4 (3.92%)	15 (15.2%)	12 (18.5%)	22 (10.2%)	11 (16.2%)	
MUNO	116 (21.1%)	24 (23.5%)	21 (21.2%)	8 (12.3%)	44 (20.5%)	19 (27.9%)	
MUOB	256 (46.6%)	60 (58.8%)	41 (41.4%)	28 (43.1%)	105 (48.8%)	22 (32.4%)	
<b>Age Group</b>							$\chi^2=46.72$ 7.5E-5
G1 (16-22)	137 (25.0%)	9 (8.8%)	27 (27.3%)	15 (23.1%)	64 (29.8%)	22 (32.4%)	
G2 (23-34)	154 (28.1%)	21 (20.6%)	35 (35.4%)	21 (32.3%)	57 (26.5%)	20 (29.4%)	
G3 (35-42)	90 (16.4%)	17 (16.7%)	13 (13.1%)	9 (13.8%)	38 (17.7%)	13 (19.1%)	
G4 (43-55)	108 (19.7%)	36 (35.3%)	13 (13.1%)	11 (16.9%)	38 (17.7%)	10 (14.7%)	
G5 (55-82)	60 (10.9%)	19 (18.6%)	11 (11.1%)	9 (13.8%)	18 (8.37%)	3 (4.41%)	

<sup>a</sup>Chi-squared test for independence. Statistical significance is determined at P<0.05. T2DM=Type 2 diabetes mellitus. HT=Hypertension. Age ranges are provided per age group.

To explore meta-inflammatory processes that may underlie racial-ethnic differences in metabolic risk, linear discriminant analysis (LDA) models were generated using MU status as a response variable and biomarkers as predictors. The biomarkers included were GLP-1, IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-10, IL-12, IL-13, IL-18, IL-3, IL-6, IL-8 (CXCL8), Insulin, MCP-1 (CCL2), PYY, TNF- $\alpha$ , TNF-RI, and VEGF-A, with age, gender, included as covariates (**Table 3.2**). For most obesity groups per race-ethnicity (OB:racial-ethnic group), meta-inflammatory LDA modeling demonstrated a highly accurate classification capacity for MU status in most NO and OB groups (AUC >0.9). This accuracy was notably highest for NHPIs, with an AUC

score of 0.961 in the NO group and 0.969 in the OB group. Reduced accuracy was observed in Part NHPIs (NO AUC=0.782; OB AUC=0.828) and the NO Asian group (AUC=0.863). The discrepancy in LDA model accuracy was the largest between the NHPI and Part NHPI groups. The importance of each feature in the distinction between MH and MU risk groups varied in strength and direction across OB:racial-ethnic groups.

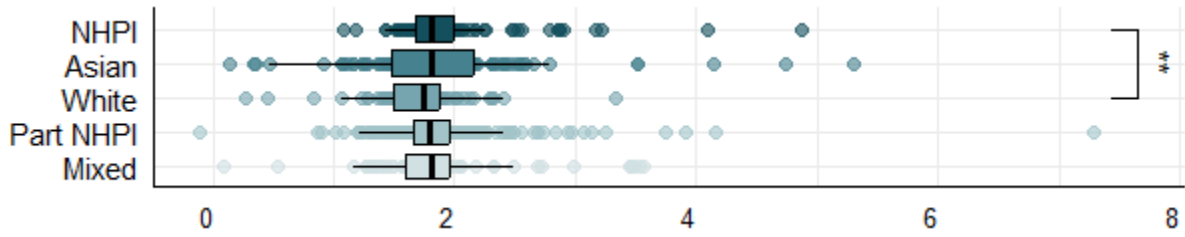
**Table 3.2** LDA loadings for meta-inflammatory biomarkers in the distinction between MH and MU status for NO and OB groups for each race-ethnicity.

	NHPI		Asian		White		Part NHPI		Mixed	
	NO	OB	NO	OB	NO	OB	NO	OB	NO	OB
Age	0.029	0.038	-0.001	-0.005	<b>0.133</b>	-0.014	0.060	0.052	<b>0.139</b>	0.004
BRI	<b>0.345</b>	0.035	<b>-0.254</b>	<b>0.395</b>	<b>0.833</b>	<b>0.465</b>	<b>0.167</b>	0.017	<b>-0.148</b>	0.082
Gender	<b>0.202</b>	<b>0.610</b>	<b>0.440</b>	<b>0.168</b>	<b>-1.898</b>	<b>-1.546</b>	<b>-0.713</b>	<b>-0.551</b>	<b>-4.133</b>	-0.033
GLP-1	0.000	-0.001	0.001	0.002	0.001	-0.005	0.000	0.000	0.000	-0.008
IFN- $\alpha$	<b>0.719</b>	<b>-1.020</b>	<b>-0.890</b>	<b>0.215</b>	<b>-0.616</b>	0.027	<b>0.995</b>	<b>-0.155</b>	<b>0.685</b>	<b>0.605</b>
IFN- $\gamma$	0.060	-0.012	<b>0.155</b>	-0.089	-0.045	<b>-0.172</b>	-0.056	-0.027	<b>-0.115</b>	0.090
IL-10	<b>0.130</b>	0.014	0.000	<b>-0.419</b>	<b>0.163</b>	<b>0.149</b>	<b>-0.679</b>	-0.061	<b>1.367</b>	<b>-0.429</b>
IL-12	<b>0.331</b>	<b>0.245</b>	<b>-0.834</b>	<b>-0.480</b>	<b>0.915</b>	<b>-2.402</b>	0.002	<b>0.976</b>	<b>-1.843</b>	<b>-1.758</b>
IL-13	<b>-0.567</b>	0.012	<b>0.216</b>	0.097	<b>0.184</b>	<b>0.315</b>	<b>-0.201</b>	<b>-0.176</b>	<b>-0.224</b>	<b>0.484</b>
IL-18	-0.001	0.000	0.000	0.002	-0.002	0.002	0.000	0.002	-0.005	-0.004
IL-1 $\beta$	<b>-0.685</b>	<b>0.566</b>	<b>0.880</b>	<b>0.146</b>	<b>0.880</b>	<b>-0.484</b>	0.048	<b>-0.132</b>	-0.020	<b>0.397</b>
IL-3	-0.038	0.000	-0.004	0.032	0.006	-0.004	0.000	-0.003	-0.013	0.025
IL-6	-0.024	<b>0.163</b>	<b>0.148</b>	-0.020	<b>0.219</b>	0.099	0.023	0.062	<b>0.282</b>	-0.099
IL-8	0.068	<b>-0.105</b>	-0.045	-0.018	-0.060	0.080	0.053	-0.059	<b>-0.120</b>	<b>0.153</b>
MCP-1	0.008	-0.006	-0.022	-0.006	-0.022	-0.001	-0.007	0.000	-0.009	-0.008
PYY	0.001	0.002	-0.002	-0.026	0.024	0.006	-0.001	0.002	0.013	0.014
TNF- $\alpha$	<b>-0.110</b>	0.069	0.067	-0.004	0.017	<b>-0.209</b>	0.091	0.024	<b>0.170</b>	<b>-0.256</b>
TNFRI	0.000	0.000	0.000	-0.001	-0.002	0.000	0.000	0.000	0.000	0.000
VEGF-A	0.004	0.000	-0.003	0.000	0.026	0.008	0.001	0.002	-0.013	0.006
AUC	0.961	0.969	0.863	0.927	1.000	0.907	0.782	0.828	0.987	0.946

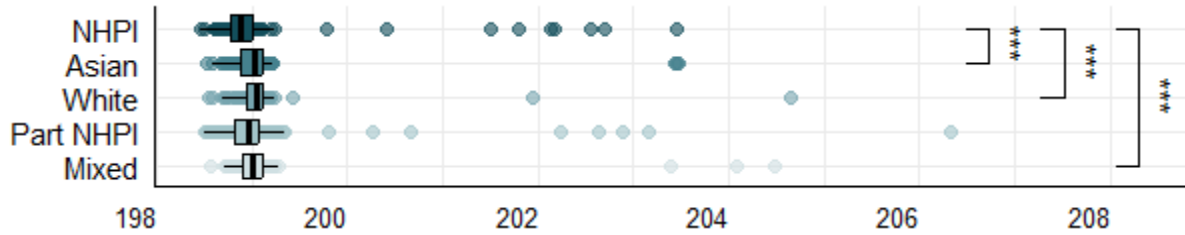
LDA loadings  $>|0.1|$  are bolded. Receiver operating characteristic (ROC) areas under the curve (AUC) values are also presented for each model. Positive values are positively associated with MU status. Negative values are associated with MH status.

Gender, IFN- $\alpha$ , and IL-12 significantly contributed to the distinction between MH and MU for nine out of ten groups (LDA coefficient  $>|0.1|$ ). For NHPIs, IL-13, TNF- $\alpha$ , and IL-1 $\beta$  were important for accurate classification and associated more closely with MH status than with MU in NO individuals, meaning that higher concentrations of these biomarkers corresponded with a higher probability of being classified as MH. Of these three biomarkers, only IL-1 $\beta$  significantly contributed to MU status classification in OB NHPIs, which was MU-associated. IL-13 exhibits anti-inflammatory effects by suppressing pro-inflammatory polarization of adipose tissue-resident macrophages (ATMs), a mechanism corresponding to its capacity to ameliorate insulin resistance in adipose tissue[171]. TNF- $\alpha$  and IL-1 $\beta$  are infamous drivers of adipose tissue inflammation and the progression of insulin resistance. This corresponds with the similarly MU-associated IL-6 in OB NHPIs, which also plays a major role in persistent adipose inflammation[171]. Anti-inflammatory cytokines that play a generally protective role in glucose homeostasis, IFN- $\alpha$ , and IL-10, significantly contributed to MU classification for NO NHPIs[172].

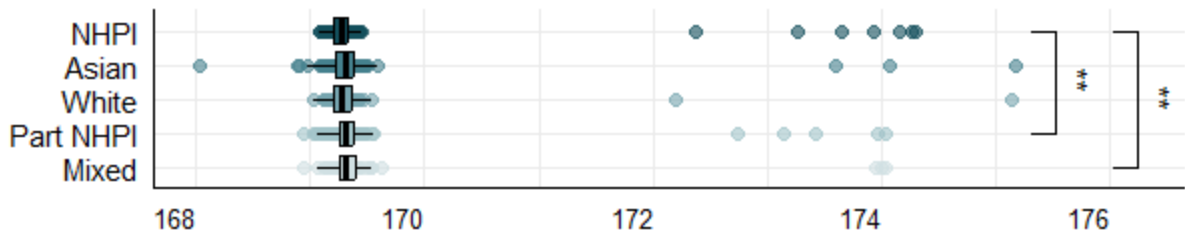
**A. IL-1 $\beta$**  (pg/ml; adjusted for age and gender)



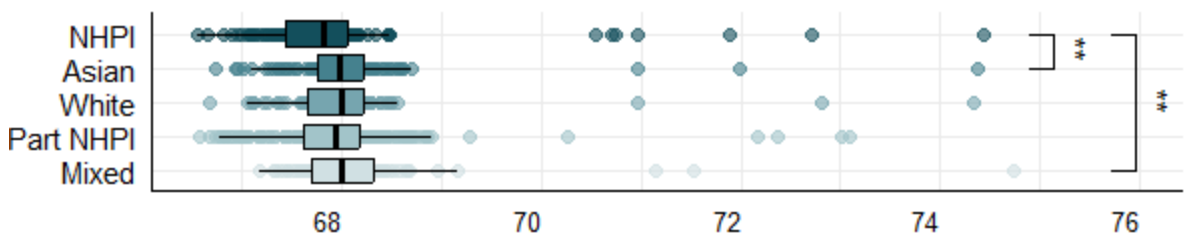
**B. GLP-1** (pg/ml; adjusted for age and gender)



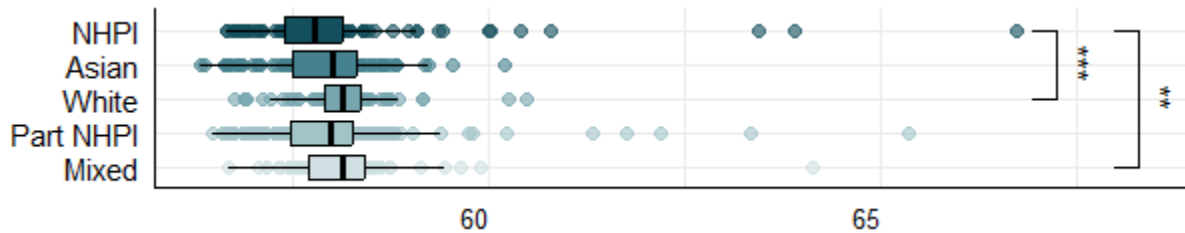
**C. PYY** (pg/ml; adjusted for age and gender)



**D. MCP-1** (pg/ml; adjusted for age and gender)



**E. IL-3** (pg/ml; adjusted for age and gender)



**Figure 3.1** Racial-ethnic differences in meta-inflammatory biomarkers **(A)** IL-1 $\beta$ ; **(B)** GLP-1; **(C)** PYY; **(D)** MCP-1; **(E)** IL-3. Results are only shown for significant pairwise comparisons.

**Table 3.3** Plasma concentrations (pg/ml) of meta-inflammatory determinants of MU risk status by racial-ethnic group.

	IL-1 $\beta$		IL-6		IL-8		IL-10		IL-13		IFN- $\gamma$		TNF- $\alpha$		IFN- $\alpha$		IL-12		
	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	Med	IQR	
<b>Total</b>	4.0E-7 <sup>a</sup>							0.046 <sup>a</sup>											
MHNO	1.835	0.605	16.452	4.206	6.530	3.052	1.393	0.241	5.716	2.186	7.989	7.868	5.518	2.121	3.274	0.788	0.764	0.617	
MHOB	2.157	0.784	16.055	4.722	6.529	3.492	1.405	0.367	5.707	1.795	9.736	9.446	5.481	2.660	3.382	1.031	0.838	0.573	
MUNO	2.005	0.519	15.982	4.211	6.701	2.312	1.387	0.251	5.472	1.639	9.358	8.010	5.736	2.020	3.193	0.904	0.694	0.647	
MUOB	2.132	0.519	16.630	4.339	6.893	2.548	1.388	0.295	5.536	1.308	10.728	9.551	5.942	1.975	3.247	0.874	0.802	0.604	
<b>NHPI</b>	0.043 <sup>a</sup>							0.027 <sup>a</sup>				0.030 <sup>a</sup>							
MHNO	1.888	0.419	16.229	4.016	6.388	0.683	1.390	0.416	6.391	2.465	6.212	3.709	4.763	1.900	3.008	0.507	0.722	0.678	
MHOB	2.117	0.644	15.373	66.029	15.482	18.231	1.456	0.101	6.797	24.342	14.325	11.789	6.638	4.893	4.014	1.842	0.651	0.297	
MUNO	2.024	0.445	16.476	6.541	6.989	3.055	1.393	0.355	5.352	2.223	10.235	9.409	6.031	1.343	3.193	1.115	0.731	0.727	
MUOB	2.196	0.505	16.476	4.869	6.682	1.732	1.388	0.244	5.433	1.647	10.738	5.760	5.953	2.014	3.110	0.681	0.841	0.568	
<b>Asian</b>	0.010 <sup>a</sup>																		
MHNO	1.575	0.717	16.115	8.417	5.050	3.805	1.387	0.258	5.327	2.455	7.770	9.619	5.664	2.113	3.363	0.741	0.761	0.424	
MHOB	2.408	0.835	16.807	2.682	5.966	3.585	1.426	0.361	5.692	1.174	12.013	10.047	5.154	1.585	3.655	0.875	0.947	0.542	
MUNO	2.024	0.868	17.972	4.956	6.636	2.844	1.356	0.342	5.645	1.909	12.685	8.166	5.736	3.568	3.435	1.069	0.816	0.678	
MUOB	2.201	1.234	16.975	3.646	6.931	4.554	1.347	0.311	5.464	2.356	12.725	13.444	5.238	2.205	3.265	1.445	0.897	0.628	
<b>White</b>							0.027 <sup>a</sup>												
MHNO	1.835	0.731	17.718	3.389	7.858	2.414	1.423	0.281	5.811	1.142	8.931	11.713	5.664	2.904	3.395	0.650	0.663	0.892	
MHOB	1.984	0.425	16.476	3.911	5.943	2.732	1.714	0.389	5.225	2.317	9.181	5.487	4.938	1.947	3.363	0.930	1.127	0.279	
MUNO	1.988	0.346	15.046	2.959	6.206	0.935	1.358	0.112	5.381	1.963	6.749	3.956	5.331	1.446	2.957	0.427	0.488	0.547	
MUOB	2.049	0.541	16.085	2.919	6.716	2.657	1.393	0.224	5.611	2.256	9.944	8.449	5.899	2.869	3.337	0.930	0.819	0.597	
<b>Part NHPI</b>	0.041 <sup>a</sup>						0.040 <sup>a</sup>												
MHNO	1.935	0.511	16.085	3.449	6.448	2.848	1.353	0.248	5.707	1.716	7.989	6.611	5.405	2.302	3.132	0.730	0.722	0.476	
MHOB	2.148	0.576	15.795	4.634	6.365	2.431	1.388	0.136	5.764	1.526	7.627	7.819	4.930	2.389	3.202	1.225	0.699	0.453	
MUNO	2.005	0.363	15.736	3.699	6.654	1.990	1.352	0.291	5.468	1.126	8.620	6.737	5.771	2.198	3.239	0.732	0.646	0.431	
MUOB	2.113	0.367	16.679	3.975	6.854	2.105	1.404	0.342	5.570	1.300	10.156	9.930	5.953	1.860	3.271	0.659	0.764	0.621	
<b>Mixed</b>																			
MHNO	1.854	0.313	17.240	1.812	6.790	2.165	1.405	0.156	5.832	3.039	8.687	4.942	5.651	1.176	3.656	0.438	0.904	0.671	
MHOB	2.006	1.014	14.547	5.807	6.359	4.669	1.380	0.439	5.507	1.222	8.181	13.473	6.214	3.974	3.047	1.293	0.850	0.556	
MUNO	1.878	0.788	14.780	5.457	5.999	3.893	1.388	0.154	6.128	2.155	7.879	7.272	3.791	2.570	3.265	0.736	0.954	0.817	
MUOB	2.130	0.617	17.211	4.405	7.611	4.975	1.372	0.166	5.457	0.714	10.779	9.182	5.698	2.027	3.076	0.699	0.738	0.530	

<sup>a</sup>Significant P-value for the Kruskal-Wallis rank-sum test. Nonsignificant P-values are not shown. Med=Median. IQR=Interquartile range.

Notably, BRI was an important discriminator for MU status in every group except for NHPI, Part NHPI, and Mixed OB individuals. Additionally, although age is a major metabolic risk factor in NHPs, LDA modeling indicated that age was not as significant a determinant of MU as was meta-inflammation. GLP-1, IL-18, IL-3, MCP-1, PYY, TNFRI, and VEGF-A were not strongly distinctive in the separation between MH and MU status. Median values and interquartile ranges (IQR) for factors indicated as significant from LDA are summarized in **Table 3.3**.

### **3.4.2 Racial-Ethnic Differences in Meta-Inflammatory Biomarkers**

To evaluate racial-ethnic differences in inflammation, we compared plasma concentrations of each target biomarker after adjusting for age and gender. NHPs had higher levels of IL-1 $\beta$  compared to Whites, which corresponds with their intergroup differences in MU risk (**Figure 3.1A**). NHPs also had significantly lower levels of GLP-1 compared to the Asian, White, and Mixed groups (**Figure 3.1B**), corresponding to a higher risk for insulin resistance in the NHP group than others. PYY was also significantly reduced in NHPs compared to Part NHPs and the Mixed group (**Figure 3.1C**).

Contrary to expectations, however, NHPs tended to have lower plasma concentrations of MCP-1 (**Figure 3.1D**), a major driver of sustained meta-inflammation in adipose tissue. The difference was significant in comparison to the Asian and Mixed groups. IL-3 was also reduced in NHPs compared to White and Mixed groups (**Figure 3.1E**). There were no other significant pairwise differences between racial-ethnic groups for meta-inflammatory biomarker concentration.

After selecting significant biomarkers from LDA modeling, logistic regression modeling was used to investigate their relationship with MU status in each racial-ethnic group (**Table 3.4**). For the Asian and White groups, individual predictor variables were not significantly associated with MU status, though age was significant for the Mixed group ( $\beta=0.097$ ;  $P=0.10$ ). For Part/NHPs, MU status was positively associated with age (Part NHP  $\beta=0.058$ ,  $P<0.001$ ; NHP  $\beta=0.056$ ,  $P=0.039$ ) and BRI (Part NHP  $\beta=1.513$ ,  $P<0.001$ ; NHP  $\beta=2.327$ ,  $P=0.007$ ). The lack of association observed in the White group was contrary to expectations, as anthropometric indices tended to be more strongly associated with metabolic risk in the White group compared to the NHP group (see *Section 2.4*).

Aside from age and BRI, meta-inflammatory biomarkers were significantly associated with MU status for Part/NHPs. For both groups, IL-13 was negatively associated with MU (Part NHP  $\beta=-0.199$ ,  $P=0.022$ ; NHP  $\beta=-0.241$ ,  $P=0.017$ ), and for NHPs, IL-6 was positively associated with MU (NHP  $\beta=0.048$ ;  $P=0.034$ ). Notably, the magnitude of the  $\beta$  coefficient for IL-13 was greater than that for age in both groups.

**Table 3.4** Logistic regression between significant meta-inflammation factors and MU status.

	NHPI		Asian		White		Part NHPI		Mixed	
	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P	$\beta$	P
INT	-0.366	0.838	0.021	0.987	0.967	0.665	-2.069	0.095	-3.609	0.155
Age	<b>0.056</b>	<b>0.039</b>	-0.006	0.712	0.027	0.295	<b>0.058</b>	<b>0.000</b>	<b>0.097</b>	<b>0.010</b>
BRI	<b>2.327</b>	<b>0.007</b>	0.792	0.110	1.248	0.073	<b>1.513</b>	<b>0.000</b>	0.447	0.612
Gender	-0.013	0.986	0.139	0.770	-0.699	0.294	-0.523	0.154	-0.804	0.298
IFN- $\alpha$	0.309	0.439	0.063	0.837	-0.826	0.169	0.391	0.215	0.897	0.245
IFN- $\gamma$	0.041	0.105	-0.004	0.899	-0.012	0.835	-0.009	0.769	-0.032	0.620
IL-10	-0.134	0.807	-0.491	0.383	-0.171	0.545	-0.742	0.113	-0.074	0.920
IL-12	0.559	0.390	-0.590	0.223	-0.908	0.179	0.389	0.332	-0.863	0.323
IL-13			-0.057	0.322	0.011	0.902			-0.049	0.499
IL-1 $\beta$	-0.229	0.762	0.515	0.097	1.341	0.123	-0.218	0.287	0.484	0.492
IL-6	<b>0.048</b>	<b>0.034</b>	0.020	0.472	0.035	0.385	0.048	0.138	0.012	0.672
IL-8	-0.137	0.173	-0.013	0.799	-0.091	0.397	0.024	0.371	0.064	0.720
TNF- $\alpha$	-0.111	0.533	0.034	0.547	-0.120	0.586	0.149	0.115	-0.293	0.139
	N=102		N=99		N=65		N=215		N=68	
	R <sup>2</sup> =0.211		R <sup>2</sup> =0.017		R <sup>2</sup> =0.137		R <sup>2</sup> =0.218		R <sup>2</sup> =0.198	
	28.7%		10.5%		22.8%		21.9%		29.5%	

$\beta$ =Estimated regression model coefficient. INT=Intercept. %=Deviance explained. Significant  $\beta$  coefficients and their P-values are bolded.

### 3.5 Discussion

Obesity-related meta-inflammation is a major risk factor for metabolic dysfunction, which underlies many of the chronic cardiometabolic complications that are disproportionately prevalent in Native Hawaiian and other Pacific Islander (NHPI) populations. Linear discriminant analysis (LDA) was used to identify plasma biomarkers that significantly contribute to the separation between metabolically healthy (MH) and metabolically unhealthy (MU) states. LDA modeling was highly accurate in classifying MU risk within this cohort and was especially accurate among NHPs. After comparing plasma concentrations of these biomarkers across racial-ethnic groups, logistic regression models were used to explore their relationship with MU further.

Most notably, after adjusting for age and gender, the MCP-1 plasma concentrations were significantly lower in NHPs than in other groups (**Figure 3.1D**). As an adipokine, MCP-1 secretion is upregulated in dysregulated adipocytes, in turn, recruiting and polarizing M1 pro-inflammatory macrophages adipose tissue, where they may become tissue-resident in sustained obesity-related inflammation[173]. IL-3, also secreted by adipocytes and significantly lower in NHPs (**Figure 3.1E**), can promote the recruitment and survival of eosinophils, which play a role in M2 (anti-inflammatory) macrophage polarization [174]. This eosinophil support may also promote glucose tolerance in obesity[175]. As neither biomarker contributed significantly to the LDA model, prevalence trends in MCP-1 or IL-3 do not necessarily correspond with MH or MU status in NHPs. However, their reduced overall prevalence may lend to racial-ethnic differences in metabolic health risk, and further investigation is necessary to elucidate these relationships.

The biomarkers identified by LDA and logistic regressions highlighted meta-inflammatory associations with metabolic risk *within* racial-ethnic groups - most notably in NHPI and Part NHPI groups. For both groups, IL-13 was negatively associated with MU status (**Table 3.4**). IL-13 has demonstrated some capacity to ameliorate diet-induced inflammation and macrophage infiltration into adipose tissue, thereby mitigating meta-inflammatory processes for developing insulin resistance[176]. In NHPIs, IL-6 was positively associated with MU status. IL-6 tends to be overexpressed in adipose tissues of insulin-resistant individuals, and has been shown to induce insulin resistance in adipocytes; a cycle which may exacerbate the adverse effects of meta-inflammation in obesity[177]. Interestingly, both IL-13 and IL-6 are synthesized locally not only in adipose tissue but also in skeletal muscle, where they both exert insulin-sensitizing effects in response to exercise[178,179]; but metabolically damaging effects of IL-6 correspond with chronic inflammation rather than acute stimulation.

BRI was most strongly associated with MU status in Part/NHPIs; an association which was not as strongly observed for other racial-ethnic groups. It may be possible that BRI is a better indicator for central adiposity within these communities than in other groups, but further investigation is necessary to validate the relationship between anthropometrics, central adiposity, and meta-inflammation in Part/NHPI populations.

The biomarkers that were *not* further explored may be informative about the changes in metabolic risk *between* racial-ethnic groups. Additionally, the LDA model was performed for each OB:racial-ethnic group with MU as a response variable; it may also be valuable to explore the meta-inflammatory discrepancies between NO and OB status for each MU:racial-ethnic group to investigate the relationship between meta-inflammation and obesity.

Among the biomarkers that were differentially prevalent between racial-ethnic groups were GLP-1 and PYY. These biomarkers are primarily gut-derived, metabolic hormones that may influence appetite control[180], insulin sensitivity[181,182], and obesity-related inflammation[183]. As gut bacterial short-chain fatty acid (SCFA) synthesis may stimulate GLP-1 and PYY secretion[184], racial-ethnic differences in the gut microbiome may partially contribute to the intergroup differences in biomarker concentrations.

Racial-ethnic differences in the association between the quantified biomarkers and metabolic health risk suggest that obesity-related meta-inflammation may be a stronger predictor or risk factor for cardiometabolic outcomes in NHPI and Part NHPI populations than in other racial-ethnic groups. Namely, metabolic risk was negatively associated with IL-13 and positively associated with IL-6 in NHPI individuals. Furthermore, the strength and direction of the association between BRI and metabolic risk in these communities suggest that obesity-related meta-inflammation may be more strongly associated with metabolic outcomes in Part/NHPIs compared to other groups, although further investigation is necessary to elucidate these trends in the context of adiposity.

## Chapter 4. Gut Bacterial Associations with Metabolic Risk

### 4.1 Abstract

**Background:** Obesity-related metabolic diseases are disproportionately prevalent in Native Hawaiian and other Pacific Islander (NHPI) communities. The gut microbiome is increasingly recognized for its impact on metabolic health but gut bacterial associations with disparate cardiometabolic outcomes are relatively uncharacterized for NHPIs.

**Methods:** Stool samples were collected at clinic events to investigate community-specific associations between gut bacteria and metabolic health risk. Participants were categorized by self-identified racial-ethnic group: NHPI (N=51), Asian (N=144), White (N=65), Part NHPI (N=154), and multiethnic (“Mixed”; N=74). The Ion 16S Metagenomics Kit was used to prepare libraries for sequencing on the Ion S5 NGS platform. Differential abundance analyses were performed using ANCOM-BC2, from which log-fold change (LFC; natural log) values were also obtained to describe gut bacterial prevalence trends.

**Results:** At the phylum level, NHPIs had the highest prevalence of Pseudomonadota (Proteobacteria; 18.07%) and the lowest prevalence of Thermodesulfobacteriota (Desulfobacterota; 4.67%) compared to other racial-ethnic groups. At the genus level, NHPIs had the highest prevalence of *Eggerthella*, *Blautia*, *Megamonas*, *Veillonella*, *Lachnoclostridium*, *Lactobacillus*, *Fusobacterium*, *Haemophilus*, *Klebsiella*, and *Mannheimia* and the lowest prevalence of *Ruminiclostridium*, *Barnesiella*, *Prevotella*, *Herbaspirillum*, *Desulfovibrio*, and *Akkermansia* compared to other racial-ethnic groups. In NHPIs, unclassified members of *Clostridiales* (LFC=1.059; q=0.002), *Flavonifractor* (LFC=0.0766; q=0.038), *Megasphaera* (LFC=2.586; q<0.001), and *Turcibacter* (LFC=0.811; q=0.047) were positively associated with A1c levels, while *Mannheimia* (LFC=-1.070; q=0.011) and *Megamonas* (LFC=-1.322; q=0.003) were negatively associated with A1c. In Part NHPIs, A1c levels were associated positively with unclassified members of *Clostridiales* Family XI. *Incertae Sedis* (LFC=1.948; q<0.001) and negatively with *Paraprevotella* (LFC=-1.026; q=0.042). Among significant results in these groups, gut bacterial associations with A1c were stronger than those with age, body roundness index (BRI), or blood pressure. There were no significant associations between gut bacterial genera and A1c levels in the Asian, White, or Mixed groups.

**Conclusions:** At the phylum and genus levels, gut bacteria that were more prevalent in NHPIs compared to other groups have been previously linked to inflammation, obesity, metabolic dysfunction, or gastrointestinal complications. Those that were less prevalent in NHPIs compared to other groups tended to be beneficial, or conditionally beneficial. Additionally, specific taxa differentially associated with metabolic health risk measures between racial ethnic groups, as their associations varied in strength and direction. Further research is necessary to verify the impact of racial-ethnic differences in the gut microbiome on racial-ethnic differences in metabolic risk.

## 4.2 Introduction

Native Hawaiians and Pacific Islander (NHPI) populations shoulder a disproportionately high burden of obesity-related cardiometabolic diseases, for which the gut microbiome is an increasingly viable therapeutic target. While certain gut bacteria are frequently linked to some of these disorders, including type 2 diabetes mellitus (T2DM) and hypertension (HT), a definitive consensus on their roles remains elusive due to conflicting reports in current literature (**Table 1.1**; **Table 4.1**).

**Table 4.1** Relative abundance trends for specific gut bacteria in T2DM patients compared to nondiabetic controls. Typical Gram stain results are provided for listed taxa, although exceptions exist for each taxon.

<b>Taxon</b>	<b>Gram</b>	<b>Functional relevance</b>	<b>Prevalence trends in T2DM</b>
<b>Actinobacteria*</b>	( + )	Produces fungicidal and antibiotic metabolites[42,43]	.Negatively correlated [185,186] .Higher in T2DM[185,187]
<i>Bifidobacterium</i>	( + )	Attenuates LPS-induced NF-kB activation and subsequent IL-8 secretion in intestinal epithelial cells[48]	.Higher in T2DM[185,187]
<b>Bacteroidetes*</b>	( - )	Can produce H <sub>2</sub> S from host mucosal glycans, which causes epithelial damage [55,56]	.Lower in T2DM[52,187,188] .No difference[185] .Higher in T2DM[189]
<i>Bacteroides</i>	( - )	Stimulates macrophages and monocytes to secrete TNF- $\alpha$ by LPS-mediated pathways[190]	.Higher in T2DM[191] .No difference[185] .Lower in T2DM[192]
<i>Prevotella</i>	( - )	Improved glucose metabolism in mice with high fiber diet but not in mice with HFD[65]; enhanced capacity for fiber metabolism[193]	.Lower in T2DM[32,52,194] .Higher in T2DM[191]
<b>Firmicutes*</b>	( + )	Enables high-calorie absorption from dietary nutrients, promoting weight gain[68]	.Higher in T2DM[52,192] .No difference[187]
<i>Blautia</i>	( + )	Modulate cytokine production; induce pro and anti-inflammatory responses; varies at the species level[85]	.Higher in T2DM[185,192]
<i>Faecalibacterium*</i>	( + )	Produces anti-inflammatory metabolites that inhibit IL-8 and IL-6 production and induce IL-10 production in PBMCs[75]	.Lower in T2DM[185,191]
<i>Lactobacillus</i>	( + )	Produces histamine, essential vitamins, competes with opportunistic pathogens for colon epithelial adhesion[195]	.Negatively correlated [185] .No difference[185] .Higher in T2DM[34,52,191]
<i>Roseburia*</i>	( + )	Primary degrader of $\beta$ -mannans (plant cell wall polysaccharides)[86]	.No difference[185] .Lower in T2DM[191]
<b>Proteobacteria*</b>	( - )	Modulate host immune responses via sustained, low-grade inflammation[87]	.Lower in T2DM[52,185] .No difference[187] .Higher in T2DM[192]
<b>Verrucomicrobia</b>	( - )	Major mucin-degrading bacteria; associated with anti-inflammatory cytokine expression[90]	.Lower in T2DM[52] .Higher in T2DM[196]
<i>Akkermansia</i>	( - )	Promotes intestinal barrier integrity & GLP-1 secretion, reduces adipocyte differentiation[197,198]	.Lower in T2DM[199]

\*Taxa known to contain butyrate-producing bacteria. Phylum names are bolded.

For instance, the relative abundance of Actinobacteria in T2DM patients compared to nondiabetic controls is reported to be higher in some studies[187] and lower in others[186], even negatively correlating with A1c in other publications[43]. The relative abundance of Firmicutes has been reported to be higher[52] or lower in T2DM patients than in nondiabetic controls[185], while some studies find no such significant differences upon intergroup comparison[43].

These generalizability challenges underscore the need for community-specific research for health-disparate NHPI populations. In 1974, Moore and Holdeman characterized gut microbiome composition in a cohort of 20 Japanese-Hawaiian men aged 60-80 years[200]. Fecal bacteria were isolated, cultured, and identified using techniques described in the Anaerobe Laboratory Manual[201]. This study resulted in the discovery and first description of the *Coprococcus* genus[202], which has since been recognized for its probiotic potential in ameliorating obesity, gastrointestinal disorders, and meta-inflammation[203]. Despite this breakthrough finding, NHPIs remain underrepresented in gut microbiome research, especially in those with disaggregated results. NHPI-inclusive study findings published between 2014 and 2024 are summarized in **Table 4.2**. Studies employing 16S-based techniques for gut microbiome profiling were prioritized for presentation.

Five studies were included in the present review. One study measured gut bacterial associations with hepatic adiposity in multiethnic cohort (MEC) study participants from Hawai'i and Nevada[204]. One study was a methodological evaluation of SparseMCMC\_HD as an analytical tool for causal mediation between gut bacteria and BMI disparity[205]. Two studies were conducted in NHPI-enriched communities in Hawai'i[45,206]. One study compared the prevalence of pathogenic bacteria in two Torres Strait Islands, Waiben and Mer[207].

In the recent MEC study, the non-alcoholic fatty liver disease (NAFLD) state was designated at liver fat >5.5%, measured by abdominal MRI[204]. Beta-binomial regression models were then used to estimate associations between the prevalence of specific gut bacteria and the NAFLD state. Obesity is a major risk factor for NAFLD due to its influence on fat accumulation, lipid metabolism, and meta-inflammation, which can cause hepatic cell injury and further metabolic dysfunction[208].

In Native Hawaiians, NAFLD is associated positively with gut bacterial genera *Parasutterella* and *Megamonas*[204]. *Megamonas* was frequently associated with obesity, meta-inflammation, and MetS in other populations[83]. However, the metabolic impact of *Parasutterella* may not necessarily be discerned through measures of its abundance[209]. *Parasutterella* may be a significant succinate producer, which plays a complex role in functional and dysfunctional metabolism[209,210]. In fact, *Parasutterella* was negatively associated with obesity in a Madrid cohort[61], demonstrating intergroup differences in its functional relevance. A positive association between *Lactobacillus* and NAFLD was observed for Native Hawaiian and African American groups[204]. While this contradicts its presumed role in alleviating

metabolic disorders via meta-inflammatory modulation[211], *Lactobacillus* has also been identified as an obesity-associated genus in Western populations[212].

In Native Hawaiians only, NAFLD is associated negatively with *Idiomarina*, *Klebsiella*, *Negativibacillus*, and *Intestinimonas*. *Idiomarina* is characterized by its extremophilic strains isolated from deep-sea sediment and submarine volcanoes[213,214]. Some members of *Klebsiella* have been implicated as causal determinants of gastrointestinal diseases[215]. In a meta-inflammatory context, this implication is inconsistent with its reduced prevalence in Hawaiians with NAFLD. *Negativibacillus* is enriched by consuming ultra-processed foods, which is linked to obesity, dyslipidemia, dysglycemia, and CVD[216]. This link makes its negative association with NAFLD in Native Hawaiians unexpected. The opposite trend was observed in White individuals with NAFLD from the same study, suggesting racial-ethnic differences in the metabolic impact of *Negativibacillus*. *Intestinimonas* may be protective against diet-induced dysbiosis as a major butyrate producer in lysine-rich environments[217]. Its negative association with NAFLD may indicate that butyrate is especially relevant to metabolic outcomes in Native Hawaiians compared to other racial-ethnic groups.

The MEC study also used WGS metagenomic sequencing techniques to assess gut bacterial metabolic capacities directly[204]. In Native Hawaiian women, butyrate kinase (BUK) was inversely associated with percent liver fat. Consistent with these findings, BUK gene expression was negatively correlated with A1c test results in the NHPI-enriched cohort[45]. While a high-fiber diet is typically considered butyrogenic[218], proportional dietary vegetable intake did not significantly influence BUK gene abundance or expression. In the same NHPI-enriched cohort, one study found that *Akkermansia* was negatively correlated with BUK gene abundance and expression[45], and another found that *Akkermansia* correlated negatively with self-esteem (SE)[206]. While butyrogenic pathways may be metabolically relevant for NHPI health and well-being in this population, further research is necessary to elucidate direct connections between butyrate production and health outcomes.

One of the papers cited above was a methodological evaluation of SparseMCMC\_HD as an analytical tool for causal mediation between gut bacteria and BMI disparity[205]. Data obtained from the American Gut Project (AGP) were used to analyze disparities between Caucasian, Asian, and Pacific Islander (API) populations. In this study, Caucasians had a significantly higher BMI than APIs. Additionally, the proportion of Asian to NHPI representation in this API group is unclear. While disaggregated findings are not reported in this publication, SparseMCMC\_HD may be a valuable tool for assessing gut bacterial mediation in the context of NHPI health disparities.

**Table 4.2** Significant metataxonomic trends in NHPI-inclusive research.

	Community	Gut bacterial taxa	Metataxonomic trends		
[204]	Native Hawaiians from Honolulu, HI or Los Angeles, CA	<i>Parasutterella</i> <sup>*</sup>	Positively associated with NAFLD (liver fat >5.5%)		
		<i>Megamonas</i> <sup>*</sup>			
		<i>Lactobacillus</i>			
				<i>Idiomarina</i> <sup>*</sup>	Negatively associated with NAFLD (liver fat >5.5%)
				<i>Klebsiella</i> <sup>*</sup>	
				<i>Negativibacillus</i> <sup>*</sup>	
				<i>Intestinimonas</i> <sup>*</sup>	
		<i>Erysipelatoclostridium</i>			
		<i>Clostridium</i> <sup>a</sup>			
[205]	US-residing API	<i>A. muciniphila</i>	Positively mediates BMI disparity		
		<i>B. adolescentis</i>	Negatively mediates BMI disparity		
[45]	NHPI-enriched cohort in Honolulu, HI	Fusobacteria <sup>b</sup>	Positively correlated with BMI		
		Lentisphaerae <sup>c</sup>	Negatively correlated with BMI		
		<i>Bifidobacterium</i>			
		Deferribacteres <sup>d</sup>	Positively correlated with A1c score		
		<i>Lactococcus</i>			
		<i>Mucispirillum</i>			
		<i>Shigella</i>			
		Bacteroidetes <sup>e</sup>	Negatively correlated with A1c score		
		<i>Bifidobacterium</i>			
		<i>Faecalibacterium</i>			
		Deferribacteres <sup>d</sup>	Positively correlated with age		
		Proteobacteria <sup>f</sup>			
		<i>Mucispirillum</i>			
<i>Shigella</i>					
Actinobacteria <sup>g</sup>	Negatively correlated with age				
Bacteroidetes <sup>e</sup>					
<i>Bifidobacterium</i>					
<i>Prevotella</i>					
		<i>B. adolescentis</i>			
[206]	NHPI-enriched cohort in Honolulu, HI	<i>Mitsuokella</i>	Positively correlated with SE		
		<i>Collinsella</i>			
		<i>Megasphaera</i>			
		<i>Herbaspirillum</i>	Negatively correlated with SE		
		<i>Akkermansia</i>			
		<i>Lachnoclostridium</i>			
		<i>A. muciniphila</i>			
[207]	Torres Strait Islands (Waiben and Mer)	Proteobacteria <sup>f</sup>	More prevalent in Mer		
		Euryarchaeota			
		Lachnospiraceae bacterium 8_1_57FAA	Positive ACME between sugar-sweetened beverage intake and mean arterial pressure		

<sup>\*</sup>Uniquely observed in NHPI groups compared to other groups in the study. <sup>A</sup>*Clostridium sensu stricto 1*. <sup>B</sup>Fusobacteriota. <sup>C</sup>Lentisphaerota. <sup>D</sup>Deferribacterota. <sup>E</sup>Bacteroidota. <sup>F</sup>Pseudomonadota. <sup>G</sup>Actinomycetota. NAFLD=Non-alcoholic fatty liver disease. MEC=Multiethnic Cohort. SE=Self-esteem. API=Asian and Pacific Islanders. ACME=Average causal mediation effect. Species were only listed if they were mentioned in at least one other paper included for review.

Two recent NHPI-inclusive studies were conducted in NHPI-enriched cohorts on O‘ahu, Hawai‘i[45,206]. One study reported that *Bifidobacterium* had a moderately negative correlation with age and slightly less prominent correlations in the same direction with BMI and A1c levels. This was the only taxon simultaneously associated with A1c and BMI. While this trend may suggest that *Bifidobacterium* plays a protective role in obesity-related health risks, further investigation is necessary to determine whether this trend is still observed after age adjustment. These results additionally suggest that BMI is not a particularly effective predictor of T2DM within the NHPI population. In this instance, significant BMI-related results may not be informative about obesity-related T2DM risk. However, these results may be recontextualized if BMI is verified as an effective predictor for other health outcomes in this population.

In contrast, three taxa exhibited a consistently positive correlation with BMI, A1c levels, and age: *Deferribacteres* (*Deferribacterota*), *Mucispirillum*, and *Shigella*, which were not frequently associated with obesity among other populations (**Table 1.1**). Since age may disproportionately influence cardiometabolic risk, especially within the NHPI population compared to other racial-ethnic groups[6], these taxa may mediate the age-related presentation of disparate health outcomes in a community-specific manner. *Lactococcus* also correlated positively with A1c score but not with age or BMI, although further research is necessary to uncover its metabolic relevance in this community. *Prevotella* was negatively correlated with age but was not highlighted for significant associations with other risk factors among the NHPI-inclusive cohort studies.

The study conducted on the Torres Strait Islands found that individuals from Mer had a significantly higher risk for cardiometabolic disease compared to individuals from Waiben, even after adjusting for age[207]. This increased risk was unexpected. The Waiben population tended to have a more Westernized diet and lower levels of physical activity, and there was no significant difference in vegetable consumption, alcohol/smoking, or BMI between the islands. Proteobacteria and Euryarchaeota were significantly more abundant in Mer than in Waiben, which may suggest their involvement in adverse health outcomes. This study also found that Lachnospiraceae bacterium 8\_1\_57FAA causally mediated the positive trend in mean arterial pressure with increasing intake of sugar-sweetened beverages after age and site adjustment.

Gut bacterial associations with health outcomes may be conditional and community-specific. NHPI-inclusive gut microbiome research presents distinct trends from those obtained in other studies. Further research is necessary to elucidate their metabolic relevance in these populations. Even among NHPI-inclusive research, gut bacterial health impacts are highly variable between distinct populations. Therefore, community- and individual-specific research is required for an accurate characterization of metabolically relevant metataxonomic trends.

However, NHPI-inclusive data tends to be aggregated, and NHPIs remain underrepresented in health disparities research[93]. The purpose of this study is to characterize the gut microbiome in an NHPI-enriched community in the context of racial-ethnic metabolic health disparities. NHPI-inclusive research would enable a relevant and accurate understanding of the gut bacterial impact on metabolic disease etiology in these populations, laying solid groundwork for advancing health equity.

## **4.3 Methods**

### **4.3.1 Human Subjects Data Collection**

*See Section 2.3.1: Human Subjects Data Collection*

Due to missing survey responses or variable quality in point-of-care test results, N=488 participants were included for downstream analyses in the present study. Racial-ethnic data was self-reported and categorized as follows: NHPI (N=51), Asian (N=144), White (N=65), Part NHPI (N=154), and Mixed groups (N=74).

### **4.3.2 Stool Sample Collection and Processing**

Home stool sample self-collection kits were distributed to participants upon biometric data collection. Each kit included one sample tube containing RNAlater (5 ml; a sample preservative supplied by ThermoFisher Scientific, Waltham, MA). Instructions for proper sample collection and storage (-20°C) were provided verbally and in print. Samples were submitted by mail or collected by a community facilitator within one week of biometric data collection. DNA and RNA were simultaneously isolated using the AllPrep PowerFecal DNA/RNA Kit (Qiagen Inc., Valencia, CA, USA) and stored at -80°C until further processing. Quality and concentration of nucleic acid yields were assessed using the NanoDrop Microvolume Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

### **4.3.3 16S-Based Metataxonomic Sequencing and Analysis**

DNA (40 ng) isolated from each stool sample was subjected to polymerase chain reaction (PCR) amplification targeting 16S rDNA hypervariable regions V2-4-8 and V3-6,7-9 (Ion Torrent 16S Metagenomics Kit; ThermoFisher Scientific, Warrington, England). Amplicon products were pooled (20  $\mu$ L per primer set), purified (Agencourt Ampure XP Kit; Beckman Coulter, Brea, CA, USA), and quantified using the Qubit dsDNA BR Assay (ThermoFisher Scientific, Warrington, England). 16S rDNA libraries were prepared from 150 ng of pooled amplicons (Ion Plus Fragment Library Kit; ThermoFisher Scientific, Austin, TX, USA) and barcoded using Ion Xpress Barcode Adapters (Life Technologies, Carlsbad, CA, USA). DNA libraries were pooled (80 pmol from up to 60 libraries) and loaded onto Ion 530™ chips (Ion S5 Next-Generation Sequencing System) in preparation for sequencing.

16S Metagenomics Kit analysis was performed using Ion Reporter™ Software v5.18.4.0 (ThermoFisher Scientific). Chimeric sequences were automatically identified and removed. Reads were mapped to reference databases Greengenes v13.5 and MicroSEQ ID v3.0. Gut microbiome profiles were compiled using metagenome taxonomic data via the Curated MicroSEQ(R) 16S Reference Library v2013.1. Upstream taxonomic ranks were determined by systematically comparing genus-level OTU data to the NCBI database via the classification function of the 'taxize' R package[219]. Unclassified remainders were then compared to the GBIS database using the same function. Phylum, family, and genus-level ranks were manually obtained for eleven taxa: [*Eubacterium*]*\_dolichum*[220], *Dorea*, [*Paraprevotellaceae*], [*Ruminococcus*]*\_gnavus*, *Desulfovibrio\_D168*, *Eschericia/Shigella\_coli/dysenteriae*, [*Eubacterium*]*\_biforme*, [*Ruminococcus*]*\_torques*, unclassified Fusobacteria, and *Candidatus Arthromitus*[221]. Taxonomy tables, OTU tables, and corresponding metadata were joined for downstream analyses using 'phyloseq'[222].

Differential abundance analyses were performed using Analysis of Compositions of Microbiomes with Bias Correction; the 'ANCOM-BC2' package[223]. ANCOM-BC applies a log transformation to OTU count data and corrects for sampling fraction and sequencing efficiency biases. Additionally, this tool allows for global and multiple pairwise comparisons for differential abundance analyses, accepts continuous and categorical covariates as fixed effects, and provides log fold change (LFC; natural log) estimates for taxonomic prevalence corresponding to each variable included in the analysis.

#### 4.3.4 Data Analysis

Intergroup comparisons of nominal distributions of biometric data were performed using Kruskal-Wallis rank-sum tests. P-values for multiple comparisons were adjusted via the Benjamini-Hochberg method. Categorical distributions were analyzed using Pearson's chi-squared tests for independence. Data was visualized via 'ggplot2', 'ggpubr,' and 'ggsignif' packages[134,135]. Microsoft Excel was used to create a bar graph illustrating the relative abundance of major gut bacterial phyla by racial-ethnic group.

## 4.4 Results

### 4.4.1 Preliminary Survey of Gut Bacterial Associations with Metabolic Risk

Since a subset of the total study cohort was included for gut microbiome analysis, **Table 4.3** summarizes sociodemographic and metabolic risk factors for the subgroup. In this cohort, distribution across gender (P=0.0198), T2DM (P=9.55E-7), BP (P=0.0011), and age groups (P=0.0015) significantly differed by race-ethnicity. However, differences in obesity-related metabolic health (MH/OB) risk were only marginally significant ( $\chi^2=21.02$ ; P=0.0501).

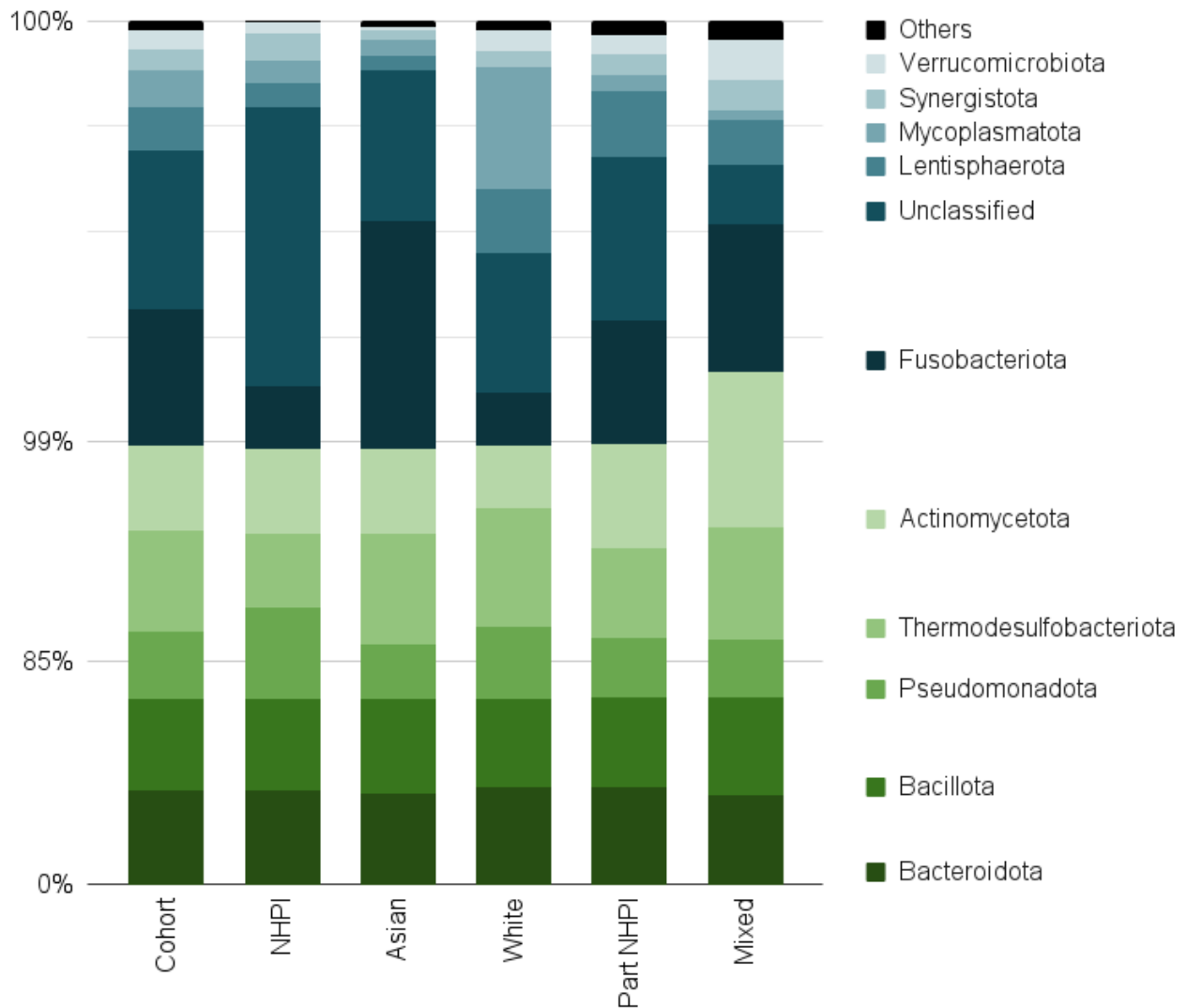
The relative abundance of major taxa was compared between groups to survey preliminary racial-ethnic differences in gut microbiome composition (**Figure 4.1**). At the phylum level, the most prevalent bacteria were Bacteroidota, Bacillota, Pseudomonadota, Thermodesulfobacteriota, and Actinomycetota, which together represented an average of 98.8% of the total gut bacterial population. Among the major phyla identified, Actinomycetota ( $W=5.984$ ,  $q=5.79E-04$ ; ANCOM-BC2, Benjamini-Hochberg P-adjustment), Bacillota ( $W=0.056$ ,  $q=1.63E-02$ ), Fusobacteriota ( $W=22.17$ ;  $q=5.32E-11$ ), Synergistota ( $W=4.509$ ;  $q=9.39E-03$ ), Thermodesulfobacteriota ( $W=6.251$ ;  $q=4.90E-04$ ), and Verrucomicrobiota ( $W=5.436$ ;  $q=2.95E-03$ ) were differentially abundant across racial-ethnic groups (**Table 4.4**).

Notably, intergroup differences in the relative abundance of Bacteroidota were nonsignificant. Among those phyla, Thermodesulfobacteriota was least prevalent in NHPIs (4.67%) and Part NHPIs (5.76%) compared to other racial-ethnic groups. Although the global intergroup differences in Pseudomonadota were nonsignificant, it was most prevalent in NHPIs compared to other groups (18.07%).

**Table 4.3** Cohort summary of sociodemographic and metabolic risk factors by racial-ethnic group.

	Total	Racial-Ethnic Groups					P <sup>a</sup>
		NHPI	Asian	White	Part NHPI	Mixed	
<b>Total (N)</b>	488	51	144	65	154	74	
<b>Gender (N)</b>							$\chi^2=11.69$ 0.0198
Male	195 (40.0%)	28 (54.9%)	44 (30.6%)	31 (47.7%)	62 (40.3%)	30 (40.5%)	
Female	293 (60.0%)	23 (45.1%)	100 (69.4%)	34 (52.3%)	92 (59.7%)	44 (59.5%)	
<b>T2DM Status (N)</b>							$\chi^2=42.81$ 9.55E-7
Nondiabetic	275 (56.4%)	21 (41.2%)	71 (49.3%)	57 (87.7%)	79 (51.3%)	47 (63.5%)	
Prediabetic	188 (38.5%)	23 (45.1%)	66 (45.8%)	8 (12.3%)	67 (43.5%)	24 (32.4%)	
Diabetic	25 (5.12%)	7 (13.7%)	7 (4.86%)		8 (5.19%)	3 (4.05%)	
<b>BP Categories (N)</b>							$\chi^2=38.88$ 0.0011
Low	4 (0.82%)		1 (0.69%)		2 (1.30%)	1 (1.35%)	
Normal	167 (34.2%)	10 (19.6%)	55 (38.2%)	25 (38.5%)	43 (27.9%)	34 (45.9%)	
Elevated	66 (13.5%)	12 (23.5%)	20 (13.9%)	12 (18.5%)	14 (9.09%)	8 (10.8%)	
HT I	121 (24.8%)	10 (19.6%)	40 (27.8%)	18 (27.7%)	35 (22.7%)	18 (24.3%)	
HT II	130 (26.6%)	19 (37.3%)	28 (19.4%)	10 (15.4%)	60 (39.0%)	13 (17.6%)	
<b>MH/OB Risk Status (N)</b>							$\chi^2=21.02$ 0.0501
MHNO	158 (32.4%)	15 (29.4%)	45 (31.3%)	26 (40.0%)	46 (29.9%)	26 (35.1%)	
MHOB	66 (13.5%)	5 (9.80%)	27 (18.8%)	11 (16.9%)	9 (5.84%)	14 (18.9%)	
MUNO	81 (16.6%)	8 (15.7%)	24 (16.7%)	8 (12.3%)	30 (19.5%)	11 (14.9%)	
MUOB	183 (37.5%)	23 (45.1%)	48 (33.3%)	20 (30.8%)	69 (44.8%)	23 (31.1%)	
<b>Age Group</b>							$\chi^2=37.98$ 0.0015
G1 (16-22)	174 (35.7%)	13 (25.5%)	38 (26.4%)	29 (44.6%)	58 (37.7%)	36 (48.6%)	
G2 (23-34)	134 (27.5%)	9 (17.6%)	56 (38.9%)	19 (29.2%)	32 (20.8%)	18 (24.3%)	
G3 (35-42)	79 (16.2%)	9 (17.6%)	21 (14.6%)	6 (9.23%)	32 (20.8%)	11 (14.9%)	
G4 (43-55)	69 (14.1%)	14 (27.5%)	19 (13.2%)	8 (12.3%)	21 (13.6%)	7 (9.46%)	
G5 (55-82)	32 (6.56%)	6 (11.8%)	10 (6.94%)	3 (4.62%)	11 (7.14%)	2 (2.70%)	

<sup>a</sup>Chi-squared test for independence. Statistical significance is determined at  $P<0.05$ . T2DM=Type 2 diabetes mellitus. HT=Hypertension. MH/OB=Obesity-related metabolic health. Age ranges are provided per age group.



**Figure 4.1** Phylum-level summary of gut microbiome composition by racial-ethnic group. The y-axis percentage values are not to scale. On average, Bacteroidota (35.7%), Bacillota (35.0%), Pseudomonadota (16.3%), Thermodesulfobacteriota (6.45%), and Actinomycetota (5.41%) were among the majority of gut bacterial phyla. The remaining 1.21% included Fusobacteriota, Unclassified bacteria, Lentisphaerota, Mycoplasmatota, Synergistota, Verrucomicrobiota, and other less prevalent phyla.

At the genus level, NHPIs had the highest prevalence of *Eggerthella*, *Blautia*, *Megamonas*, *Veillonella*, *Lachnoclostridium*, *Lactobacillus*, *Fusobacterium*, *Haemophilus*, *Klebsiella*, and *Mannheimia*. *Bilophila* was also notably prevalent in NHPIs, similar to the highest prevalence in the Mixed group. The genera that were least prevalent in NHPIs were *Ruminiclostridium* (which was similarly depleted in the White group), *Barnesiella*, *Prevotella*, *Herbaspirillum*, *Desulfovibrio*, and *Akkermansia*. Part/NHPIs had a similarly low prevalence of *Slackia*, *Cronobacter*, *Paraprevotella* (comparatively depleted in NHPIs), and *Faecalibacterium*.

**Table 4.4** Differentially abundant gut bacteria across racial-ethnic groups at phylum and genus levels.

	Total Cohort	Relative Abundance by Race-Ethnicity					ANCOM-BC2	
		NHPI	Asian	White	Part NHPI	Mixed	W	q <sup>c</sup>
<b>Actinomycetota</b>	5.409%	5.372%	5.437%	3.985%	<b>6.669%</b>	5.582%	5.984	5.79E-04
<i>Bifidobacterium</i>	4.234%	4.174%	4.650%	4.275%	<b>4.888%</b>	3.181%	8.356	4.63E-05
<i>Slackia</i>	0.014%	0.008%	0.013%	<b>0.028%</b>	0.007%	0.012%	4.908	1.17E-02
<i>Eggerthella</i>	0.051%	<b>0.085%</b>	0.036%	0.026%	0.034%	0.075%	3.959	2.07E-02
<b>Bacillota</b>	34.97%	34.79%	36.08%	33.04%	34.14%	<b>36.79%</b>	0.056	1.63E-02
<i>Acidaminococcus</i>	0.439%	0.251%	0.530%	0.374%	<b>0.833%</b>	0.206%	7.256	3.27E-04
<i>Blautia</i>	2.921%	<b>3.710%</b>	2.703%	2.599%	2.545%	3.047%	6.257	7.95E-04
<i>Catenibacterium</i>	0.739%	0.874%	0.756%	<b>0.956%</b>	0.842%	0.266%	3.909	2.06E-02
[ <i>Clostridiales</i> ] <sup>a</sup>	0.018%	0.008%	0.020%	<b>0.033%</b>	0.022%	0.006%	4.657	1.41E-02
<i>Enterococcus</i>	0.129%	0.158%	0.125%	<b>0.327%</b>	0.023%	0.013%	8.414	3.69E-04
[ <i>Eubacterium</i> ] <sup>b</sup>	0.730%	0.984%	0.743%	<b>1.211%</b>	0.529%	0.182%	3.784	2.07E-02
<i>Faecalibacterium</i>	8.938%	8.285%	9.060%	9.501%	8.315%	<b>9.527%</b>	0.016	3.76E-03
<i>Megasphaera</i>	0.304%	0.261%	0.300%	0.220%	<b>0.376%</b>	0.361%	7.922	2.55E-04
<i>Turicibacter</i>	0.119%	0.161%	0.108%	0.076%	0.084%	<b>0.164%</b>	5.763	2.77E-03
<i>Tyzzellerella</i>	0.032%	0.035%	<b>0.048%</b>	0.037%	0.027%	0.013%	5.663	3.53E-03
<i>Megamonas</i>	1.279%	<b>1.968%</b>	1.732%	1.069%	1.427%	0.199%	5.496	2.79E-03
<i>Veillonella</i>	0.086%	<b>0.236%</b>	0.057%	0.019%	0.058%	0.060%	5.333	4.90E-03
<i>Lactococcus</i>	0.050%	0.065%	0.025%	0.020%	0.025%	<b>0.114%</b>	4.853	9.05E-03
<i>Lachnoclostridium</i>	0.208%	<b>0.275%</b>	0.182%	0.149%	0.189%	0.243%	3.967	1.80E-02
<i>Ruminiclostridium</i>	0.020%	0.012%	<b>0.026%</b>	0.012%	0.022%	0.027%	3.774	3.74E-02
<i>Lactobacillus</i>	0.900%	<b>1.386%</b>	0.751%	0.631%	0.784%	0.950%	3.665	2.40E-02
<b>Bacteroidota</b>	35.69%	35.60%	34.37%	<b>37.29%</b>	36.99%	34.19%	0.150	9.06E-02
<i>Alistipes</i>	2.013%	1.786%	2.136%	1.425%	<b>2.610%</b>	2.107%	4.057	1.52E-02
<i>Bacteroides</i>	22.65%	25.13%	21.63%	19.06%	22.15%	<b>25.30%</b>	10.31	1.73E-06
<i>Barnesiella</i>	0.532%	0.333%	0.548%	0.598%	0.507%	<b>0.674%</b>	3.651	2.51E-02
<i>Paraprevotella</i>	0.390%	0.098%	0.371%	<b>0.701%</b>	0.251%	0.528%	11.89	1.73E-06
<i>Prevotella</i>	13.42%	9.890%	14.12%	<b>17.24%</b>	13.93%	11.90%	19.62	1.37E-12
<b>Fusobacteriota</b>	0.551%	0.639%	<b>0.934%</b>	0.412%	0.419%	0.351%	22.17	5.32E-11
<i>Fusobacterium</i>	0.510%	<b>0.843%</b>	0.364%	0.611%	0.255%	0.479%	15.14	4.49E-07
<b>Mycoplasmata</b>	0.088%	0.055%	0.037%	<b>0.289%</b>	0.038%	0.021%	14.56	7.32E-07
<b>Pseudomonadota</b>	16.25%	<b>18.07%</b>	15.63%	16.87%	15.32%	15.36%	0.825	9.80E-01
<i>Cronobacter</i>	0.082%	0.013%	0.083%	<b>0.259%</b>	0.015%	0.041%	6.354	2.77E-03
<i>Enterobacter</i>	0.042%	0.033%	0.033%	0.015%	0.046%	<b>0.083%</b>	4.840	1.25E-02
<i>Haemophilus</i>	0.161%	<b>0.348%</b>	0.121%	0.104%	0.134%	0.101%	6.129	1.52E-03
<i>Herbaspirillum</i>	0.343%	0.149%	0.334%	0.335%	0.387%	<b>0.511%</b>	5.612	2.79E-03
<i>Klebsiella</i>	0.215%	<b>0.347%</b>	0.242%	0.105%	0.157%	0.224%	9.347	8.31E-05
<i>Mannheimia</i>	0.207%	<b>0.343%</b>	0.131%	0.107%	0.151%	0.300%	6.591	7.53E-04
<i>Shewanella</i>	0.008%	0.008%	0.008%	0.003%	0.007%	<b>0.017%</b>	4.288	2.07E-02
<b>Synergistota</b>	0.050%	0.061%	0.022%	0.038%	0.051%	<b>0.076%</b>	4.509	9.39E-03
<b>Thermodesulfo...</b> <sup>c</sup>	6.450%	4.670%	7.076%	<b>7.510%</b>	5.757%	7.238%	6.251	4.90E-04
<i>Bilophila</i>	7.176%	8.420%	6.777%	5.341%	6.503%	<b>8.841%</b>	4.301	1.17E-02
<i>Desulfovibrio</i>	0.278%	0.081%	0.285%	0.289%	0.330%	<b>0.404%</b>	3.881	2.07E-02
<b>Verrucomicrobiota</b>	0.045%	0.028%	0.009%	0.050%	0.044%	<b>0.093%</b>	5.436	2.95E-03
<i>Akkermansia</i>	0.036%	0.011%	<b>0.056%</b>	0.031%	0.045%	0.039%	4.853	1.25E-02

<sup>a</sup>*Clostridiales* Family XIII. *Incertae Sedis*. <sup>b</sup>*Eubacterium bifforme*. <sup>c</sup>Thermodesulfobacteriota. <sup>d</sup>Adjusted P-values (Benjamini-Hochberg). Phylum names are bolded. Nonsignificant phylum-level results are presented if significant results were obtained for downstream taxa. The highest percentage for each taxon between racial-ethnic groups is bolded.

#### 4.4.2 Gut Bacterial Associations with MU Risk Factors

To explore the metabolic relevance of these differences, gut bacterial associations with age, A1c levels, BRI, and BP were assessed for each racial-ethnic group (**Table 4.5**). Four genera were consistently associated with at least one of the four risk factors across all racial-ethnic groups: *Herbaspirillum*, *Klebsiella*, *Megasphaera*, and *Turicibacter*.

***Herbaspirillum***: In NHPIs, *Herbaspirillum* was only positively associated with diastolic BP and exhibited a 7.36% increase for every mm Hg increase in diastolic BP ( $q < 0.001$ ). For the White group, *Herbaspirillum* was negatively associated with BRI (LFC -0.450;  $q = 0.042$ ) but positively associated with systolic BP (LFC 0.075;  $q = 0.049$ ). For the Asian group, *Herbaspirillum* abundance would increase slightly (3.35%;  $q < 0.001$ ) with every year of increasing age, and like in the NHPI group, it would increase by 5.13% per unit increase of diastolic BP. In the Part NHPI (LFC -0.045,  $q = 0.01$ ) and Mixed groups (LFC -0.083;  $q < 0.001$ ), *Herbaspirillum* was negatively associated with diastolic BP. Additionally, in the Mixed group, *Herbaspirillum* was negatively associated with BRI, with an estimated 25.9% reduction in abundance per unit change in BRI.

***Klebsiella***: For NHPIs (LFC 0.075;  $q = 0.013$ ) and Asians (LFC 0.042;  $q = 0.003$ ), *Klebsiella* was only associated with diastolic BP out of the four measured risk factors. *Klebsiella* was only associated with age for the White group (LFC 0.073;  $q = 0.021$ ) and with BRI in the Part NHPI group (LFC 0.222;  $q < 0.001$ ). For the Mixed group, however, *Klebsiella* was positively associated with age (LFC 0.103;  $q = 0.047$ ) and diastolic BP (LFC 0.077;  $q = 0.001$ ), but negatively associated with A1c levels (LFC -1.156;  $q < 0.001$ ).

***Megasphaera***: Uniquely in NHPIs, *Megasphaera* exhibited a strong positive association with A1c levels (LFC 2.586;  $q < 0.001$ ). For both NHPIs and Asians, *Megasphaera* was positively associated with age (NHPI LFC 0.058,  $q < 0.001$ ; Asian LFC 0.068,  $q < 0.001$ ), but its association with BRI and diastolic BP exhibited opposing directionalities between the groups. The association with BRI was negative for NHPIs (LFC -0.171;  $q = 0.004$ ) and positive for Asians (LFC 0.689;  $q < 0.001$ ), and that with diastolic BP was positive for NHPIs (LFC 0.037;  $q = 0.013$ ) and negative for Asians (LFC -0.041;  $q < 0.001$ ). For the Part NHPI and Mixed groups, significant associations between *Megasphaera* and diastolic BP similarly demonstrated opposing directionality: it was positive for Part NHPIs (LFC 0.058;  $q = 0.001$ ) and negative for the Mixed group (LFC -0.091;  $q = 0.005$ ). Notably, the abundance of *Megasphaera* was associated positively with diastolic BP for both the NHPI and Part NHPI groups but differentially associated with other MHOB risk factors.

***Turicibacter***: Uniquely for NHPIs, *Turicibacter* was positively associated with A1c levels, exhibiting an approximate 125% change per unit increase of A1c ( $q = 0.047$ ). Uniquely, in Part NHPIs, *Turicibacter* was associated positively with systolic BP (LFC 0.031;  $q = 0.024$ ). For NHPIs (LFC -0.031;  $q = 0.029$ ), Part

NHPIs (LFC -0.048; q=0.01), and the Mixed group (LFC -0.083; q<0.001), *Turicibacter* was negatively associated with diastolic BP, while the opposite trend was observed for the Asian group (LFC -0.048; q=0.001). *Turicibacter* and BRI were negatively associated in NHPIs (LFC -0.183; q=0.004) and Asians (LFC -0.465; q=0.001) but positively for Part NHPIs (LFC 0.181; q<0.001). A negative association was also observed between *Turicibacter* and age for the White group (LFC -0.023; q=0.006).

**Table 4.5** Significant log fold change (LFC) in the abundance of gut bacterial genera per unit increase of age, BRI, A1c, systolic BP, and diastolic BP by racial-ethnic group.

	Age		BRI		A1C		Systolic BP		Diastolic BP	
	LFC	q	LFC	q	LFC	q	LFC	q	LFC	q
<b>NHPI (N=51)</b>										
<i>Acidaminococcus</i>	-0.008	0.734	<b>0.265</b>	<b>0.003</b>	-0.493	0.300	0.048	0.067	<b>-0.082</b>	<b>0.000</b>
<i>Alistipes</i>	<b>-0.030</b>	<b>0.038</b>	<b>-0.555</b>	<b>0.004</b>	0.397	0.493	-0.002	1.000	<b>0.070</b>	<b>0.016</b>
<i>Allisonella</i>	<b>-0.055</b>	<b>0.002</b>	<b>0.173</b>	<b>0.018</b>	0.320	0.496	-0.024	0.365	0.008	0.703
<i>Barnesiella</i>	-0.003	0.901	0.110	0.188	-0.067	0.972	0.023	0.444	<b>-0.049</b>	<b>0.020</b>
<i>Butyricimonas</i>	-0.018	0.191	<b>-0.163</b>	<b>0.011</b>	0.192	0.760	-0.027	0.252	0.027	0.055
<i>Catenibacterium</i>	<b>-0.033</b>	<b>0.026</b>	0.053	0.607	-0.507	0.311	0.027	0.332	-0.024	0.273
[Clostridiales] <sup>‡</sup>	0.009	0.665	-0.038	0.607	<b>1.059</b>	<b>0.002</b>	-0.012	0.811	0.018	0.703
[Clostridiales XI] <sup>‡</sup>	<b>0.046</b>	<b>0.014</b>	<b>0.346</b>	<b>0.012</b>	0.573	0.368	-0.098	0.067	<b>-0.056</b>	<b>0.024</b>
[Clostridiales XIII] <sup>‡</sup>	<b>-0.108</b>	<b>0.003</b>	0.110	0.164	0.438	0.462	0.106	0.067	<b>-0.196</b>	<b>0.002</b>
<i>Desulfovibrio</i>	-0.008	0.352	0.063	0.188	-0.247	0.578	-0.058	0.054	<b>0.106</b>	<b>0.000</b>
* <i>Dorea</i>	<b>-0.031</b>	<b>0.014</b>	-0.007	1.000	-0.182	0.772	0.034	0.133	-0.018	0.374
[ <i>E. biforme</i> ] <sup>‡</sup>	<b>-0.043</b>	<b>0.002</b>	<b>0.178</b>	<b>0.018</b>	0.241	0.589	0.001	1.000	0.011	0.720
<i>Flavonifractor</i>	0.013	0.547	-0.035	0.607	<b>0.766</b>	<b>0.038</b>	-0.021	0.444	0.024	0.346
<i>Fusobacterium</i>	0.013	0.346	<b>-0.182</b>	<b>0.027</b>	-0.434	0.430	-0.012	0.811	0.029	0.171
<i>Herbaspirillum</i>	0.008	0.739	0.127	0.068	-0.301	0.496	-0.049	0.086	<b>0.071</b>	<b>0.000</b>
<i>Klebsiella</i>	-0.014	0.279	0.142	0.065	0.915	0.205	-0.071	0.067	<b>0.075</b>	<b>0.013</b>
<i>Mannheimia</i>	-0.002	0.901	<b>0.262</b>	<b>0.002</b>	<b>-1.070</b>	<b>0.011</b>	-0.024	0.365	-0.010	0.703
<i>Megasphaera</i>	<b>0.058</b>	<b>0.000</b>	<b>-0.171</b>	<b>0.004</b>	<b>2.586</b>	<b>0.000</b>	-0.050	0.067	<b>0.037</b>	<b>0.013</b>
<i>Paraprevotella</i>	<b>-0.055</b>	<b>0.001</b>	<b>-0.245</b>	<b>0.004</b>	0.187	0.777	-0.059	0.067	<b>0.089</b>	<b>0.000</b>
<i>Pseudobutyrvibrio</i>	-0.019	0.137	0.059	0.401	-0.036	1.000	0.042	0.086	<b>-0.052</b>	<b>0.007</b>
* <i>Ruminococcus</i>	<b>-0.023</b>	<b>0.038</b>	-0.045	0.471	0.013	1.000	0.006	0.931	0.009	0.729
* <i>Succinivibrio</i>	0.003	0.901	<b>-0.292</b>	<b>0.022</b>	0.406	0.493	0.052	0.163	0.006	0.894
<i>Subdoligranulum</i>	0.003	0.791	<b>0.143</b>	<b>0.047</b>	0.352	0.496	0.002	1.000	<b>-0.072</b>	<b>0.009</b>
* <i>Streptococcus</i>	<b>0.035</b>	<b>0.033</b>	-0.012	0.992	0.097	0.972	0.016	0.811	-0.032	0.317
<i>Turicibacter</i>	0.012	0.452	<b>-0.183</b>	<b>0.004</b>	<b>0.811</b>	<b>0.047</b>	0.037	0.106	<b>-0.031</b>	<b>0.029</b>
<i>Tyzzera</i>	<b>0.034</b>	<b>0.014</b>	<b>0.220</b>	<b>0.021</b>	-0.366	0.493	0.002	1.000	-0.001	1.000
<i>Megamonas</i>	<b>-0.061</b>	<b>0.003</b>	0.144	0.076	<b>-1.322</b>	<b>0.003</b>	0.049	0.106	<b>-0.141</b>	<b>0.000</b>
<b>White (N=65)</b>										
* <i>Akkermansia</i>	<b>0.049</b>	<b>0.000</b>	-0.133	0.564	0.976	0.989	0.029	0.763	0.022	0.797
<i>Alistipes</i>	<b>0.062</b>	<b>0.000</b>	-0.415	0.114	0.579	0.989	-0.135	0.049	0.253	0.006
<i>Anaerostipes</i>	<b>0.038</b>	<b>0.001</b>	0.114	0.642	0.214	0.989	0.019	0.875	-0.080	0.168
<i>Butyricimonas</i>	<b>0.020</b>	<b>0.020</b>	-0.026	0.902	-0.780	0.989	0.020	0.818	0.024	0.744
<i>Catenibacterium</i>	<b>0.047</b>	<b>0.001</b>	0.236	0.356	-1.161	0.989	0.017	0.875	0.007	0.984
[Clostridiales XI] <sup>‡</sup>	<b>-0.042</b>	<b>0.004</b>	0.273	0.356	1.096	0.989	-0.069	0.306	0.000	0.994
<i>Coprococcus</i>	<b>-0.028</b>	<b>0.004</b>	0.258	0.310	0.013	0.989	-0.010	0.879	-0.020	0.808
<i>Cronobacter</i>	<b>-0.134</b>	<b>0.000</b>	0.812	0.065	0.610	0.989	-0.007	0.972	0.076	0.312
<i>Desulfovibrio</i>	<b>0.050</b>	<b>0.000</b>	0.139	0.560	-0.746	0.989	-0.012	0.879	-0.044	0.439
<i>Dialister</i>	<b>-0.039</b>	<b>0.000</b>	-0.166	0.541	0.344	0.989	-0.009	0.898	0.056	0.312
<i>Eggerthella</i>	<b>-0.084</b>	<b>0.000</b>	0.315	0.123	1.385	0.989	-0.016	0.875	0.055	0.312
* <i>Eubacterium</i>	<b>0.028</b>	<b>0.032</b>	-0.135	0.560	-0.937	0.989	0.003	0.985	0.051	0.312

**Table 4.5** Significant log fold change (LFC) in the abundance of gut bacterial genera per unit increase of age, BRI, A1c, systolic BP, and diastolic BP by racial-ethnic group.

	Age		BRI		A1C		Systolic BP		Diastolic BP	
	LFC	q	LFC	q	LFC	q	LFC	q	LFC	q
<i>[E. bifforme]</i> <sup>‡</sup>	<b>0.062</b>	<b>0.000</b>	<b>0.794</b>	<b>0.001</b>	-1.494	0.989	0.035	0.542	<b>-0.173</b>	<b>0.002</b>
<i>Haemophilus</i>	<b>0.032</b>	<b>0.001</b>	-0.309	0.125	-1.254	0.989	0.058	0.176	-0.024	0.797
<i>Herbaspirillum</i>	0.016	0.432	<b>-0.450</b>	<b>0.042</b>	1.037	0.989	<b>0.075</b>	<b>0.049</b>	-0.006	0.984
<i>Klebsiella</i>	<b>0.073</b>	<b>0.021</b>	-0.288	0.385	0.996	0.989	-0.159	0.176	0.189	0.168
<i>Lactococcus</i>	<b>0.128</b>	<b>0.000</b>	0.059	0.884	-0.428	0.989	0.047	0.306	<b>-0.137</b>	<b>0.018</b>
<i>Mannheimia</i>	<b>0.079</b>	<b>0.000</b>	-0.129	0.560	0.068	0.989	0.014	0.879	-0.033	0.643
<i>Megamonas</i>	<b>0.024</b>	<b>0.042</b>	0.406	0.113	-1.831	0.989	-0.029	0.763	0.051	0.439
<i>Megasphaera</i>	<b>-0.093</b>	<b>0.001</b>	-0.247	0.356	1.958	0.989	-0.119	0.064	<b>0.189</b>	<b>0.037</b>
* <i>Oxalobacter</i>	<b>0.029</b>	<b>0.042</b>	-0.299	0.356	-0.296	0.989	0.026	0.816	-0.036	0.643
* <i>Parasutterella</i>	<b>-0.096</b>	<b>0.000</b>	0.192	0.541	-0.300	0.989	-0.011	0.879	0.047	0.515
<i>Phascolarcto...</i> <sup>e</sup>	<b>0.050</b>	<b>0.003</b>	0.317	0.163	-1.294	0.989	0.010	0.879	-0.081	0.156
<i>Photobacterium</i>	<b>-0.025</b>	<b>0.001</b>	0.158	0.541	0.234	0.989	0.000	0.995	0.000	0.994
<i>Ruminiclostridium</i>	<b>0.074</b>	<b>0.000</b>	<b>-0.649</b>	<b>0.025</b>	0.178	0.989	<b>0.092</b>	<b>0.049</b>	-0.039	0.515
<i>Subdoligranulum</i>	0.011	0.160	<b>-0.590</b>	<b>0.025</b>	-0.349	0.989	-0.001	0.985	0.100	0.085
<i>Turicibacter</i>	<b>-0.023</b>	<b>0.006</b>	-0.081	0.771	-0.425	0.989	-0.043	0.306	0.010	0.939
<i>Veillonella</i>	<b>-0.026</b>	<b>0.020</b>	<b>1.361</b>	<b>0.025</b>	5.021	0.989	0.019	0.875	-0.074	0.312
<b>Asian (N=144)</b>										
<i>Acidaminococcus</i>	0.015	0.219	<b>0.357</b>	<b>0.005</b>	-0.946	0.439	0.037	0.160	<b>-0.080</b>	<b>0.000</b>
<i>Allisonella</i>	<b>-0.035</b>	<b>0.008</b>	-0.138	0.447	-1.503	0.180	0.027	0.354	0.001	0.935
<i>Anaerostipes</i>	0.000	0.998	-0.047	0.945	0.139	0.984	0.002	0.969	<b>0.023</b>	<b>0.003</b>
<i>Butyricimonas</i>	<b>0.030</b>	<b>0.008</b>	0.012	0.980	-0.224	0.984	-0.003	0.968	0.004	0.851
<i>Catenibacterium</i>	<b>-0.057</b>	<b>0.000</b>	0.187	0.433	-0.961	0.439	0.033	0.237	0.005	0.914
<i>[Clostridiales]</i> <sup>‡</sup>	0.004	0.824	0.045	0.973	-0.297	0.984	-0.019	0.402	<b>0.029</b>	<b>0.012</b>
<i>[Clostridiales XIII.]</i> <sup>‡</sup>	-0.003	0.840	-0.135	0.447	0.492	0.984	0.014	0.766	<b>0.021</b>	<b>0.006</b>
<i>Desulfovibrio</i>	-0.013	0.293	0.258	0.079	-0.418	0.984	<b>-0.059</b>	<b>0.010</b>	<b>0.079</b>	<b>0.000</b>
<i>[E. bifforme]</i> <sup>‡</sup>	-0.001	0.981	0.015	0.980	0.251	0.984	-0.028	0.237	<b>0.027</b>	<b>0.012</b>
<i>Eggerthella</i>	-0.009	0.622	<b>-0.312</b>	<b>0.016</b>	0.284	0.984	0.029	0.237	-0.015	0.304
<i>Enterobacter</i>	-0.017	0.148	-0.097	0.647	0.381	0.984	0.007	0.931	<b>0.042</b>	<b>0.000</b>
<i>Flavonifractor</i>	0.004	0.824	0.023	0.980	-0.158	0.984	-0.027	0.237	<b>0.030</b>	<b>0.012</b>
<i>Fusobacterium</i>	0.029	0.061	<b>0.525</b>	<b>0.001</b>	-1.673	0.150	<b>0.069</b>	<b>0.010</b>	<b>-0.067</b>	<b>0.000</b>
<i>Haemophilus</i>	-0.008	0.661	-0.265	0.098	0.756	0.560	0.032	0.237	<b>-0.047</b>	<b>0.000</b>
<i>Herbaspirillum</i>	<b>0.033</b>	<b>0.005</b>	-0.227	0.170	-0.069	0.984	-0.023	0.383	<b>0.050</b>	<b>0.000</b>
<i>Klebsiella</i>	0.003	0.840	-0.169	0.447	0.305	0.984	-0.009	0.931	<b>0.042</b>	<b>0.003</b>
* <i>Lachnobacterium</i>	0.011	0.355	0.133	0.447	-0.328	0.984	-0.011	0.811	<b>0.041</b>	<b>0.000</b>
* <i>Lachnospira</i>	-0.004	0.793	0.006	0.980	-0.151	0.984	-0.012	0.789	<b>0.026</b>	<b>0.002</b>
<i>Lactococcus</i>	<b>0.028</b>	<b>0.008</b>	0.087	0.695	-0.763	0.586	0.024	0.369	0.015	0.072
<i>Mannheimia</i>	<b>-0.025</b>	<b>0.049</b>	-0.033	0.980	0.317	0.984	0.006	0.944	-0.002	0.935
<i>Megamonas</i>	<b>-0.041</b>	<b>0.011</b>	<b>0.599</b>	<b>0.001</b>	-1.248	0.180	-0.034	0.237	-0.004	0.929
<i>Megasphaera</i>	<b>0.068</b>	<b>0.000</b>	<b>0.689</b>	<b>0.000</b>	-0.899	0.497	-0.007	0.931	<b>-0.041</b>	<b>0.000</b>
<i>Paraprevotella</i>	<b>0.065</b>	<b>0.000</b>	<b>-0.321</b>	<b>0.049</b>	-1.493	0.180	-0.029	0.255	<b>0.119</b>	<b>0.000</b>
<i>Photobacterium</i>	<b>-0.035</b>	<b>0.001</b>	-0.008	0.980	0.322	0.984	0.002	0.968	0.001	0.976
<i>Pseudobutyrvibrio</i>	<b>-0.031</b>	<b>0.012</b>	-0.086	0.695	-0.010	0.984	-0.004	0.958	0.015	0.387
<i>Turicibacter</i>	-0.003	0.840	<b>-0.465</b>	<b>0.001</b>	-0.216	0.984	-0.003	0.963	<b>0.048</b>	<b>0.000</b>
<i>Tyzzera</i>	<b>-0.023</b>	<b>0.022</b>	-0.114	0.577	0.658	0.722	0.005	0.944	0.017	0.088
<b>Part NHPI (N=154)</b>										
<i>Barnesiella</i>	-0.018	0.284	0.089	0.104	0.373	0.692	0.014	0.412	<b>-0.037</b>	<b>0.042</b>
<i>[Clostridiales XI.]</i> <sup>‡</sup>	<b>-0.033</b>	<b>0.001</b>	-0.044	0.338	<b>1.948</b>	<b>0.000</b>	-0.028	0.068	<b>0.039</b>	<b>0.019</b>
<i>[Clostridiales XIII.]</i> <sup>‡</sup>	-0.006	0.626	<b>-0.100</b>	<b>0.048</b>	-0.406	0.707	-0.020	0.186	<b>0.054</b>	<b>0.004</b>
<i>Coprococcus</i>	-0.018	0.136	<b>0.104</b>	<b>0.035</b>	0.058	0.998	0.015	0.311	<b>-0.038</b>	<b>0.010</b>

**Table 4.5** Significant log fold change (LFC) in the abundance of gut bacterial genera per unit increase of age, BRI, A1c, systolic BP, and diastolic BP by racial-ethnic group.

	Age		BRI		A1C		Systolic BP		Diastolic BP	
	LFC	q	LFC	q	LFC	q	LFC	q	LFC	q
<i>Dialister</i>	<b>-0.028</b>	<b>0.029</b>	0.089	0.181	0.009	0.998	0.030	0.063	-0.022	0.294
<i>Eggerthella</i>	<b>-0.043</b>	<b>0.000</b>	<b>-0.113</b>	<b>0.012</b>	1.041	0.050	0.002	0.940	0.017	0.294
<i>Flavonifractor</i>	<b>0.038</b>	<b>0.001</b>	<b>-0.110</b>	<b>0.016</b>	0.089	0.998	-0.007	0.766	0.018	0.425
<i>Fusobacterium</i>	0.001	0.972	<b>0.148</b>	<b>0.005</b>	0.256	0.948	0.030	0.063	<b>-0.057</b>	<b>0.001</b>
<i>Haemophilus</i>	-0.012	0.297	<b>0.185</b>	<b>0.000</b>	-0.037	0.998	0.000	0.999	<b>-0.034</b>	<b>0.010</b>
<i>Herbaspirillum</i>	-0.010	0.509	0.035	0.670	-0.785	0.093	0.025	0.098	<b>-0.045</b>	<b>0.010</b>
* <i>Holdemania</i>	<b>0.023</b>	<b>0.029</b>	0.074	0.097	0.501	0.572	-0.003	0.869	-0.009	0.599
<i>Klebsiella</i>	-0.002	0.965	<b>0.222</b>	<b>0.000</b>	-0.141	0.998	-0.004	0.835	0.016	0.358
* <i>Lachnoclostridium</i>	-0.013	0.284	-0.009	0.932	-0.057	0.998	0.024	0.068	<b>-0.037</b>	<b>0.010</b>
* <i>Lactobacillus</i>	-0.024	0.092	<b>0.191</b>	<b>0.001</b>	0.474	0.540	0.024	0.134	-0.020	0.358
<i>Lactococcus</i>	-0.010	0.356	<b>0.150</b>	<b>0.001</b>	0.939	0.065	-0.004	0.835	-0.003	0.905
* <i>Leuconostoc</i>	-0.015	0.176	<b>0.179</b>	<b>0.001</b>	0.391	0.707	0.004	0.823	<b>-0.032</b>	<b>0.035</b>
<i>Megasphaera</i>	0.014	0.277	0.018	0.902	0.001	0.998	-0.010	0.602	<b>0.058</b>	<b>0.001</b>
<i>Paraprevotella</i>	-0.017	0.176	<b>0.105</b>	<b>0.047</b>	<b>-1.026</b>	<b>0.042</b>	-0.001	0.940	-0.005	0.836
<i>Photobacterium</i>	0.000	0.972	<b>0.170</b>	<b>0.001</b>	0.382	0.707	0.001	0.949	<b>-0.044</b>	<b>0.004</b>
* <i>Roseburia</i>	<b>-0.027</b>	<b>0.029</b>	0.008	0.943	0.003	0.998	0.006	0.794	0.007	0.786
* <i>Senegalimassilia</i>	-0.007	0.583	<b>0.095</b>	<b>0.047</b>	1.112	0.061	0.015	0.333	<b>-0.065</b>	<b>0.001</b>
<i>Shewanella</i>	<b>0.025</b>	<b>0.030</b>	0.023	0.702	-0.032	0.998	<b>0.041</b>	<b>0.024</b>	<b>-0.088</b>	<b>0.000</b>
* <i>Slackia</i>	0.003	0.888	<b>0.094</b>	<b>0.035</b>	-0.182	0.998	-0.004	0.835	0.018	0.219
<i>Subdoligranulum</i>	0.002	0.937	-0.064	0.133	0.394	0.707	-0.023	0.106	<b>0.038</b>	<b>0.009</b>
<i>Turicibacter</i>	-0.002	0.965	<b>0.181</b>	<b>0.000</b>	-0.461	0.654	<b>0.031</b>	<b>0.024</b>	<b>-0.048</b>	<b>0.001</b>
<i>Veillonella</i>	-0.007	0.626	<b>0.284</b>	<b>0.000</b>	-0.029	0.998	<b>0.036</b>	<b>0.024</b>	<b>-0.039</b>	<b>0.010</b>
* <i>Victivallis</i>	<b>0.045</b>	<b>0.001</b>	-0.002	0.952	0.011	0.998	0.009	0.602	<b>-0.042</b>	<b>0.010</b>
<b>Mixed (N=74)</b>										
<i>Acidaminococcus</i>	0.066	0.270	-0.014	0.930	-2.162	0.741	-0.044	0.654	<b>0.054</b>	<b>0.011</b>
<i>Butyrivimonas</i>	-0.053	0.337	-0.015	0.917	0.802	0.991	-0.013	0.939	<b>0.054</b>	<b>0.006</b>
<i>Catenibacterium</i>	-0.002	0.990	0.036	0.905	-0.443	0.991	-0.050	0.531	<b>0.075</b>	<b>0.002</b>
[ <i>Clostridiales</i> ] <sup>a</sup>	-0.005	0.990	-0.079	0.546	0.203	0.991	-0.018	0.893	<b>0.036</b>	<b>0.049</b>
[ <i>Clostridiales XIII.</i> ] <sup>b</sup>	0.001	0.990	-0.264	0.074	1.999	0.991	-0.004	0.993	<b>0.061</b>	<b>0.025</b>
<i>Cronobacter</i>	0.040	0.523	<b>-0.321</b>	<b>0.009</b>	0.865	0.991	0.013	0.939	0.004	0.903
<i>Dialister</i>	-0.036	0.530	<b>0.287</b>	<b>0.003</b>	0.191	0.991	-0.021	0.893	0.016	0.531
<i>Enterobacter</i>	0.217	0.174	<b>-0.992</b>	<b>0.028</b>	-3.042	0.991	0.314	0.133	-0.032	0.249
* <i>Enterococcus</i>	<b>0.141</b>	<b>0.042</b>	<b>-0.636</b>	<b>0.001</b>	0.212	0.991	0.025	0.893	0.025	0.209
[ <i>E. bifforme</i> ] <sup>b</sup>	0.003	0.990	0.169	0.101	-0.658	0.991	-0.064	0.373	<b>0.095</b>	<b>0.000</b>
<i>Fusobacterium</i>	<b>0.204</b>	<b>0.042</b>	-0.229	0.103	0.109	0.991	-0.197	0.133	<b>0.197</b>	<b>0.001</b>
* <i>Granulicatella</i>	0.109	0.337	-0.372	0.103	-2.382	0.991	-0.136	0.373	<b>0.092</b>	<b>0.048</b>
<i>Haemophilus</i>	0.052	0.337	<b>-0.454</b>	<b>0.000</b>	0.103	0.991	-0.028	0.893	0.011	0.696
<i>Herbaspirillum</i>	0.053	0.337	<b>-0.300</b>	<b>0.009</b>	-0.897	0.991	0.084	0.133	<b>-0.083</b>	<b>0.000</b>
<i>Klebsiella</i>	<b>0.103</b>	<b>0.047</b>	<b>-1.156</b>	<b>0.000</b>	1.430	0.991	0.028	0.893	<b>0.077</b>	<b>0.001</b>
<i>Mannheimia</i>	0.000	0.998	<b>-0.248</b>	<b>0.009</b>	0.122	0.991	-0.028	0.893	0.036	0.052
<i>Megamonas</i>	0.009	0.990	0.129	0.533	-1.069	0.991	-0.049	0.610	<b>0.144</b>	<b>0.000</b>
<i>Megasphaera</i>	0.037	0.541	0.065	0.683	1.183	0.991	0.040	0.784	<b>-0.091</b>	<b>0.005</b>
* <i>Oscillibacter</i>	-0.030	0.539	0.036	0.754	-0.400	0.991	-0.044	0.482	<b>0.049</b>	<b>0.002</b>
<i>Paraprevotella</i>	-0.039	0.539	<b>-0.318</b>	<b>0.050</b>	4.973	0.339	-0.012	0.939	<b>-0.126</b>	<b>0.004</b>
<i>Phascolarcto...</i> <sup>e</sup>	-0.032	0.539	0.049	0.754	-0.113	0.991	-0.041	0.482	<b>0.062</b>	<b>0.021</b>
* <i>Prevotella</i>	0.002	0.990	0.195	0.246	-0.088	0.991	-0.092	0.133	<b>0.150</b>	<b>0.000</b>
<i>Ruminiclostridium</i>	0.032	0.683	-0.389	0.053	2.086	0.991	0.166	0.133	<b>-0.154</b>	<b>0.005</b>
<i>Shewanella</i>	-0.010	0.990	-0.204	0.101	0.502	0.991	0.075	0.373	<b>-0.064</b>	<b>0.011</b>
<i>Subdoligranulum</i>	0.050	0.364	<b>-0.308</b>	<b>0.009</b>	-0.194	0.991	-0.025	0.893	0.005	0.878

**Table 4.5** Significant log fold change (LFC) in the abundance of gut bacterial genera per unit increase of age, BRI, A1c, systolic BP, and diastolic BP by racial-ethnic group.

	Age		BRI		A1C		Systolic BP		Diastolic BP	
	LFC	q	LFC	q	LFC	q	LFC	q	LFC	q
* <i>Sutterella</i>	-0.036	0.523	0.056	0.754	-0.691	0.991	-0.012	0.939	<b>0.083</b>	<b>0.005</b>
<i>Turcibacter</i>	0.041	0.482	0.176	0.074	-0.133	0.991	0.023	0.893	<b>-0.083</b>	<b>0.000</b>
<i>Tyzzarella</i>	0.006	0.990	0.041	0.754	-0.496	0.991	-0.004	0.993	<b>-0.055</b>	<b>0.043</b>
<i>Veillonella</i>	0.056	0.337	<b>-0.496</b>	<b>0.000</b>	-0.278	0.991	0.003	0.993	<b>-0.041</b>	<b>0.027</b>

\*Uniquely significant for one racial-ethnic group. <sup>a</sup>Unclassified member of Clostridiales; <sup>b</sup>Clostridiales Family XI. *Incertae Sedis*; <sup>c</sup>Clostridiales Family XIII. *Incertae Sedis*. <sup>d</sup>*Eubacterium bifforme*; unclassified genus. <sup>e</sup>*Phascolarctobacterium*. Cells containing significant results are bolded.

In NHPIs, uniquely significant genera included *Dorea*, *Ruminococcus*, *Streptococcus*, and *Succinivibrio*. *Dorea* was negatively associated with age, and its abundance was estimated to decrease by 3.05% per year with increasing age (LFC=-0.031; q=0.014). Similarly, *Ruminococcus* was negatively associated with age, but not as strongly (LFC=-0.023; q=0.038). In contrast, *Streptococcus* was positively associated with age, and its abundance increases by approximately 3.56% each year (LFC=0.035; q=0.033). *Succinivibrio* had a strongly negative association with BRI, and its abundance decreased by 25.3% per unit increase in BRI (LFC=-0.292; q=0.022). Interestingly, each of these taxa was significantly associated with only one of the four risk factors investigated.

Notably, *Megamonas* and *Megasphaera* were associated simultaneously with age, A1c levels, and diastolic BP. Moreover, for each genus, these associations were consistent in directionality. *Megamonas* was negatively associated with all three, and its strongest association was with A1c, exhibiting a 70.86% reduction per unit increase of A1c level (LFC=-1.322; q=0.003). Although trends in *Megasphaera* were previously discussed, its NHPI-specific trends are notable. *Megasphaera* positively associated with each of the three risk factors. Its strongest association was also with A1c levels, exhibiting a 1227.7% increase per unit change of A1c (q<0.001). At the same time, *Megasphaera* was negatively associated with BRI, decreasing by -15.72% per unit increase in BRI (q=0.004).

Other taxa that were significantly associated with A1c in NHPIs were [*unclassified*] Clostridiales, *Flavonifractor*, *Mannheimia*, and as previously mentioned, *Turcibacter*. *Flavonifractor* was positively associated with A1c levels, increasing by 115.1% (q=0.038) per unit change. In Part NHPIs, *Flavonifractor* was positively associated with age (LFC=0.038; q=0.001) and negatively associated with BRI (LFC=-0.11; q=0.016), and positively associated with diastolic BP in the Asian group (LFC=0.03; q=0.012). Members of *unclassified* Clostridiales were strongly associated with A1c levels in NHPIs (q=0.002). Its adverse association with metabolic outcomes was consistent for the Asian and Mixed groups, exhibiting a moderate, positive association with diastolic BP for both groups (Asian LFC=0.029, q=0.012; Mixed LFC=0.036, q=0.049).

In NHPIs, *Mannheimia* exhibited a strongly negative association with A1c in NHPIs, decreasing by 65.7% per unit increase in A1c level ( $q=0.011$ ). At the same time, it had a strongly positive association with BRI, increasing by 30.0% per unit increase in BRI (LFC=0.262;  $q=0.002$ ). *Mannheimia* was also positively associated with age in Whites (LFC=0.079;  $q<0.001$ ), negatively associated with age in Asians (LFC=-0.025;  $q=0.049$ ), and negatively associated with BRI in the Mixed group, decreasing in abundance by 21.96% per unit change in BRI (LFC=-0.248;  $q=0.009$ ).

For Part NHPIs, uniquely significant genera included *Roseburia*, *Holdemania*, *Victivallis*, *Lachnoclostridium*, *Leuconostoc*, *Senegalimassilia*, *Lactobacillus*, and *Slackia*. With each year increase, *Roseburia* decreased by 2.66% (LFC=-0.027;  $q=0.029$ ), while *Holdemania* increased in prevalence by 2.33% (LFC=0.023;  $q=0.029$ ). *Victivallis* also increased in prevalence per year change in age by 4.6% (LFC=0.045;  $q=0.001$ ) but also decreased in prevalence by 4.11% per unit increase in diastolic BP (LFC=-0.042;  $q=0.010$ ). *Lachnoclostridium* also negatively associated with diastolic BP (LFC=-0.037;  $q=0.01$ ), while *Leuconostoc* and *Senegalimassilia* would decrease in prevalence by 3.15% (LFC=-0.032;  $q=0.035$ ) and 6.29% (LFC=-0.065;  $q=0.001$ ) per unit change in diastolic BP, respectively. *Leuconostoc* and *Senegalimassilia* were also both positively associated with BRI (LFC=0.179/ $q=0.001$  and LFC=0.095/ $q=0.047$ , respectively), trends that were similarly observed in *Lactobacillus* (LFC=0.191,  $q=0.001$ ) and *Slackia* (LFC=0.094;  $q=0.035$ ).

In Part NHPIs, *Paraprevotella* decreased in abundance by 64.2% per unit change of A1c (LFC=-1.026;  $q=0.042$ ) but increased in prevalence by 11.07% per unit increase in BRI (LFC=0.105;  $q=0.047$ ). While *Paraprevotella* was not significantly associated with A1c in NHPIs, it was negatively associated with age (LFC=-0.055;  $q=0.001$ ) and BRI (LFC=-0.245;  $q=0.004$ ), but positively associated with diastolic BP (LFC=0.089;  $q<0.001$ ). Similar associations were observed for *Paraprevotella* in the Asian group, as its prevalence is estimated to decrease with increasing BRI (LFC=-0.0321;  $q=0.049$ ) and increase with diastolic BP (LFC=0.119;  $q<0.001$ ). In contrast to trends observed in NHPIs, *Paraprevotella* is expected to increase in prevalence by 6.72% per year change in age (LFC=0.065;  $q<0.001$ ). Uniquely in the Mixed group, the association between *Paraprevotella* and diastolic BP was negative (LFC=-0.126;  $q=0.004$ ).

Like *Paraprevotella*, members of *Clostridiales Family XI. Incertae Sedis* were also associated with A1c levels in Part NHPIs (LFC=1.948;  $q<0.001$ ), increasing in abundance by 601.5% per unit change. In Part NHPIs, *Clostridiales Family XI. Incertae Sedis* members were also negatively associated with age (LFC=-0.033;  $q=0.001$ ) and positively associated with diastolic BP (LFC=0.039;  $q=0.019$ ). These trends were opposite of those observed for the NHPI group, as *Clostridiales Family XI. Incertae Sedis* members are expected to increase in prevalence with age (LFC=0.046;  $q=0.014$ ) and decrease in prevalence per unit change in diastolic BP (LFC=-0.056;  $q=0.024$ ). This taxon was also negatively associated with age in the White group (LFC=-0.042;  $q=0.004$ ).

## 4.5 Discussion

Due to high levels of intra-taxonomic variability at the phylum level, analyses were focused at the genus level. Each of the genera that were more prevalent in NHPIs than in other groups, *Eggerthella*[224], *Blautia*, *Megamonas*[83], *Veillonella*[225], *Lachnoclostridium*[226], *Lactobacillus*, *Fusobacterium*[89], *Haemophilus*[88], *Klebsiella*[215], and *Mannheimia*[227], have been previously linked to inflammation, obesity, T2DM, hypertension, gastrointestinal complications, or other adverse health outcomes. *Blautia* has been observed to engage in both pro and anti-inflammatory processes, though it tends to be enriched in T2DM[185,192]. *Haemophilus* is associated with intestinal inflammation, and *Lactobacillus* even produces histamine[195], which may disrupt metabolic functioning if sustained. *Klebsiella* was even positively associated with diastolic BP for the NHPIs in this study cohort (**Table 4.3**).

Although, these bacteria may not be strictly detrimental to NHPI health. In the NHPI group, *Fusobacterium* was negatively associated with BRI. For Part NHPIs, *Fusobacterium* and *Lachnoclostridium* were negatively associated with diastolic BP. In NHPIs, *Megamonas* was negatively associated with age, A1c, and diastolic BP. Although *Mannheimia* was positively associated with BRI, it was negatively associated with A1c. However, it is possible that *Mannheimia* was depleted among MU individuals. It is possible that these bacteria may conditionally contribute to better or worse metabolic outcomes. Additionally, it is likely that previous reports regarding the metabolic relevance of these taxa originated from NHPI-exclusive study populations, and NHPI-specific relationships with these taxa are poorly characterized.

The genera that were least prevalent in NHPIs compared to other groups tended to be beneficial or anti-inflammatory. Among them were *Ruminiclostridium*, *Barnesiella*, *Prevotella*, *Herbaspirillum*, *Desulfovibrio*, and *Akkermansia*. *Ruminiclostridium* is reportedly linked to pathways involving blood pressure regulation and is depleted in hypertensive cases[228]. *Barnesiella* is known to produce short-chain fatty acids (SCFAs) involved in glucose homeostasis and anti-inflammatory functions and is typically negatively associated with obesity[39,61,66]. Within this study cohort, *Ruminiclostridium* was not significantly associated with any clinical measures, but *Barnesiella* was negatively associated with diastolic BP for NHPIs and Part NHPIs.

*Prevotella's* metabolic relevance may be conditional: it is known to induce inflammation[57] but is also negatively correlated with intestinal permeability[51]. Notably, *Prevotella* has been shown to aid in glycemic control under high-fiber dietary conditions but not in high-fat diet (HFD) conditions[65]. Interestingly, the only significant association between *Prevotella* and metabolic risk factors was observed in the Mixed group for diastolic BP (LFC 0.150; P<0.001). *Akkermansia* has been associated with intestinal barrier integrity, GLP-1 secretion, and reduced adipocyte differentiation[197,198]. At the same time, gut bacterial production of short-chain fatty acids (SCFAs) can induce GLP-1 secretion, and

*Akkermansia* has been observed to be negatively associated with fecal SCFA concentrations [51]. *Desulfovibrio* has been previously linked to a number of metabolic complications, but it may also be conditionally beneficial, as it tends to be enriched in the prevalence of other beneficial bacteria and is correlated with lipid homeostasis[229]. In NHPIs, however, *Desulfovibrio* was positively associated with diastolic BP. While *Herbaspirillum* is likely pathogenic[230], it was negatively associated with diastolic BP in Part NHPIs. These discrepancies indicate that metataxonomic data alone are insufficient to elucidate the metabolic relevance of conditionally beneficial taxa.

Gut bacteria also differentially associated with metabolic risk factors between racial-ethnic groups. *Megamonas* was negatively associated with A1c and diastolic BP in the NHPI group but was positively associated with BRI in the Asian group and diastolic BP in the Mixed group. Additionally, taxa that were significantly associated with metabolic risk measures in NHPIs, including *Butyricimonas*, *Desulfovibrio*, *Klebsiella*, *Mannheimia*, and *Turicibacter*, were only significantly associated with age for the White group.

Compared to other racial-ethnic groups, gut bacterial trends in NHPIs correspond with reports from previous literature regarding proinflammatory taxa. In these communities, metabolic health risk may be sustained by meta-inflammation induced by gut bacteria, and this risk is higher for NHPIs compared to other groups. However, the observed associations between gut bacteria and metabolic risk measures were not strictly consistent with previous literature. This may indicate ethnic-specific, or perhaps community-specific, relationships between the gut microbiome and health outcomes. Additionally, previous reports of proinflammatory tendencies among these taxa were likely determined in NHPI-exclusive study populations. Further investigation is necessary to determine the metabolic relevance of differentially abundant taxa in these communities and to elucidate their relationship with meta-inflammatory processes.

## Chapter 5. Conclusion

### 5.1 Summary of Findings

#### 5.1.1 Anthropometric Predictors of Obesity-Related Metabolic Outcomes

For Aim 1, we hypothesized that anthropometric indices are predictive of health risk and that ethnic-specific obesity thresholds were lower for NHPI populations compared to those established for other racial-ethnic groups. Body roundness index (BRI) emerged as the most consistent and effective predictor of metabolically unhealthy (MU) status across gender and racial-ethnic groups. Ethnic-specific BRI thresholds were then used to stratify groups by obesity-related metabolic health (MH/OB) risk.

Indeed, anthropometric indices were technically predictive of health risk. However, study findings contradicted the initial hypothesis regarding the lower threshold for obesity status designation in NHPIs. Metabolic risk-related anthropometric thresholds tended to be *higher* for NHPIs compared to other racial-ethnic groups. This discrepancy may be partially attributed to racial-ethnic differences in the utility of anthropometric indices. Although BRI was slightly more effective than BMI in estimating health risk, indices were generally less effective predictors for NHPIs than in other groups.

#### 5.1.2 Meta-Inflammatory Health Risk Factors

In Aim 2, we hypothesized that meta-inflammatory profiles are associated with metabolic health risk and that meta-inflammatory biomarkers may tend toward a more proinflammatory profile in NHPIs compared to other racial-ethnic groups. Linear discriminant analysis (LDA) models were used to obtain a set of meta-inflammatory biomarkers that significantly contributed to the distinction between MH and MU status within each racial-ethnic group. After age adjustment, plasma concentrations of these biomarkers were then compared between racial-ethnic groups. Aside from IL-1 $\beta$ , the biomarkers that were deemed significant by LDA did not significantly differ between groups. After obtaining nonsignificant results, intergroup comparisons proceeded for the biomarkers that were *not* identified as significant by LDA.

Plasma concentrations of generally anti-inflammatory and regulatory metabolic hormones GLP-1 and PYY were lower in NHPIs compared to other groups, consistent with expectations. IL-3, which may indirectly promote insulin sensitivity in adipose tissues, was also reduced in NHPIs, and a reduction in anti-inflammatory capacity is consistent with the initial hypothesis. However, MCP-1, a primary driver of pro-inflammatory recruitment and polarization of adipose tissue-resident macrophages, was also less prevalent in NHPIs compared to other groups. This directly contradicted the initial hypothesis.

MCP-1 did not significantly contribute to the distinction between MH and MU status in NHPIs but did significantly differ between racial-ethnic groups. This discrepancy may indicate that one set of biomarkers

(identified by LDA: IFN- $\alpha$ , IFN- $\gamma$ , IL-10, IL-12, IL-13, IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$ ) may be involved in the distinction between MH and MU status *within* racial-ethnic groups, but that another set of biomarkers (GLP-1, IL-18, IL-3, MCP-1, PYY, TNFRI, and VEGF-A) may be involved in metabolic health risk differences *between* racial-ethnic groups. Additionally, logistic regression models based on significant meta-inflammatory biomarkers (selected by LDA) were more effective in predicting MU status in NHPs than in other racial-ethnic groups. Altogether, these results could suggest that meta-inflammation is more strongly associated with metabolic health risk for NHPs than for other racial-ethnic groups.

### 5.1.3 Gut Bacterial Associations with Metabolic Health Risk

For Aim 3, it was hypothesized that gut bacterial metataxonomic profiles are associated with type 2 diabetes mellitus (T2DM) and hypertension (HT) risk and that NHP individuals will exhibit a higher relative abundance of taxa previously linked to T2DM and HT compared to other racial-ethnic groups.

The taxa that were more prevalent in NHPs compared to other groups have each been previously linked to inflammation, obesity, metabolic dysfunction, or gastrointestinal complications. Each of the genera that were more prevalent in NHPs than in other groups, *Eggerthella*[224], *Blautia*, *Megamonas*[83], *Veillonella*[225], *Lachnoclostridium*[226], *Lactobacillus*, *Fusobacterium*[89], *Haemophilus*[88], *Klebsiella*[215], and *Mannheimia*[227], have been previously linked to inflammation, obesity, T2DM, hypertension, gastrointestinal complications, or other adverse health outcomes. The genera that were least prevalent in NHPs compared to other groups tended to be beneficial or anti-inflammatory. Among them were *Ruminiclostridium*, *Barnesiella*, *Prevotella*, *Herbaspirillum*, *Desulfovibrio*, and *Akkermansia*. In this sense, study findings were consistent with the initial hypothesis.

Gut bacteria also differentially associated with metabolic risk factors between racial-ethnic groups. *Megamonas* was negatively associated with A1c and diastolic BP in the NHP group but was positively associated with BRI in the Asian group and diastolic BP in the Mixed group. Additionally, taxa that were significantly associated with metabolic risk measures in NHPs, including *Butyricimonas*, *Desulfovibrio*, *Klebsiella*, *Mannheimia*, and *Turicibacter*, were only significantly associated with age for the White group.

## 5.2 Study Limitations

Self-reported racial-ethnic data was used to stratify participants into single and multiethnic categories. The Part NHP group was considerably larger than others, and NHP-specific trends may have been obscured by the heterogeneity of this group. “Single” ethnic categories were similarly heterogeneous. The NHP group included members from Tongan, Sāmoan, Hawaiian, Micronesian, and Melanesian communities, and the Asian group included communities from South, Southeast, East, and West Asia. Self-reported racial-ethnic data is valuable because it may indirectly contextualize social, behavioral, and environmental factors that are typically unreported otherwise. However, a different strategy for collecting

self-reported racial-ethnic data may procure study findings that are more meaningful and relevant to specific communities.

Another limitation of this study is sampling. Members of the NHPI group tended to be older than others. Age may play a significant role in exacerbating disparate health outcomes, and statistical age adjustment may not capture biological trends as accurately as more inclusive sampling may have. Additional limitations were introduced by the nonuniform prevalence of obesity-related metabolic risk (MH/OB) status across racial-ethnic groups. At some points during data analysis, sample sizes were reduced to N=4 NHPI individuals in the MHOB group and N=8 White individuals in the MUNO group, limiting analytical options and constraining the interpretation of results. Although ethnic-specific definitions for MHOB were obtained, it is unclear whether the low representation per risk group necessarily allowed for an accurate risk assessment for the broader community.

The analytical approaches selected for this project also had their limitations. For Aim 1, binary predictors, including ethnic-specific obesity group stratification based on anthropometric indices, are not ideal for application in receiver operating characteristic (ROC) analyses[120]. For Aim 2, there was a high degree of variability in results obtained from the multiplex immunoassay. Major outliers were observed for targets including IL-18, TNF- $\alpha$ , TNFR1, and VEGF-A, especially for the NHPI group, but outlier exclusion would have further reduced small sample sizes per MHOB group. For Aim 3, LFC values obtained from ANCOM-BC analysis may indicate depletion or may be augmented due to a low number of observations upon intergroup comparison. Insufficient data for differential abundance analyses may similarly indicate depletion in one or more groups being analyzed.

### **5.3 Future Directions**

Better anthropometric predictors for metabolic risk beyond those assessed in the present study may exist. However, the general relationship between anthropometric measures and metabolic risk may be weaker for NHPIs than for other groups. Thus, future directions may include examining the direct relationship between adiposity and metabolic risk in NHPIs using imaging-based techniques like Dual-Energy X-ray Absorptiometry (DXA) or magnetic resonance imaging (MRI). Further assessments of anthropometric associations with adiposity may help to recontextualize findings from previous NHPI-inclusive studies in terms of obesity-related physiology.

To expand on meta-inflammatory analyses, future directions may include a greater focus on intergroup differences in biomarker prevalence. Additionally, since inflammatory biomarkers were not significantly more prevalent in NHPIs, it may be possible that lower levels of systemic inflammation are sufficient for the presentation of MU status in NHPIs. Findings from these studies may elucidate NHPI-specific trends

in obesity-related disease etiology, especially if they were analyzed together with direct measurements of central adiposity.

While meta-inflammatory associations may be inferred based on metataxonomic data and previous literature, community-specific relationships may contradict expected results. Gut bacteria may differentially associate with metabolic outcomes, and their specific associations with meta-inflammatory biomarkers are relatively uncharacterized in NHPI populations. Additionally, metataxonomic data alone may not be fully representative of the metabolic influence of specific gut bacteria. Thus, future studies may consider characterizing direct relationships between metagenomic data, gut bacterial metabolites, and meta-inflammation within this community. This may uncover the impact of the gut microbiome on the presentation of metabolic outcomes.

## **5.4 Conclusion**

Indirect measures of fat accumulation inaccurately characterize obesity-related health risk in ethnically diverse populations. Such misrepresentation is especially detrimental for health-disparate communities, including understudied Indigenous populations like NHPIs. Community-specific research is necessary to generate an appropriate and metabolically relevant definition of obesity in these populations.

Meta-inflammation may associate more strongly with metabolic health risk for NHPIs than for other racial-ethnic groups, potentially highlighting racial-ethnic differences in obesity-related disease etiology. It may be possible that meta-inflammation may disproportionately affect metabolic risk in NHPIs compared to other populations.

Consistent with those findings, at phylum and genus levels, gut bacteria that were more prevalent in NHPIs compared to other racial-ethnic groups are closely linked to inflammation and metabolic dysfunction. However, these taxa may not necessarily be detrimental to metabolic health, as their associations with metabolic risk factors (including age, A1c levels, BRI, and blood pressure) were not strictly health-adverse.

Overall, racial-ethnic differences in obesity, meta-inflammation, and the gut microbiome may contribute to racial-ethnic differences in metabolic health risk. Further NHPI-inclusive, community-centric research is necessary to characterize these relationships effectively.

## Notes

Sections 1.1, 1.2, 2.2, and 4.2 contain excerpts adapted from the following publication:

Wells RK, Torres A, Mau MK, Maunakea AK. Racial-Ethnic Disparities of Obesity Require Community Context-Specific Biomedical Research for Native Hawaiians and Other Pacific Islanders. *Nutrients* 2024; 16(24):4268. <https://doi.org/10.3390/nu16244268>

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