UNIVERSITY OF CALIFORNIA

Los Angeles

Moving Toward Precision:

Understanding the Heterogeneity of Obesity

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science

in Clinical Research

by

Kin Wai Tony Hung

2020

ABSTRACT OF THE THESIS

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Master of Science in Clinical Research

University of California, Los Angeles, 2020

Professor Janet S. Sinsheimer, Chair

Background: Obesity is a global health pandemic that has been linked to detrimental health and socioeconomic impact. Growing evidence have recognized obesity as a spectrum of metabolic imbalances with complex biopsychosocial interactions including the brain-gut axis. A precision understanding on obesity while at its infancy is necessary to accelerate reduction of its health burden.

Methods: With our aim to better understand the biopsychosocial interactions at the transitional junction of obesity development, we conducted a cross sectional study in overweight and obese individuals. Univariate and multivariate logistic regression models were used to examine obesity and its association with sociodemographic, clinical, and dietary-behavioral factors. Biological interactions including the gut microbiome, gut amino acids and brain structural volumes were also examined. Microbial data were analyzed for alpha diversity, beta diversity, and relative abundance of taxa. Amino acids and brain structural volumes were analyzed using multiple ANOVA. Interactions were tested by Pearson correlations and corrected for multiple hypothesis.

Results: Among 130 participants, there were higher odds of obesity if individuals were Hispanic [Adjusted Odds Ratio (AOR) 1.70, p = 0.0089], and married (AOR 1.63, p = 0.036). Compared to non-Hispanic, Hispanic had a significantly different microbiome profile (p = 0.046) with lower microbial species richness (Chao1) (p = 0.032) and evenness (Shannon) (p = 0.0029). A predominance of the phylum Firmicutes was positively correlated to American diet consumption (p = 0.036) while negatively correlated to Hispanic ethnicity (p = 0.021). Fourteen of twenty gut amino acids including all essential amino acids were increased among Hispanics (p < 0.05). Brain structural volumes in reward regions were decreased especially if individuals were Hispanic (pallidum, p = 0.036; brainstem, p = 0.011), married (left thalamus, p = 0.024), or consumed an American diet (brainstem, p = 0.043).

Conclusions: Hispanic expressed a unique gut microbial signature, which was associated with obesity despite sociodemographic, clinical, and dietary differences. Gut amino acids and brain structural volumes may further differentiate Hispanic ethnic differences and warrant future research. Addressing ethnic disparities guided by biologic phenotypes may unlock novel understanding of obesity heterogeneity and transform its impact on obesity care.

The thesis of Kin Wai Tony Hung is approved.

David Elashoff

Emeran Mayer

Arpana Gupta

Janet S. Sinsheimer, Committee Chair

University of California, Los Angeles

2020

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Table 1. Baseline participant characteristics

	Overall (n = 130)		Overweight (n = 62)		Obese (n = 68)		*P*
Characteristic	No	%	No	%	No	%	
Age							0.17
Less than 30y	59	45.4%	32	51.6%	27	39.7%	
30y or older	71	54.6%	30	48.4%	41	60.3%	
Gender							0.093
Female	87	66.9%	37	59.7%	50	73.5%	
Male	43	33.1%	25	40.3%	18	26.5%	
Ethnicity							0.014*
Hispanic	52	40.0%	18	29.0%	34	50.0%	
Non-Hispanic	78	60.0%	44	71.0%	34	50.0%	
Education							0.51
College Graduate	37	29.8%	19	32.8%	18	27.3%	
Non-College Graduate	87	70.2%	39	67.2%	48	72.7%	
Annual Income							0.88
Less than $70K	64	55.7%	31	56.4%	33	55.0%	
$70K or More	51	44.3%	24	43.6%	27	45.0%	
Marital Status							0.028*
Married	30	25.6%	9	16.4%	21	33.9%	
Not Married	87	74.4%	46	83.6%	41	66.1%	
Waist to Hip Ratio‡							0.043*
Obese	37	43.9%	15	44.1%	22	68.8%	
Normal	29	56.1%	19	55.9%	10	31.3%	
Dietary Pattern							0.031*
American Diet	99	76.2%	42	67.7%	57	83.8%	
Non-American Diet	31	23.8%	20	32.3%	11	16.2%	

*P-value < 0.05
‡Waist to Hip ratio adjusted by gender obesity cut off of >0.9 for male >0.85 for female

Table 2. Univariate and multivariate analyses of biopsychosocial characteristics associated with obesity

	Univariate analyses			Multivariate analyses		
Characteristic	Un-AOR	95% CI	*P* Value	AOR	95% CI	*P* Value
Age						
Less than 30y	0.79	0.55 - 1.12		–	–	
30y or older (reference)	–	–	0.17			
Gender						
Female	1.37	0.94 - 2.00	0.093	–	–	
Male (reference)	–	–				
Ethnicity						
Hispanic	1.56	1.08 - 2.26	0.014*	1.70	1.13 - 2.54	0.0089*
Non-Hispanic (reference)	–	–		–	–	
Education						
College Graduate	0.88	0.59 - 1.30	0.51	–	–	
Non-College Graduate (reference)	–	–				
Annual Income						
Less than $70K	0.97	0.67 - 1.42	0.88	–	–	
$70K or More (reference)	–	–				
Marital Status						
Married	1.62	1.02 - 2.54	0.028*	1.63	1.02 - 2.60	0.036*
Not Married (reference)	–	–		–	–	
Waist to Hip Ratio						
Obese	1.67	1.00 - 2.79	0.043*	–	–	
Normal (reference)	–	–				
Dietary Pattern						
American Diet	1.57	1.02 - 2.41	0.031*	–	–	
Non-American Diet (reference)	–	–				

*P-value < 0.05

Table 3. Multivariate subgroup analyses of ethnicity and marital status associated with obesity

	Hispanic			Non-Hispanic		
Characteristic	AOR	95% CI	*P* Value	AOR	95% CI	*P* Value
Marital Status						
Married	–	–		1.81	1.01 - 3.24	0.036*
Not Married (reference)	–	–		–	–	
	Married			**Not Married**		
Characteristic	AOR	95% CI	*P* Value	AOR	95% CI	*P* Value
Dietary Pattern						
American	5.00	1.47 - 17.02	0.0019*	–	–	
Non-American (reference)	–	–		–	–	
Gender						
Female	–	–		1.63	1.00 - 2.68	0.04*
Male (reference)	–	–		–	–	

*P-value < 0.05

Table 4. Odd ratio of obesity based on biopsychosocial and dietary risk factors

	Hispanic (AOR 1.7)		Non-Hispanic (AOR 1)	
Married (AOR 1.81)	American Diet (AOR 5) 15.4	Non-American Diet (AOR 1) 3.1	American Diet (AOR 5) 9.1	Non-American Diet (AOR 1) 1.8
Not Married (AOR 1)	Female (AOR 1.63) 2.8	Male (AOR 1) 1.7	Female (AOR 1.63) 1.6	Male (AOR 1) 1

Table 5. Association of Firmicutes:Bacteroidetes with sociodemographic and dietary characteristics

	Firmicutes:Bacteroidetes		
Characteristic	Ratio	95% CI	*P* Value
Age			
Less than 30y	1.15	0.89 - 1.40	0.97
30y or older (reference)	1.16	0.63 - 1.68	
Sex			
Female	0.99	0.80 - 1.18	0.27
Male (reference)	1.49	0.61 - 2.35	
Race			
Hispanic	0.78	0.62 - 0.94	0.021*
Non-Hispanic (reference)	1.40	0.90 - 1.91	
Education			
College Graduate	1.22	0.85 - 1.58	0.87
Non-College Graduate (reference)	1.17	0.73 - 1.60	
Annual Income			
Less than $70K	0.95	0.74 - 1.17	0.37
$70K or More (reference)	1.11	0.83 - 1.39	
Marital Status			
Married	0.89	0.63 - 1.16	0.24
Not Married (reference)	1.09	0.87 - 1.31	
Waist to Hip Ratio			
Obese	1.37	0.41 - 2.34	0.77
Normal (reference)	1.22	0.71 - 1.74	
Dietary Pattern			
American Diet	1.26	0.87 - 1.66	0.036*
Non-American Diet (reference)	0.78	0.58 - 1.00	
Obesity			
Overweight	1.06	0.79 - 1.33	0.57
Obese	1.24	0.69 - 1.79	

*P-value < 0.05

Table 6. Association of Firmicutes or Bacteroidetes predominance with obesity by ethnicity or dietary characteristics

	Bacteroidetes Predominance			Firmicutes Predominance		
	Hispanic (%)	Non-Hispanic (%)	p	Hispanic (%)	Non-Hispanic (%)	p
Overweight	15 (41.7%)	24 (57.1%)	0.17	3 (18.8%)	20 (58.8%)	0.0062*
Obese	21 (58.3%)	18 (42.9%)		13 (81.3%)	14 (41.2%)	

	Bacteroidetes Predominance			Firmicutes Predominance		
	American Diet (%)	Non-American Diet (%)	p	American Diet (%)	Non-American Diet (%)	p
Overweight	25 (43.9%)	14 (66.7%)	0.07	17 (41.5%)	6 (66.7%)	0.17
Obese	32 (56.1%)	7 (33.3%)		24 (58.5%)	3 (33.3%)	

*P-value < 0.05

Table 7. Multiple one-way ANOVA p-values of characteristics associated with amino acids adjusted for FDR

		Characteristics				
Pathways	Metabolites	Hispanic Race	Marital Status	American Diet	F:B Ratio	Obesity
Amino Acid Metabolism	Glycine	0.026*	0.86	0.30	0.78	0.73
	Serine	0.030*	0.86	0.30	0.78	0.73
	Threonine	0.045*	0.89	0.30	0.78	0.85
	Alanine	0.030*	0.86	0.30	0.78	0.97
	Aspartate	0.10	0.89	0.95	0.78	0.73
	Asparagine	0.59	0.86	0.92	0.78	0.73
	Glutamate	0.033*	0.89	0.37	0.78	0.73
	Glutamine	0.15	0.86	0.30	0.78	0.97
	Histidine	0.045*	0.86	0.89	0.78	0.73
	Lysine	0.026*	0.86	0.30	0.78	0.73
	Phenylalanine	0.030*	0.86	0.30	0.78	0.73
	Tyrosine	0.030*	0.86	0.89	0.78	0.73
	Tryptophan	0.045*	0.86	0.30	0.78	0.73
	Leucine	0.030*	0.86	0.30	0.78	0.73
	Isoleucine	0.026*	0.86	0.30	0.78	0.73
	Valine	0.026*	0.86	0.30	0.78	0.73
	Methionine	0.026*	0.86	0.30	0.97	0.85
	Cysteine	0.99	0.86	0.30	0.97	0.73
	Arginine	0.47	0.97	0.30	0.78	0.73
	Proline	0.24	0.86	0.36	0.78	0.97

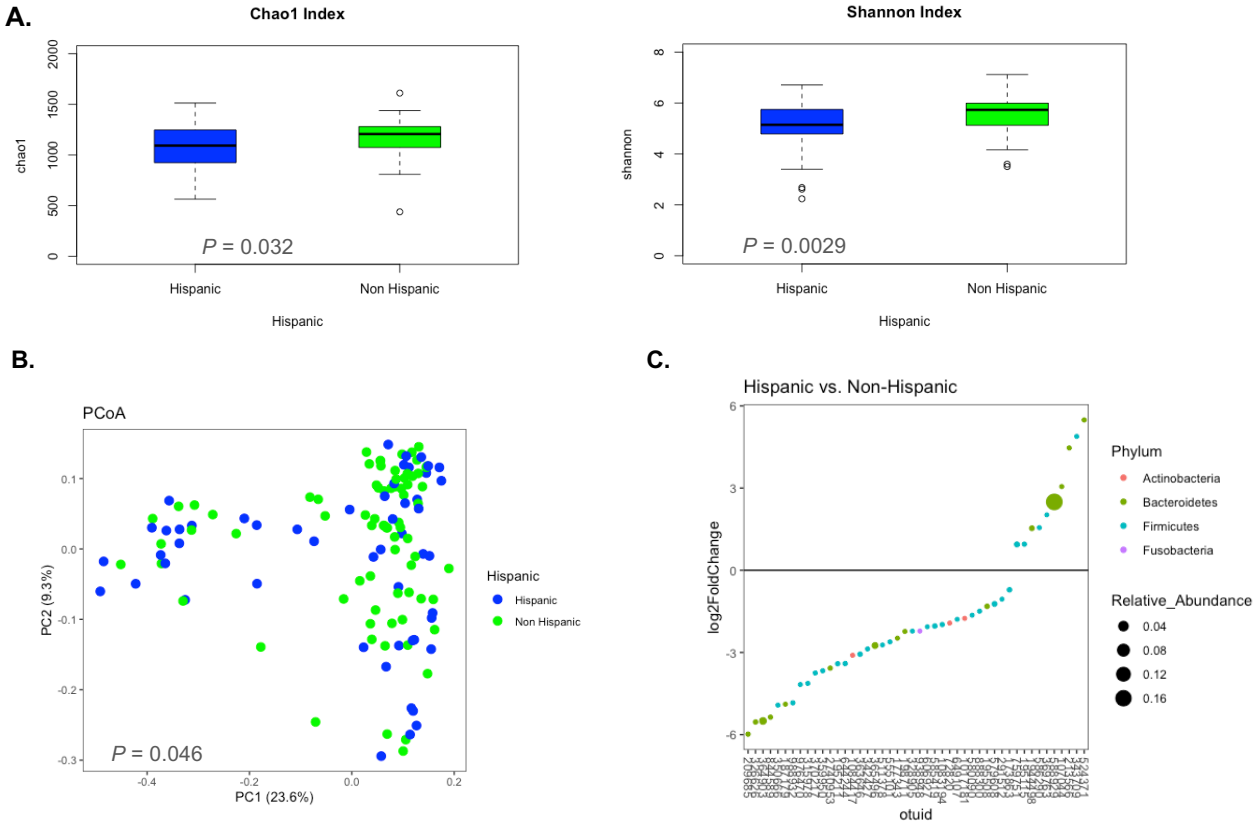
*P-value < 0.05

Table 8. Multiple one-way ANOVA p-values of characteristics associated with brain structural volumes adjusted for FDR

		Characteristics				
Pathways	Metabolites	Hispanic Race	Marital Status	American Diet	F:B Ratio	Obesity
Brain Structure Volume	Left Thalamus	0.08	0.024*	0.52	0.99	0.22
	Right Thalamus	0.22	0.23	0.47	0.99	0.24
	Left Caudate	0.23	0.35	0.95	0.71	0.31
	Right Caudate	0.23	0.35	0.95	0.71	0.31
	Left Putamen	0.95	0.92	0.76	0.60	0.77
	Right Putamen	0.95	0.92	0.76	0.60	0.84
	Left Pallidum	0.036*	0.87	0.88	0.97	0.99
	Right Pallidum	0.036*	0.87	0.68	0.97	0.99
	Left Hippocampus	0.96	0.64	0.37	0.80	0.24
	Right Hippocampus	0.96	0.64	0.74	0.80	0.16
	Left Amygdala	0.83	0.44	0.98	0.39	0.85
	Right Amygdala	0.83	0.30	0.98	0.11	0.85
	Left Accumbens	0.93	0.31	0.87	0.59	0.48
	Right Accumbens	0.93	0.24	0.87	0.61	0.48
	Brain Stem	0.011*	0.54	0.043*	0.52	0.39

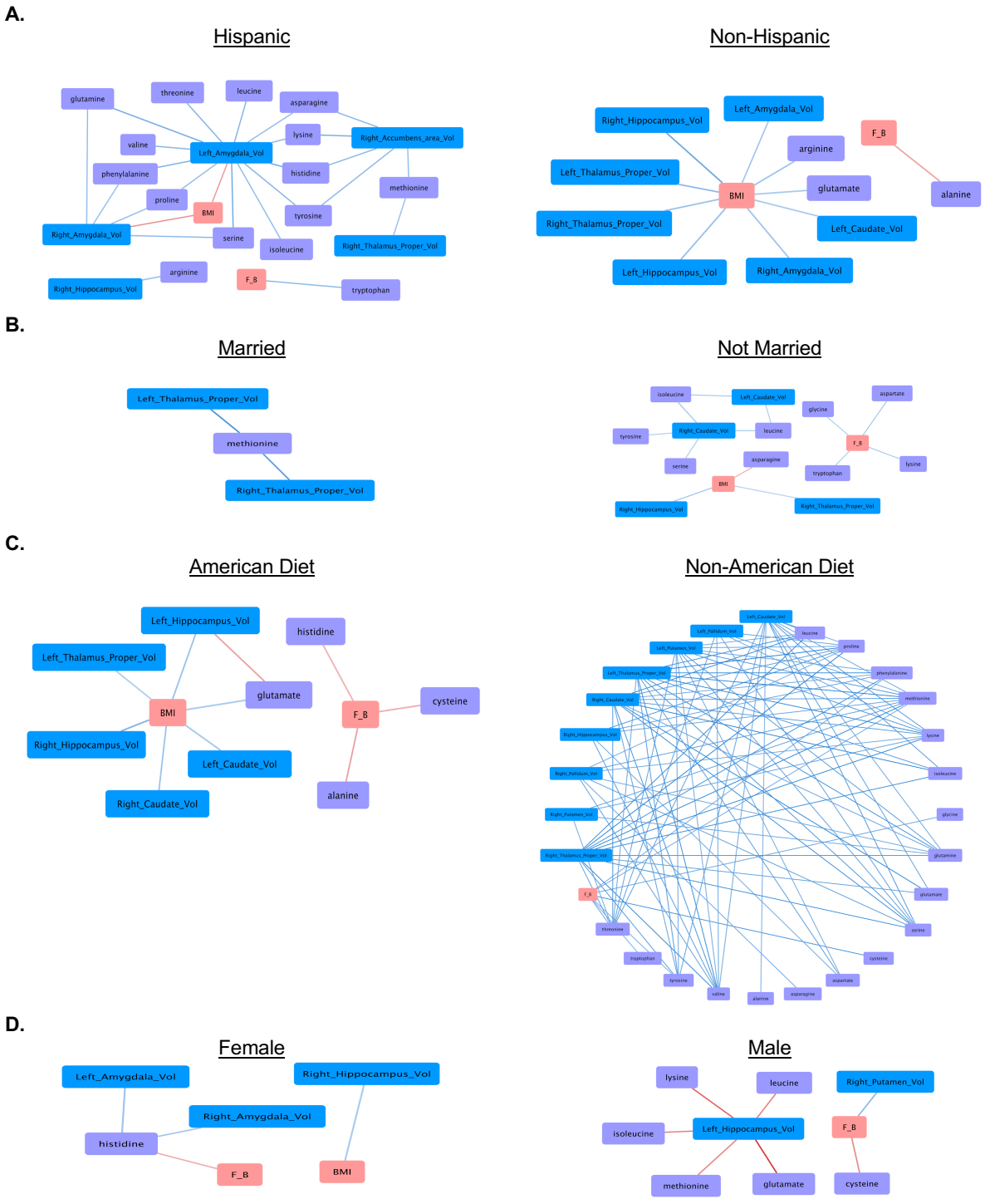
*P-value < 0.05

Figure 1. Microbial Profiles of Hispanic versus Non-Hispanic



A. Alpha Diversity. Chao1 and Shannon Indexes illustrated in box plots of Hispanic vs. non-Hispanic. **B.** Beta Diversity. Principal coordinates analysis plots of microbial composition of Hispanic vs. non-Hispanic. P-value is shown for the difference in root square Jensen-Shannon divergence distance matrix. **C.** Differential expression of microbial genera associated with Hispanic vs. non-Hispanic

Figure 2. Pearson correlations of significant characteristics



Notable differences in correlation patterns were observed in four significant covariates**: A.** Hispanic vs. non-Hispanic, **B.** Married vs. not Married, **C.** American diet vs. non-American diet, and **D.** Female vs. Male. Continuous mapping displayed positive (red) or negative (blue) correlations with darker color representing stronger correlation. All correlations depicted are $q < 0.05$. Color boxes index amino acid (purple), brain structure (blue), F:B ratio (red), BMI (red)

ACKNOWLEDGMENT

This research was supported in part by grants from the following:

National Institutes of Health:

K23 105628 (AG)

ULTR001881/DK041301 (UCLA CURE/CTSI Pilot and Feasibility Study; AG)

R01 DK048351 (EAM)

Pilot funds provided for brain scanning by the Ahmanson-Lovelace Brain Mapping Center

UCLA Specialty Training and Advanced Research (STAR) Program

Special thanks to the mentors and colleagues who have supported me on this research:

Arpana (Annie) Gupta, Ph.D.

Emeran Mayer, M.D., Ph.D.

David Elashoff, Ph.D.

Janet S. Sinsheimer, Ph.D.

Tien Dong, M.D.

Chelsea Chen

Introduction:

Obesity is a heterogenous, chronic condition that has reached pandemic proportions over the past 50 years.[1,2] Defined as excessive fat accumulation diagnosed at a body mass index (BMI) ≥ 30 kg/m^2, obesity has been associated with an increased risk of mortality, accounting for 19% of premature deaths, and is a major risk factor for other noncommunicable diseases including cardiovascular diseases, diabetes mellitus, and cancer.[3,4,5] From 1975 to 2016, the global prevalence of obesity has nearly tripled from 3.2% to 11.1% in adult men and from 6.4% to 15.3% in adult women.[6,7] In the United States alone, over 88 million adults (42.4%) are estimated to be obese, of which non-Hispanic blacks (49.6%) have the highest age-adjusted prevalence of obesity, follow by Hispanics (44.8%), non-Hispanic Whites (42.2%) and non-Hispanic Asians (17.4%).[8] The heterogeneity in obesity prevalence between and within countries have been explained by not only ethnicity, but also socio-economic differences.[9,10] For instance, disparities in obesity prevalence between neighboring countries might be explained by exposure to obesogenic or “Western-American” diet (high energy content, high sugar and fat, and low in fiber).[11] Furthermore, prior studies that evaluate disparities in obesity prevalence in the United States have found that factors such as cultural norms, poverty, indicators related to the food environment (i.e. access to supermarkets or fast food restaurants), gender, marital status, and other demographic groups are also associated with obesity outcomes.[12] Indeed, the obesogenic environmental and societal risk factors are multifaceted, and include dietary influences, social determinants, societal infrastructures, public health policies and beyond.[13,14]

Current research supports the fundamental pathogenesis of obesity as an excessive energy imbalance overtime predisposed by genetic and epigenetic susceptibility and regulated by metabolic hemostasis.[15,16] Notably, studies have highlighted a key regulatory role of the brain-gut-microbiome (BGM) signaling in obesity development.[17,18,19,20,21,22] Signaling from the brain influences many gastrointestinal processes, including the gut microbiome.[23] Alterations in the brain’s key reward and emotional regulation regions may also contribute to dysregulation of

appetitive behaviors and predisposition to obesity.[24,25] Conversely, signals from the gut microbiota can alter neural signaling to the brain.[26,27] While the exact mechanisms of BGM axis remain incompletely understood, emerging evidences have suggested that gut microbiota output of amino acids in part influence neurodevelopmental processes and contribute to the development of metabolic disorders.[28,29] In particular, fecal metabolites, including branched chain amino acids (BCAA), aromatic amino acids (AAA), certain amino acids, as well as their downstream metabolic byproducts, have been shown to influence glucose homeostasis and insulin resistance.[30] Additionally, alteration in gut microbial composition and diversity, or dysbiosis, have been associated with obesity. Other microbial signatures such as the Firmicutes:Bacteroidetes (F:B) ratio have been observed in obese individuals to potentially reflect differences in their metabolic profiles.[31] Indeed, the heterogeneity of obesity and its complex causes are increasingly been recognized as reflected by a proposal to change the International Code of Diseases (ICD) classification of obesity from “endocrine, nutritional and metabolic diseases” to an overarching parent category instead based on arrays of its multifaceted etiologies, degree of adiposity and health risks.[32,33] Albeit, significant questions remain about the relationships by which how in conjunction these biopsychosocial factors interact with BGM axis and contribute to obesity, and thus warrant further investigation.

With our aim to better understand the heterogeneity of obesity at the transitional junction of obesity development, we conducted a cross sectional study in healthy overweight and obese individuals to examine the biopsychosocial interactions and to identify potential BGM biomarkers of obesity. Studied variables included sociodemographic (age, gender, ethnicity, education, income, and marital status), clinical (waist to hip ratio), and dietary-behavioral factors (dietary pattern). Gut microbiome, fecal metabolites, and brain structural volumes data were investigated as potential BGM biomarkers. Our study aimed to test the hypotheses that 1. interactions between covariates differed between overweight individuals compared to obese

individuals, and 2. gut microbiome, fecal metabolites, and brain structural volumes would characterize differential biophenotypes among the studied variables.

Methods:

Study Population

The sample was comprised of 130 right-handed individuals, between the age of 18-50 years old without significant medical or psychiatric conditions. Participants were recruited at the University of California Los Angeles (UCLA) Center for Neurobiology of Stress and Resilience (CNSR) between July 2015 and August 2019, through advertisements posted in the UCLA and Los Angeles community. Medical and psychiatric conditions were screened using a standardized screening sheet and a physical exam by a trained registered nurse. All participants were classified as healthy after a clinical assessment that included a modified Mini-International Neuropsychiatric Interview Plus 5.0.[34] Exclusion criteria included substance abuse, pregnancy, tobacco dependence (half a pack or more daily), abdominal surgery, vascular risk factors, weight loss surgery, excessive exercise (more than 1 hour every day and marathon runners) or psychiatric illness. Even though often associated with increased BMI, participants with hypertension, diabetes or metabolic syndrome were excluded to reduce heterogeneity of the population. Also, participants with eating disorders, including digestive or eating disorders such as anorexia or bulimia nervosa were excluded for the same reason. Participants taking medications that interfere with the central nervous system or regular use of analgesic drugs were excluded. Participants were also excluded if they had been on antibiotics or probiotics with 3 months of recruitment.

All participants were in the overweight and obese category. In accordance to the World Health Organization (WHO) definition, BMI = 25 - 29.9 kg/m2 was defined as overweight, and ≥ 30 kg/m2 was obese. No participants exceeded 400 lbs due to Magnetic resonance imaging (MRI) scanning weight limits.

Written informed consent were obtained from all participants prior to surveys and data collection. All procedures complied with the principles of the Declaration of Helsinki and were approved by the Institutional Review Board at our institution (IRB # 16-000187).

Data Collection and Processing

Anthropometrics data

Our study included baseline data collection of participants' sociodemographic information, body measurements and dietary consumption pattern. Sociodemographic data included information on age, gender, ethnicity, education, annual income, and marital status. We dichotomized age as "less than 30 years" (<30y) or "30 years or older" (≥30y), gender as "male" or "female," ethnicity as "Hispanic" or "non-Hispanic," education as "college graduate" or "non-college graduate," annual income as "less than $70K" or "$70K or more," and marital status as "married" or "not married." Body measurements collected include weight (kilograms), height (centimeters), and waist and hip circumferences (centimeters). Waist to hip ratio were then computed based on the body measurements and was dichotomized as "obese" or "normal", adjusted by the gender obesity cut-off of > 0.9 for male and > 0.85 for female.

Diet Habits

Dietary pattern was reported in a dietary consumption questionnaire, which asked participants to select the diet(s) that best reflect what they consume on a regular basis. Qualitative dietary pattern was reported as American diet or other diets, which we dichotomized as "American diet" or "non-American diet." American diet was defined as diet characterized by of high consumption of processed foods such as frozen and packaged foods as well as pasta and breads; meats, including red meat, fish, eggs, and dairy products were also consumed; vegetables and fruits were consumed but not in large quantiles. Other diets and descriptions include Mediterranean, paleo, vegetarian, gluten free, diary free, low FODMAP (fermentable oligo-, di-, monosaccharides and polys), or other diets. Questionnaire was established by the UCLA CNSR.

Fecal specimen

Fecal specimens were requested from eligible participants for microbiome and metabolite characterization. Participants were each provided with a fecal collection kit that included gloves, a collection hat, a preservative vial with an attached spoon, and an addressed stamped envelope. Participants were instructed to mail in fecal sample after collection. Fecal specimens were stored in -80^{o}C freezer till sample processing. Fecal samples were aliquoted under liquid nitrogen.

Microbiome Characterization: 16S Ribosomal RNA Sequencing

Genomic DNA was extracted from 0.5 mL duodenal aspirate using the Powersoil kit as per the manufacturer's instructions (MO BIO, Carlsbad, CA, USA). The V4 region of 16S ribosomal RNA (rRNA) genes was amplified and underwent paired end sequencing on an Illumina MiSeq (San Diego, CA, USA).[35] The 253 base pair reads were processed using QIIME version 1.9.1 with default parameters.[36] Sequence depth ranged from 37,860 to 631,287 sequences per sample. Operational taxonomic units (OTUs) were picked against the May 2013 version of the Greengenes database, prefiltered at 97% identity. The OTUs were removed if they were present in fewer than 10% of samples. Alpha diversity (i.e. diversity within a sample) were calculated in QIIME using OTU-level data rarefied to 37,860 sequences.

Fecal Amino Acids Characterization

Samples were also shipped to Metabolon for processing and analysis as a single batch on their global metabolomics and bioinformatics platform. Data was curated by mass spectroscopy using established protocols and software as previously described.

Brain Magnetic Resonance Imaging

MRI Acquisition

Whole brain structural data was acquired using a 3.0T Siemens Prisma MRI scanner (Siemens, Erlangen, Germany). Detailed information on the standardized acquisition protocols, quality control measures, and image preprocessing were previously published. The following

structural acquisition protocol was used: High resolution T1-weighted images were acquired: echo time/ repetition time (TE/TR) =3.26ms/2200ms, field of view (FOV)=220×220mm slice thickness=1mm, 176 slices, 256×256 voxel matrices, and voxel size=0.86×0.86×1mm.

Quality Control and Preprocessing of images:

Structural images were included based on compliance with acquisition protocol, full brain coverage, minimal motion (Gibbs ringing), absence of flow/zipper, and minor atrophy/vascular degeneration. Preprocessing for quality control involved bias field correction, co-registration, motion correction, spatial normalization, tissue segmentation, Maximum relative motion thresholds for translation and rotation for each direction (x, y, and z) were set at 2mm and 2°, respectively. No subjects presented with serious adverse imaging artifacts and no subjects exceeded motion thresholds.

Structural Image Parcellation:

T1-image segmentation and regional parcellation were conducted using FreeSurfer v.5.3.0 following the nomenclature described in the Destrieux and Harvard-Oxford subcortical atlas. This parcellation results in the labeling of 165 regions, 74 bilateral cortical structures, 7 subcortical structures, the midbrain, and the cerebellum.

Brain Regions of Interest

Based on previous research, regions of interest were restricted to core regions of the reward and emotional regulation network (basal ganglia: caudate nucleus, globus pallidum, putamen, thalamus, nucleus accumbens, amygdala, hippocampus, and brainstem [including the substantia nigra/SN and ventral tegmental area/VTA]), as these regions have been implicated in brain-gut axis alterations associated with obesity.

Statistical Analysis

We conducted univariate and multivariate logistic regression models to estimate the unadjusted and adjusted odds ratios for covariates associated with the primary outcome, respectively. Primary outcome was overweight or obese categorization, for which we

dichotomized individuals as “overweight” (BMI = 25 - 29.9 kg/m^2) or “obese” (BMI ≥ 30 kg/m^2). Multivariate models were performed by minimizing the Bayesian information criterion (BIC) using backward stepwise method. Subgroup multivariate analyses were performed on significant covariates. Descriptive statistics were calculated as frequencies and percentages. Statistical significance for all analyses was set at $P < 0.05$. All statistical analyses were conducted using JMP PRO (Mac, version 14.0).

Biological interactions including the gut microbiome, amino acid metabolism and brain structural volumes were also examined against primary outcome and covariates. Microbial data using 16S rRNA sequencing were analyzed for alpha diversity, beta diversity, and association of taxa abundance. Alpha diversity refers to metrics of diversity within a community (i.e. patient sample), which pertain to the total number of species (richness) or how evenly distributed the members of a community are among the species present (evenness).[37] For our study, we used Chao1 (a metric of richness) and Shannon index (a metric of evenness) with 97% OTUs representing the equivalent of species. The significance of differences in alpha diversity was calculated by two-tailed t test. Beta diversity refers to comparison of microbial composition across communities (i.e. patient samples) based upon which species are present/absent or their relative abundances.[38] In our study beta diversity was calculated using root square Jensen-Shannon divergence distance, a phylogenetic metric that compares the fraction of a phylogenetic tree that is covered by the species present in one sample compared to another and visualized by principal coordinates analysis in R. Adonis, a permutational ANOVA, was carried out using 10,000 permutations to test for differences in root-square Jensen distances across the various covariates. Association of microbial genera with color grade and significant covariates were evaluated using DESeq2 in R-studio, which uses an empirical Bayesian approach to shrink dispersion and fit non-rarified count data to a negative binomial model.[39] This method has previously been shown to be robust for detecting differences in the abundances of microbes in 16S rRNA datasets.[40] P-values for differential abundance were converted to q

values to correct for multiple hypothesis testing (<0.05 for significance).[41] Amino acid metabolites and brain structural volumes were analyzed using multiple one-way ANOVA. Parameters were controlled and corrected for multiple hypothesis testing by false discovery rate (FDR). Finally, Pearson correlations were performed with gut microbial (F:B ratio), fecal amino acids, brain structural volumes, and sociodemographic continuous variables (age and BMI). Correlations were analyzed among but not within types and were separated by significant categorical covariates (e.g. “Hispanic” vs. “non-Hispanic”). P-values for correlation were converted to q values to correct for multiple hypothesis testing (<0.05 for significance). Significant correlations ($q < 0.05$) were used to build multi-partite interaction networks for visualization. Continuous mapping displayed positive (red) or negative (blue) correlations with darker color representing stronger correlation.

Results:

Baseline Participant Characteristics

Among 130 studied participants, 62 (48%) were overweight and 68 (52%) were obese. About half of all participants were ≥30y (55%) and earned an annual income less than $70K (56%). Majority of them were female (67%), non-Hispanics (60%), non-college graduate (70%), and not married (74%). Forty-four percent (44%) of participants had an obese waist to hip ratio, while 56% had a normal ratio. Most consumed typical American diet (76%), while one quarter consumed a non-American diet (24%), which include Mediterranean, paleo, vegetarian, gluten free, diary free, low FODMAP, or other diets. Compared to the overweight participants, obese participants were more likely to be Hispanic (50% vs. 29%, p =0.014), to be married (34% vs. 16%, p=0.028), to have an obese waist to hip ratio (69% vs. 44%, p=0.043), and to consume an American diet (84% vs. 68%, p=0.031). Distribution of age, gender, education, and annual income were similar between the overweight and obese participants. Baseline characteristics are displayed in *Table 1*.

Odds of Obesity

In univariate analyses (*Table 2*), individuals were found to have higher odds of obesity if they were Hispanic [Odds Ratio (OR) 1.56, p=0.014], were married (OR 1.62, p=0.028), had an obese waist to hip ratio (OR 1.67, p=0.043), and consumed an American diet (OR 1.57, p=0.031). Controlling for all covariates, multivariate analyses (*Table 2*) found that individuals had higher odds of being obese if they were Hispanic [Adjusted OR (AOR) 1.70, p = 0.0089] or married (AOR 1.63, p = 0.036). In subgroup analyses (*Table 3*), Hispanics had higher odds of obesity independent of other covariates, whereas non-Hispanics had higher odds of obesity if they were married (AOR 1.81, p=0.036). Among married individuals, those who consumed an American diet had higher odds of being obese (AOR 5.00, p=0.0019). For individuals who were not married, female had higher odds of obesity (AOR 1.63, p=0.04). Combined obesity OR of significant covariates were computed based on AOR from subgroup analyses (*Table 4*). The highest combined obesity OR (15.4) was observed with combined risk factors of Hispanic ethnicity (AOR 1.7), married (AOR 1.81), and American dietary consumption (AOR 5). The lowest combined obesity OR (1) was seen with combined factors of Non-Hispanic ethnicity (AOR 1), not married (AOR 1), and male gender (AOR 1).

Microbiome analysis

Microbial composition as represented by root square Jensen-Shannon divergence distance, a measure of phylogenetic similarity between samples, showed a statistically significant differences in the microbiome of samples of Hispanic participants compared to the non-Hispanic participants (p=0.046) (*Figure 1B*). A trend toward statistically significant differences in the relative abundance of the gut microbiota between participants with annual income below $70K and above $70K (p=0.066). Compared to the non-Hispanic, Hispanic participants had a significantly lower microbial species richness (Chao1) (p = 0.032) and evenness (Shannon) (p = 0.0029) (*Figure 1A*). Participants who were ≥30y also had a significantly lower microbial species richness (Chao1), and a trend toward a lower microbial

species evenness (Shannon) (p=0.099). Participants had similar beta and alpha diversity independent of other covariates (p>0.05) or obesity status (obese or overweight).

Analysis of relative abundance of microbes at the phylum levels confirmed taxonomic shifts by Hispanic ethnicity (*Figure 1C*). Compared to non-Hispanic, Hispanic had relatively fewer Firmicutes and more Bacteroidetes (p=0.021), corresponding to a negative correlation of F:B ratio (*Table 5*). In contrast, participants who consumed primarily an American diet as opposed to a non-American diet had relatively more Firmicutes and fewer Bacteroidetes (p=0.036), corresponding to a positive correlation of F:B ratio. Among individuals with Bacteroidetes predominance (F:B ratio <1), neither Hispanic ethnicity (p=0.17) nor American dietary consumption (p=0.07) were significantly associated with being overweight or obese, albeit a trend toward statistical significance was observed (*Table 6*). On the contrary, among individuals with Firmicutes predominance gut microbiome (F:B ratio>1), odds of obesity was significantly magnified among the Hispanic (p=0.0062), while odds of obesity was similar independent of American dietary consumption (p=0.17).

Amino Acid Metabolites

Fourteen of twenty fecal amino acids including all essential amino acids were increased among Hispanic ($p < 0.05$). These include BCAA (leucine, isoleucine, valine), AAA (phenylalanine, threonine, tryptophan), other essential amino acids (histidine, lysine, methionine), and certain non-essential amino acids (glycine, tyrosine, serine, alanine, glutamate). Presence of fecal amino acids was not statistically different among other studied covariates including marital status, consumption of American diet, F:B ratio, and obesity status. Amino acid metabolites analyses are displayed in *Table 7*.

Brain Structural Volumes

Regional brain structural volumes were decreased among Hispanic (pallidum, $p = 0.036$; brainstem, $p = 0.011$), married (left thalamus, $p = 0.024$), and individuals who consumed an American diet (brainstem, $p = 0.043$). Compared to non-Hispanic, Hispanic participants also had

a trend toward a decreased in thalamus volumes (p=0.08). Hippocampus and amygdala structural volumes showed trend toward association with obesity status (p=0.16) and F:B ratio (p=0.11), respectively. Accumbens, caudate and putamen structural volumes were not significantly different among any studied covariates or obesity status. Brain structural volumes analyses are displayed in *Table 8*.

Correlations

Notable differences in correlation patterns were observed in the four significant covariates (*Figure 2*): A. Hispanic vs. non-Hispanic, B. married vs. not married, C. American diet vs. non-American diet, and D. female vs. male. First, Hispanic and non-Hispanic had opposite correlation patterns of 1. BMI with brain reward regions and 2. F:B ratio with fecal amino acids. In Hispanic, BMI was positively correlated to the brain emotional regulatory region (amygdala), and F:B ratio was negatively correlated to fecal amino acid (tryptophan). In non-Hispanic, BMI was negatively correlated to the brain satiety and reward regions (amygdala, hippocampus, thalamus, caudate), and F:B ratio was positively correlated with fecal amino acid (alanine).

Second, unique correlation patterns were found in individuals who were not married. Specifically, BMI was positively associated with fecal amino acid (asparagine) and negatively correlated to brain satiety and reward regions (hippocampus, thalamus). Being not married was also found to have negative correlation of F:B ratio and fecal amino acids (tryptophan, glycine, aspartate, lysine).

Correlation patterns of F:B ratio and fecal amino acids were opposite based on dietary patterns. For individuals who consumed an American diet, F:B ratio was positively correlated to fecal amino acids (cysteine, alanine, histidine). Whereas for individuals who consumed a non-American diet, F:B ratio was negatively correlated to fecal amino acids (tryptophan, threonine, glycine, methionine, phenylalanine, cysteine, tyrosine). A positive correlation of brain satiety region (hippocampus) and fecal amino acid (glutamate) was observed for American diet consumption, whereas negative correlations of multiple brain regions (hippocampus, caudate,

thalamus, pallidum, putamen) with fecal amino acids (tryptophan, threonine, glutamine, methionine, phenylalanine, cysteine, tyrosine, valine, alanine, asparagine, aspartate, serine, glutamate, isoleucine, lysine, proline, leucine) were found for non-American diet consumption.

Finally, notable gender differences were found in correlation pattern of fecal amino acids with brain reward regions. In females, fecal amino acid (histidine) was negatively correlated to brain reward region (amygdala). Whereas in males, fecal amino acids (lysine, leucine, isoleucine, methionine, glutamate) were positively correlated to brain satiety region (hippocampus).

Discussion:

In our cross-sectional study of 130 healthy participants, Hispanic and married individuals were associated with obesity despite other sociodemographic, clinical, and dietary differences. Significant interacting factors including female gender and American dietary consumption were found to further heighten the odds of obesity in our subgroup analyses. Intriguingly, Hispanic individuals expressed unique gut microbial, fecal amino acids and brain structural signature, while married individuals were found to have decreased thalamus structural volume. Individuals who consumed an American diet were found to have distinct Firmicutes predominance microbiome and decreased brainstem volumes, but its independent association to obesity was subjugated to other covariates. Correlation patterns suggest complex BGM interactions among significant obesogenic biopsychosocial characteristics. Taken together, our study provides findings that inform further precision understanding of obesity heterogeneity.

Consistent with previous literatures, our findings support the understanding of the complex pathogenesis of obesity and underscore the obesity risks among the Hispanic and married.[42,43,44,45,46] According to the Center for Disease Control (CDC), Hispanic Americans are 1.2 to 1.8 times more likely to be obese than non-Hispanic whites across all age groups.[47] The disproportional obesity prevalence in turn contributes to the significant health disparities among

the Hispanic community.[48,49,50,51,52,53] On the contrary, studies have suggested that marriage associates with weight gain, but also promotes overall health.[54,55] Certainly, a multitude of other factors might confound the underlying risks and influence the health burden of obesity.[56,57] Our subgroup analyses, for instance, found that female gender and consumption of a typical American diet potentiate the odds of obesity among the Hispanic and married. For the Hispanic, prior studies have hinted at contributing causes of obesity including higher rates of unemployment, higher levels of food insecurity, and poor access to healthcare resources.[58,59,60] Marital association to obesity might be subjected to economic, cultural, or psychosocial influences beyond that of our studied covariates.[61] Acknowledging that our understanding remains generalized, however, the challenge will be to devise obesity prevention and management strategies that are individualized.

Recent efforts toward a more precision understanding of obesity have directed our attention to the gut microbiome.[62,63] In our study, we found that individuals who are obese compared to overweight have similar microbial profiles, suggesting that these two “metabolic states” might not be detected by the gut microbiome alone. In contrast, distinct microbial profiles characterized by lower microbial species richness (Chao1) and evenness (Shannon) were observed in Hispanic individuals, suggesting dysbiosis might be a potential link to the observed ethnic differences. Indeed, dysbiosis have been described to associate with many other chronic conditions such as diabetes, inflammatory bowel diseases, colorectal cancer, and even aging.[64,65,66,67] In our study, individuals who were ≥30y of age had lower microbial diversity than those <30y; whether this is contributing to or is a consequence of aging is yet to be elicited. Certainly, the lower microbial diversity observed in Hispanic and older individuals suggests correlation to dysbiosis and thus warrant further investigation.

Intriguingly, we observed a distinct microbial predominance of the phylum Firmicutes or Bacteroidetes among individuals who consume an American diet or who are Hispanic, respectively. The significance of this observation, to be sure, is debatable.[68,69,70,71] Several

reports though have suggested that a relative reduction in the phylum Bacteroidetes and a proportional increase in Firmicutes are characteristic of metabolic syndrome such as in obesity.[72,73,74] In our analyses, we observed that among the Bacteroidetes predominance individuals with neither being Hispanic nor consumption of an American diet is associated with obesity. On the contrary, among the Firmicutes predominance individuals, the odds of being obese for Hispanic is magnified. In other words, having a Bacteroidetes predominance gut microbiome appears to exhibit a “protective” effect to dampen the odds of obesity, while having a Firmicutes predominance gut microbiome “detrimentally” heighten the odds. Notably, we observed no significant difference in the relative abundance of Firmicutes to Bacteroidetes between the overweight and obese, suggesting that once again any microbial difference between the obese and overweight might be too small to be detected. Acknowledging that there have also been contradictory studies, the relative abundance of Firmicutes to Bacteroidetes is nevertheless a simple and potentially a meaningful microbial signature that can assist in further differentiating heterogeneity of obesity.[75,76]

As the studies of the gut microbiome have led us to recognize the significance of the microbial composition, they have also inspired research efforts into the fecal metabolites.[77,78] By examining the metabolic byproducts of macro- and micronutrient, studies of the metabolites or metabolomics attempt to address not what but how the microbiome contribute to the host body homeostasis.[79] While the exact mechanisms are yet to be elucidated, prior studies have suggested that the gut microbiome alter the bioavailability and distribution of free amino acids in the gastrointestinal tract, and in turn modulate synthesis of short-chain fatty acids (SCFA) and thus host metabolism.[80,81] Interestingly, certain amino acids such as the BCAA and AAA are found to be more closely associated with metabolic disorders.[82,83] Alteration of plasma BCAA metabolism, for instance, was found to result in accumulation of toxic metabolites, which subsequently trigger mitochondrial dysfunction and stress signaling associated with insulin resistance.[84,85] In our study, we found that Hispanic, when compared to non-Hispanic, had

greater abundance of distinct fecal amino acids, including BCAA (leucine, isoleucine, valine), AAA (phenylalanine, threonine, tryptophan), other essential amino acids (histidine, lysine, methionine), and certain non-essential amino acids (glycine, tyrosine, serine, alanine, glutamate). Amino acids profiles were similar among other studied covariates. Taken together, one might infer that an increase in fecal amino acids abundance suggests either an increase in amino acids synthesis and/or uptake, or a decrease in amino acids breakdown. Questions, however, remained as to the underlying cause and implications of such findings. Although causality might not be confirmed, metabolomics seems to bring us closer to a more precise understanding of perhaps ethnic differences of obesity that invites further investigation.

Equally intriguing are our findings of reduced brain structural volumes among the individuals with obesity risk factors. We have known for decades that obesity has been linked to a number of underlying neurobiological changes. In particular, prior studies have revealed that individuals who are obese exhibited smaller cortical thickness and total cerebral volume.[86] In a prospective observational study of 12,087 participants, total body fat (TBF) in men is negatively associated with all subcortical gray matter volumes (thalamus, caudate nucleus, putamen, globus pallidus, hippocampus, and nucleus accumbens, except for amygdala), while TBF in women is negatively associated with globus pallidus volume.[87] Literatures suggest that a reduction in neuronal fiber bundle length, which has been found to correlate with elevated BMI, is believed to contribute to the brain atrophy.[88] In our study, brain structural volumes were decreased among the Hispanic (pallidum, $p = 0.036$; brainstem, $p = 0.011$), married (left thalamus, $p = 0.024$), and individuals who consumed an American diet (brainstem, $p = 0.043$). These regions of volume reduction correspond to the brain reward network, suggesting potential neurobehavioral associations to these obesity risk factors.[89] Interestingly, brain structural volumes are not significantly different between the overweight and obese, which parallel to their resemblance of gut microbiome and fecal metabolites. Certainly, our understanding is still at its

infancy, but our findings present a promising perspective to understand the heterogeneity of obesity and to guide future translational and functional studies.

Notably, a probable cross-link among our findings that merit further exploration is the brain-gut axis. In our correlation analyses we find evidence to support the intricate BGM connection to obesity. Among the four significant covariates (Hispanic ethnicity, marital status, American dietary consumption, and gender), significant correlation networks were found, including correlations between 1. BMI with brain reward regions (BMI-Brain), 2. BMI with fecal amino acids (BMI-AA), 3. fecal amino acids with gut microbiome (AA-GM), and 4. brain reward regions with fecal amino acids (Brain-AA). Our analyses support a plausible hypothesis that a complex relational network exists among fecal amino acids, dysbiosis (per F:B ratio), brain reward network and obesity. To be sure, correlational relationships do not necessarily prove causation, and their interpretations might be challenging given possibility of confounders. However, correlations do provide valuable insights into the direction and strength of the studied relationships, which may be useful for hypotheses generation in future investigations. As such, several interesting observations of the correlational network are noteworthy to discuss:

First, a positive BMI-Brain correlation with the amygdala (emotion regulatory region) was observed for the Hispanic, whereas negative BMI-Brain correlations with the hippocampus (satiety region) were found across number of factors including non-Hispanic, female, being not married, and consumption of an American diet. Acknowledging the heighten obesity risk of the Hispanics, a positive BMI-Brain correlation suggests dysregulation of the brain emotional network might play a contributing role its disproportional obesogenic risk. On the contrary, negative BMI-Brain correlations with the hippocampus might suggest potential negative feedback of satiety signaling or hippocampus volume reduction due to obesity. Second, from the perspective of the microbiome, all positive AA-GM correlations involve cysteine, alanine, or histidine, whereas negative AA-GM correlations involve tryptophan. Notably, opposite AA-GM correlations between and within two contrasting factors are observed: 1. American diet (positive)

vs. non-American diet (negative) and 2. Hispanic (negative) vs. non-Hispanic (positive). The opposite AA-GM correlations within and between dietary pattern and ethnicity might suggest opposite mechanistic pathogenesis of obesity that depend on Firmucutes and Bacteroidetes influence on fecal amino acids. For instance, Firmucutes predominance association to American dietary consumption might promote fecal cysteine, alanine, or histidine production and in part contribute to its obesogenic potentials, or Bacteroidetes predominance association to Hispanic ethnicity and its negative correlation to fecal tryptophan might influence ingestive behavior, appetite, and metabolic homeostasis.[90] Third, glutamate has direct negative correlation to BMI, whereas asparagine has direct positive correlation to BMI. Interestingly, a recent study that aimed to identify metabolic pattern associated with obesity found that plasma concentration of amino acids including glutamate, alanine, proline, tyrosine, and BCAAs were higher in the obese participants, while asparagine and serine were higher in non-obese participants.[91] Consistent to this observation, our study suggests that balancing the gastrointestinal absorption and execution of amino acids such as glutamate and asparagine might have direct correlation to obesity. Finally, we found that positive Brain-AA correlations commonly involve the hippocampus (brain satiety region) with glutamate, whereas negative Brain-AA correlations involve many other brain regions (amygdala, hippocampus, thalamus, accumbens, caudate) with commonly BCCA (leucine, isoleucine, valine), AAA (phenylalanine, threonine, tryptophan) and methionine.[92] The direct and indirect interactions of fecal amino acids and brain regions are though complex, making interpretation of our finding challenging. For instance, synaptic glutamate signaling in brain includes multiple interacting receptors, modulating cotransmitters and distinct regional dynamics that have been implicated in anxiety, stress, memory, and certain psychiatry disorders.[93] An animal study has suggested that high fat diets trigger neurochemical changes through glutamatergic transmission, leading to a desensitization of NMDA receptors within the hippocampus, which might account for cognitive deficits.[94]

Indeed, we have come a long way in understanding the heterogeneity of obesity, but we ought to recognize that obesity is not only heterogenous in its pathophysiology, but also is in its manifestations and its response to therapies.[95] For instance, individuals with similar body weight or BMI have been shown to exhibit markedly different co-morbidities and levels of health risk.[96,97] Although majority of individuals with obesity will develop conditions such as diabetes or cardiovascular disease, a minority proportion will remain free of cardiometabolic comorbidities during their lifetime and even metabolically healthy.[98] Furthermore, the heterogeneity of obesity is complicated by the recognition that the current "one-size-fits-all" treatment approaches, including pharmacotherapy, diet and lifestyle interventions, are often hit-or-miss with highly variable efficacies and outcomes.[99] Only until recently, studies have begun to reveal distinct clinical subtypes of obesity that have differential responses to pharmacotherapy.[100] While still at its infancy, precision obesity care is undoubtably on the horizon.[101]

Several limitations need acknowledgement. First, our cross-sectional study design limited our ability to establish temporal or causal relationships between the studied covariates and the primary outcome of obesity status. A prospective observational study design might provide enhanced power and temporal association to our observations, but feasibility of such design is challenging. Our sample size of 130 participants is notable but a larger sample size can yield greater power given multiple hypothesis testing and correction. In addition, our microbiome, metabolites, and brain structural volumes data contain missing data, which confound interpretation. Given the multifactorial nature of obesity, not all relevant variables might be represented by our covariates. Subject to recall or social desirability bias, self-reported dietary pattern can be strengthened with actual dietary consumption data, albeit collection of actual diet consumption data presents its own challenge. Continuous covariates (i.e. age, waist to hip ratios) and outcome variables (i.e. obesity status instead of BMI) were dichotomized with the advantage for ease of statistic computation, but power to detect statistical significance difference can be compromised.[102] our Lastly, our participants are recruited confined to a

community at Los Angeles and our analyses are limited to the population of healthy individuals who are overweight or obese, hence limiting generalizability.

Nevertheless, our findings present a noteworthy perspective in understanding how heterogeneity of obesity is influenced by biopsychosocial risk factors and might be precisely differentiated through gut microbial, fecal metabolites and brain structural characterization. We conclude to accept our hypotheses and find consistent evidence to suggest Hispanic and married individuals are associated with obesity despite sociodemographic, clinical, and dietary differences. Notably, Hispanic might express a unique gut microbial, fecal amino acids, and brain structural volumes signature that warrant future research. Microbial characterization in particular is an emerging predictive marker for therapeutics and might also serve as selection biomarker in obesity practices and clinical trials. By addressing ethnic disparities guided by precision phenotypes, we may potentially unlock novel understanding of obesity heterogeneity and transform its impact on obesity care.

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