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**An Analysis on How Housing Status Influences the Gut-Brain-Axis for populations
in and around the Skid Row Area of Los Angeles, California**

A Thesis Presented
By

Mauricio Guzman

And to the W.M. Keck Science Department
Of Claremont McKenna, Pitzer, and Scripps Colleges
In partial fulfillment of
the degree of Bachelor of Arts

A Senior Thesis Project in Combined Human Biology and Economics
24 April 2023

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Acknowledgements

To the children:

To begin with, I dedicate this senior thesis project to the **children of color** who have suffered from extreme poverty— who were forced to become victims of systemic racism and systemic violence, have been forced into faulty political systems that have not guaranteed anything but paucity, and have been impacted by illness and death as a result of their low socioeconomic status (SES). This paper is for you. I hear you. I hope that I continue to reverberate your existence and most importantly, catalyze efficacious and preventative solutions in the veins of public policy. I know the issues presented in this paper run deeply in our country and I encourage nothing but the deconstruction of the systems that birth inequity.

To my Mother:

Mamá, muchas gracias por todo. Eres literalmente la mejor madre que alguien puede pedir. Si no fuera por ti y tu resiliencia, literalmente no estaría aquí hoy. Aunque sé que asistir a Pitzer fue una trayectoria difícil para nosotros, mira, lo logré. Y solo lo voy seguir logrando. Espero poder hacerte sentir orgullosa de mí, mamá. Todo esto de verdad me costó. Pero, como me has enseñado a creer en mí mismo-- qué puedo hacer cualquier cosa a lo que me proponga-- lo hice. Soy un académico por tu humildad, por tu esfuerzo, y por tu voluntad. Y por eso, te agradezco. Te quiero mucho, mamá. ¡Lo hicimos!

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Abstract

Over recent years, the “microbiota-gut-brain axis” (MGBA) has garnered significant attention in the scientific community. Specifically, perturbations of the MGBA via stress and dietary intake have been linked to a wide-range of diseases including gastrointestinal diseases, metabolic diseases, mood disorders, and cognitive diseases. However, most studies have been solely conducted on mice models and have yet to consider the more complex, intricate systems that impact the human body. In addition, researchers have yet to consider the populations who may be most susceptible to chronic stress and negative dietary outcomes. Drawing from the fields of medical sociology, non-invasive human biology, and economics, we construct a mixed methodological approach to researching unhoused and housed populations in and around the Skid Row area of Los Angeles, California using a gut biomarker indicative of obesity and its associations to neuroinflammation and cognitive decline.

Keywords: Literally homeless, housed, unhoused, Gut-Brain-Axis, gut microbiota, microbiome, neuroinflammation, neurological disease, Skid Row, Los Angeles, obesity, diet, gut

Introduction

The human body is a complex superorganism that hosts trillions of microorganisms including bacteria, fungi, archaea, viruses, and unicellular eukaryotes that all work together to support the development of the host and sustain life (“DOE Explains,” 2023). Consequently, with this collectivist work and in combination with the human genome, the development of major organs form such as those seen within the gastrointestinal tract (also known as the gut). The gut comprises the mouth, pharynx, esophagus, stomach, small and large intestine, rectum and anus that allow one to swallow, digest, absorb material, and material not absorbed is excreted feces (National Cancer Institute, 2011). However, over the recent years, scientists have focused more particularly on a niche communication network within the gut-- a community that links the genome, various microbial species, and the environment. This niche community is referred to as the gut microbiota (Thomas, 2022). The gut microbiota garnered significant attention in research due to its apparent association with health and mortality outcomes among human populations, which lends credibility to Hippocrates' assertion that "all diseases begin in the gut" (Thomas, 2022).

The Development of the Gut Microbiome

As research suggests, the gut microbiota is pre-established from the earliest stages of life. For instance, a study found that the pre-determination of gut microbiota actually begins in the uterus, as bacterial byproducts are transferred through the placenta from the birthing parent (Belli, 2020). Once an infant is born, the mode of delivery also plays a role in determining the gut microbiota, as studies show that vaginally-born infants and cesarean-born infants have distinct gut microbiota and metabolite profiles (Coelho et al., 2023; Li et al., 2021). After birth, the infant's diet can further shape the gut microbiota, with differences seen in infants who are

breast-fed vs formula-fed (Laursen et al., 2017). Moreover, microbiota composition may be structured by factors such as early exposure to antibiotic use, the birthing parent's health during pregnancy, and geographic environments such as hospital settings, which have been associated with the "spread of pathogens" and "early atopic manifestations" (Thomas, 2022, pg. 3).

Although many fluctuations occur in the early stages of life, researchers generally believe that a stabilized, "adult-like microbial framework is achieved" after the age of three and comprises two major bacterial phyla: *Bacteroidetes* and *Firmicutes* that encompass about 90% of the total microbial makeup of the gut (Thomas, 2022, pg. 1 and 5). The main role of normal gut microbiota is to "host nutrient metabolism, xenobiotic and drug metabolism, [maintain] structural integrity of the gut mucosal barrier, [assist with] immunomodulation, and [protect] against pathogens" (Jandhyala, S. M., 2015, ¶ 1).

Physical Disease, Microbiota-Gut-Brain-Axis, and Mood

Even though the gut microbiome is pre-established in early life, it is becoming increasingly apparent that significant exogenous variables may disrupt gut microbiota homeostasis or the gut microbial makeup, leading to dire health consequences. While the exact mechanisms of these disturbances are still being unraveled, studies show that imbalances in the gut microbiota can be linked to a range of conditions such as functional gastrointestinal diseases including but not limited to: functional constipation, irritable bowel syndrome (IBS), diarrhea, and fecal incontinence, metabolic diseases, and liver and joint-related diseases (Bezek et al., 2020; Harrison and Taren, 2018; Mukhtar et al., 2019). What is perhaps even more intriguing is the growing body of evidence suggesting that gut microbiota composition can also lead to neurological decline, cognitive impairment, cognitive illnesses, and mood disorders (Appleton, 2018; Solas et al., 2017). Hence, scientists coined the term "microbiota-gut-brain-axis" where

“main inputs of the gut-brain-axis (GBA)” stem from the microbiota residing within the gut and ultimately influence the brain (Mukhtar et al., 2019, \mathbb{P} 3).

More specifically, many studies conducted by independent research groups show that gut dysbiosis disrupts monoamines in mice, which are more notably recognized as neurotransmitter molecules like serotonin, dopamine, etc. Disruption of these neurotransmitters contribute to depressive symptomatology (Appleton, 2018). Other mice studies suggest that some associations exist between rodent aggression and perturbations of the gut microbiota (Kwiatkowski, 2022). Nonetheless, research that exclusively employed mouse models (such as those who utilized Germ-Free conditions) suggested emotional and behavioral dysregulation, but such studies may be limited in their direct application to the human gut microbiome.

Obesity as an Inflammatory Disease, the Diet, and its links to the Gut-Brain-Axis

With the increasing prevalence of mental illnesses and cognitive disorders due to the demographic transition of ultra-urbanization, scientists recognized that imbalances of the microbiota-gut-brain-axis tend to be correlated with neurological illnesses, disorders and deficits such as deficits in learning, memory, and executive functioning, autism, Alzheimer's disease (AD), dementia, Parkinson's disease, schizophrenia, and anxiety (Cryan et al., 2019; Srivastava, 2009). Precisely, obesity, as a metabolic disease, is attributed to this cognitive impairment, and is specifically seen as a medial cause for AD, and dementia (Solas et al., 2017). The pathophysiological mechanism that is gaining scientific recognition is the inflammatory dysregulatory cascade caused by obesity and diet that produces “low-grade inflammation in peripheral tissues and the circulation” (Solas et al., 2017, \mathbb{P} 2). Low-grade inflammation in the

peripheral tissues is thought to spread to the brain and potentially cause brain atrophy (Solas et al., 2017).

With this information, obesity is not only considered a metabolic disease but can also be considered an inflammatory disease that is elicited by fat (aka adipose tissue) and the gut microbiota (Solas et al., 2017). Specifically, high-fat diets lead to the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , feeding-related peptides (leptin and resistin), plasminogen activator inhibitor 1, C-reactive protein and most notably, interleukins (IL)-1 β and IL-6 that can ultimately contribute to neuroinflammation and disease (Solas et al., 2017). Thus, one could see how diet composition needs to be recognized as an important factor for dysbiosis of the microbiota-gut-brain-axis as it elevates pro-inflammatory cytokines in the circulation (causing systemic and central inflammation), which ultimately leads to cognitive impairment (Solas et al., 2017).

Stress, Systemic Inflammation, and the Gut-Brain-Axis

Although not widely understood, recent studies found that stress had an impactful contribution on the microbiota-gut-brain-axis. One murine study found that mice exposed to social stress (by the means of interacting with an aggressive mouse for a period of six hours) had differing microbial composition and pro-inflammatory cytokine levels compared to the control group. Specifically, mice had decreased *Bacteroides* in their gut microbiome and had increased levels of circulating IL-6 (Bailey et al., 2011). For human studies, few studies suggest the direct relationship of stress on the human gut microbiome. However, researchers suggested that stress, within itself, is a causal mechanism for systemic inflammation. For instance, a meta-analysis found a correlation between low socioeconomic status (SES) and elevated levels of

pro-inflammatory cytokines IL-6 and C-reactive protein (Muscatell et al., 2020). These findings suggest a link between chronic stress and systemic inflammation and can contribute to the scientific community's broader understanding of systemic inflammation and its catalysis of neuroinflammation and neurodegeneration (Muscatell, 2020; Sun and Shimada, 2022). As such, further exploration needs investigate the direct impacts of chronic stress and psychosocial stress on human populations (i.e. unemployment, homelessness, extreme poverty, social defeat, social exclusion, etc.), evaluating its impacts on the gut microbiota, and developing mathematical models to predict gut microbiome outcomes for human populations, specifically for those who are severely or multi-vulnerable.

Harrison and Taren proposed a framework of analysis that suggests how poverty can shape the microbiota and further exacerbate metabolic disease for those residing in high-income countries (Harrison and Taren, 2018). Notably, the authors highlight that obesity is most prevalent for individuals who are of low-socioeconomic status, which may be a direct consequence of the changes in their microbiota composition and the external factors that contribute to these microbial shifts (Harrison and Taren, 2018). Interestingly, the authors consider the “calories-per-dollar-trade-off” in which individuals who are of low socioeconomic status have a *constrained choice* of consuming foods that provide the most caloric energy and satiation for the lowest price. Specifically, the foods most likely to be consumed are those high in fat or those that are considered to be “refined carbohydrates,” while “fresh” foods that are “nutrient-dense” or high in dietary fiber are likely to be excluded from a poorer individual's consumption. Consequently, high fiber foods are a crucial component in reducing obesity-induced inflammation (Harrison and Taren, 2018). Ultimately, the authors argue that diet is a key factor that is driven by socioeconomic class and thus, only few wealthy populations in

the Western society may solely reap the benefits of the bioavailability of foods that can contribute to the amelioration of metabolic and chronic disease (Harrison and Taren, 2018).

As such, researchers need to take into account a biopsychosocial framework in how they examine the microbiota while refraining from experimental approaches and extrapolations that may not be directly applicable to the human experience. Thus, factors that need to be further taken into consideration are the contributions of human stress on the microbiota, the impact of calorically-dense diets on the microbiota, and most importantly, the susceptible populations that are most impacted by these elements, which are driven by socio-structural forces. By having a biopsychosocial framework to understand health outcomes, not only does one understand the biological drives that cause disease and illness but also accounts for the social and environmental contexts with which individuals are situated that influence disease and illness. Therefore, research that includes a multifaceted approach to our understanding of the gut-brain-axis is crucial and beneficial for providing trauma-informed care and presenting solutions that are curated for vulnerable populations who are most susceptible to differential dietary outcomes and chronic life stressors.

Specific Aims and Innovations

This study provides an innovative research proposal that includes a mixed methodological approach to research (embedding a biopsychosocial framework that is not traditionally seen within scientific research), which aims to subdue the paucity of this style of analysis within existing research to date. Additionally, this project focuses on a particular population more narrowly in hopes to spark conversation surrounding efficacious treatment options for those who are most susceptible to dietary inequities, chronic life stress, and

differential gut microbiota outcomes. Furthermore, I will engage with the local-level analysis of the gut microbiota for individuals residing in Los Angeles, California, specifically within the Skid Row area, which has also not been seen in research to date. Notably, this proposal will include both quantitative and qualitative research methods used prominently in the fields of sociology, biology, and economics, and will propose the most ethical recruitment methods when learning from transient, vulnerable populations (seen to date) drawing from both the fields of medical sociology and non-invasive, human biology.

Particularly, I focus on the gut-brain-axis for an *unhoused* population in California in which chronic stress may be exacerbated and dietary intake may be poor and include a proposed econometrics, logistic regression analysis to determine how the gut microbiome composition may be affected by these socio-structural elements. Additionally, I introduce a bacterial biomarker indicative of obesity through a microbial ratio, which may suggest susceptibility to cognitive illness in the future for vulnerable individuals. Lastly, I discuss the future directions and limitations of my analysis to preserve and further the preciseness of my study.

Proposed Mixed Methodologies

The Non-Extractionist Approach

Before considering working and engaging with vulnerable populations, it is important to first recognize the historical context of the health equity research enterprise. In Dr. Petteway's poem, titled *Adjustment*, Petteway states:

The adult spine, aka backbone, is composed of 24 segments. Separately, each segment is incapable of animating our bodies. Communities of color, low-income communities, and other marginalized groups represent the backbone of the health equity research

enterprise-- it literally cannot exist without our bodies and what they are subjected to in the face of structural inequality. [Therefore,] this poem... draws attention to the settler-colonial, racial-capitalist, and extractivist logics of racial and health equity discourses dominated by narratives produced by mostly White scholars... [and] suggests that-- more than anything else-- this model of practice is what's most in need of adjustment (Petteway, 2022).

Considering this analogy, Petteway “challenges the field’s dominant knowledge production paradigm” and highlights the extractionist approach within health equity research (Petteway, 2022). Petteway argues that health equity researchers need not only to reconsider who authors discussion of scholarship but also the ways in which research is conducted. Petteway offers a counter-perspective that illuminates the dehumanizing and unethical approaches used in traditional scientific methodology and typically by White scholars. Thus, for this research proposal, I aim to re-evaluate the ways in which I engage with such populations by actively refraining from harmful language (i.e. subjects vs. participants) within writing and will consider my own social positionality as I engage with the community I am learning from. Additionally, I hope to place myself in the position of the learner, rather than the teacher, and will do my best in providing social support throughout the progress of the study. Recognizing the vulnerability of the unhoused community I partner with is crucial to research. My aim of this study is to introduce Petteway’s counter-perspective in the best way possible as to challenge the dehumanizing and unethical approaches that stem from inflexible scientific knowledge paradigms. Keeping this in mind, the remainder of this methodology section will focus on three primary areas within the study design: ethical recruitment and considerations, community-based data collection, and a proposed logistic regression model for the gut microbiota.

Study Design

In order to conduct this study, I propose to work with the following two populations: unhoused individuals of Skid Row and housed individuals near the Skid Row area, both of which will follow two different recruitment methods. Differing recruitment methods for this study is an essential part of the biopsychosocial framework because it considers “the increased *Biopsychosocial Risk Factors*” that may be faced by the unhoused population of the study. Particularly, there may be increased engagement with prostitution, illicit drug use, self-harm, criminal activity, panhandling, sexual abuse, etc. caused by the increased susceptibility to socio-structural inequity and inadequate healthcare (Kincaid, 2019). And, in combination with these factors, the social stigmatization of these behaviors may exacerbate a population to become hidden or hard-to-reach (HTR) in the societal setting but also, within the academic research setting (Kincaid, 2019; Gray, 2015). Thus, different recruitment methods need to be considered that push away from the “one-size-fits-all” approach and the recruitment process needs to be restructured to be a culturally-competent one (Kincaid, 2019; Strehlau, 2017). While I acknowledge that conducting research on marginalized populations is inherently unethical and extractivist within itself, I also recognize the potential benefits of studying these communities in terms of advancing protectionist policies and promoting social change. Therefore, I work to reframe the narrative of research by aligning my intentions of the study to solely be in benefit for the marginalized group I work with and to be as flexible as possible.

Literature Surrounding the Recruitment and Retention Process of HTRP for Research Studies

The literature surrounding recruitment and retention of unhoused people in research studies have proposed some practical challenges when working with these hard-to-reach

populations (HTRP). Ellard-Gray and others have suggested some of the following challenges while working with HTR populations: defining the sample frame, mistrust of the researcher/research process, fear of the lack of anonymity/confidentiality, and discrepancies with informed consent (Ellard-Gray, 2015; Booth, 1999). Since homeless individuals may be transient and there exists differential tiers to homelessness, defining the sample in a study may become complicated in a research study. In conjunction with this issue, homeless individuals or HTRP can also experience academic mistrust, affecting their willingness to participate in a study, since historical violations by researchers have been conducted in the past (Ellard-Gray, 2015).

Furthermore, those who identify within a socially stigmatized group or who engage with socially stigmatized behaviors (i.e. intravenous drug-users; sexually marginalized individuals) may be either 1) avoidant and fearful of contacting those outside of their social group and/or 2) may be unwilling to identify themselves to the researcher due to privacy concerns (Ellard-Gray, 2015).

Thus, the anonymity and confidentiality of research tangent to participant concerns needs to be carefully considered within the methodology. Finally, Booth identifies concerns in regards to informed consent of homeless individuals. Specifically, Booth argues that informed consent may become complicated for individuals who are drug-users/ intoxicated, suffer from severe mental health issues or are under-age and therefore, research conducted on homeless populations should consider such concern (Booth, 1999).

Nonetheless, within the same literature, the authors provide some unique ethical considerations that may assuage these challenges. Firstly, researchers should consider a well-defined sample frame that can include a method of recruitment that may be site-specific and utilizes careful language to appropriately capture or “narrow down” their population of interest (Ellard-Gray, 2015). Moreover, one needs to consider building rapport, “hanging-out” in the

community, collaborating with community partners, and absolute research-transparency before and throughout the process of recruitment/the progress of the study. By considering a relationship-building framework with the participant, historical mistrust of said individual may be lessened and, in turn, may be an effective way to retain participants (Ellard-Gray, 2015; Booth, 1999). Furthermore, when conducting research involving vulnerable populations, it is imperative to prioritize confidentiality. Confidentiality can be achieved by de-identifying data through the use of pseudonyms instead of real names, securely storing data, and maintaining discretion when engaging with illegal activities (Ellard-Gray, 2015). Moreover, having clear language in the informed consent forms, strict eligibility requirements for the study, and allowing the participant to withdraw from the study at any time are also critical considerations when researching severely vulnerable populations like the homeless (Ellard-Gray, 2015; Booth, 1999). Booth suggests that researchers should also consider the following when working with unhoused populations: how the researcher presents themselves (i.e. through appropriate clothing, body language, and dialogue), utilizing safety protocols, providing welfare services for those willing to share, being culturally-sensitive and non-threatening, and asking for feedback throughout the progress of the study (Booth, 1999).

The Belmont Report states, “...honoring the choices of subjects capable of exercising autonomy and protecting subjects incapable of making their own decisions. Ethical human experimentation preserves respect for the subjects whose participation is essential to the humanitarian mission of biomedical and social science research”(Quinn, 2015, ¶ 8). Thus, for this research proposal, I must consider these frameworks of understanding crafted by field experts of HTRP research and angle my research toward beneficence/respect of the participants to maintain ethicality and sensitivity within the structure of the research study design.

Recruitment for the Housed Population near Skid Row

To begin, I define my sample population as those who identify with LA County's definition of "literally homeless". According to the County of Los Angeles Workforce Development, Aging, and Community Service, "literally homeless" is defined as:

An individual or family who lacks a fixed, regular, and adequate nighttime residence, meaning:

I. An individual or family with a primary nighttime residence that is a public or private place not designed for or ordinarily used as a regular sleeping accommodation for human beings, including a car, park, abandoned building, bus or train station, airport, or camping ground;

II. An individual or family living in a supervised publicly or privately-operated shelter designated to provide temporary living arrangements (including congregate shelters, transitional housing, and hotels and motels paid for by charitable organizations or by federal, state, or local government programs for low-income individuals)...

(September 3, 2019).

By doing so, I create a valid operational definition in regards to the term homeless/unhoused that will exclude individuals that may identify as situationally (first-time) homeless individuals (Kincaid, 2019).

Additionally, I will adopt a snowball-and-site sampling method, which has been deemed as the most ethical and only feasible sampling method when recruiting unhoused populations (Kincaid, 2019; Faugier and Sargeant, 1997). With this method, one is able to go to a geographic

site and conduct a snowball sample where unhoused individuals introduce the researcher to others in the community; rather than a researcher going into a community and randomly extracting data (Booth, 1999). Participants will be recruited from a Skid Row kitchen site, particularly the site of the community-based organization called *Midnight Mission*, which offers three meals a day to the homeless individuals of Skid Row (“Homeless Programs and Services,” 2023).

Before recruitment and during the progress of the study, I must build rapport with community members to gain trust of willing participants and actively communicate in full transparency the research process and aims of the study. All participants must be able to understand and sign the informed consent documents prior to the study, which will be determined by HTRP field experts. Furthermore, I will submit to the Institutional Review Board prior to the study to approve the study design as I will be working with a severely vulnerable population. It is important to note that my work will not have any interest in profiting off of the findings but instead, I will use the findings to catalyze conversation surrounding equity policies that can benefit the unhoused individuals of Skid Row. Lastly, participants of the study may opt-out at any given point in time and will have access to social support throughout the study.

Sample Size

I hope to capture a population of about one-hundred unhoused individuals who reside in Skid Row. The eligibility criteria for my population of interest are those who are 18 years of age or older, those who do not exhibit severe mental illness based on pre-screening assessments, do not have compromised autonomy, and most importantly, are fully willing and able to participate in the study. Additionally, I must collaborate with community organizations that will be willing

to attend the Midnight Mission site in order to collect data/distribute surveys so that data collection remains in one site. Furthermore, as a researcher, I will not be collecting fecal matter data myself since I recognize the complications in doing so. Having community clinicians doing this procedure instead would be more beneficial since these community organizations are more trusted by community members and may have already established rapport by the Skid Row unhoused community. The community health organization of interest is USC's Student Run-Clinic who have provided "high quality comprehensive care" to those who are "chronically ill" and of "underserved populations" like the homeless ("USC Student Run-Clinic: Homepage," 2023).

Materials for Sampling

- **Surveys**

For the purposes of this study, HTRP field experts will distribute two surveys in which respondents can self-report their health outcomes. The two surveys of interest will be one that captures perceived stress for individuals and another that captures the quality of dietary intake of individuals. Particularly, will be using UPenn's *Perceived Stress Questionnaire*, which is a questionnaire consisting of thirty items designed to assess stressful life events or circumstances that may trigger or exacerbate disease symptoms. The Perceived Stress survey allows for researchers to calculate an index that measures a respondent's perceived stress level indicated at the bottom of the survey (see appendix: Survey 1). I will utilize questions 1, 7, 10, 13, 17, 21, 25, and 29, accumulate their raw scores, subtract thirty, and divide by ninety to get each individual's perceived stress score index. In combination with the Perceived Stress Index Survey, HTRP field experts will distribute the *Short Healthy Eating Index Survey*, which is a questionnaire that can

be translated into a Healthy Eating Index (HEI) that measures diet quality by assessing how exactly a set of food aligns with recommendations of the *Dietary Guidelines for Americans* (see appendix: Survey 2). For this index, a score of 100 reflects the best possible score of alignment to the *Dietary Guidelines for Americans*. As an econometrician, I choose to utilize these surveys in order to create two continuous, independent variables that are based upon indexes.

I will utilize these variables to capture an individual's perceived stress and quality of diet, which are suggested to be determinants of the gut microbiome composition as suggested in the introduction.

- ***Fecal Matter Data Collection***

Additionally, community clinicians will collect stool samples from the one-hundred unhoused individuals who have met the eligibility criteria and have decided to willingly participate in the study. Stool samples will be analyzed to study the gut microbiome compositions of the participants in the study as this method is proven to be the most non-invasive procedure for gut-microbiome research (Tang, 2020).

Recruitment, Surveying, and Data Collection for the Housed Population near Skid Row

To recruit the unhoused population of the study, I will consider a random sampling of individuals who surround the Skid Row area. I consider a group who resides in the surrounding area of Skid Row in order to reduce the variability of the factors that may affect the dependent variable. For example, choosing a different housed population that resides in a different state may be affected by differing geo-policies (i.e. food and chemical policies), differing geospatial layouts and high exposure to other confounding variables. Thus, I constrain the two populations within a geo-location to limit confounding biases for the study.

A random-sampling method will be adopted for this population by using a computer generator and randomly selecting 200 addresses near the Skid Row area. For the addresses selected at random, the following materials will be mailed out: the informed consent document, the *Perceived Stress Questionnaire*, the *Short Healthy Eating Index Survey* and a stool sample kit to collect fecal matter. By sending out the materials to different addresses, it can be confirmed that the population of interest is captured and is housed by definition. My hope is to capture one-hundred participants similar to that of the unhoused group. Participants could then return the materials to the supplier free-of-charge. Similarly, participants may opt-out of the study at any given point in time and may ask any questions or concerns at any time.

Submissions of ALL Fecal Matter Data

All fecal matter kits will be mailed to *Creative Biogene* company, which analyzes and quantifies the gut microbiome through feces collection sampling. Specifically, I aim to obtain the Firmicutes to Bacteroidetes ratio, a biomarker indicative of obesity, for each individual who participates in the study (Kasai, 2015; Koliada, 2017).

Post-Data Collection

Once all data is collected, I will input all the data into Excel, de-identify the data, clean the data, and will transfer the data to STATA- 17 SE software (a software used to analyze mass data sets and perform statistical analysis).

Logistic Regression Analysis

I propose to work with a logistic regression for analysis of the *firmicutes to bacteroidetes* ratio. By using a logistic regression, I can code the dependent variable (*firmicutes to bacteroidetes* ratio) with the following hypothetical values:

If $\hat{y} > 1$ (indicative of obesity), then code as 1.

If $\hat{y} \leq 1$ (gut ratio not being disturbed or inconclusive), then code as 0.

where: \hat{y} is the *firmicutes to bacteroidetes* ratio (F:B ratio)

That is, I can construct the dependent variable (*firmicutes to bacteroidetes* ratio) to become binary— one outcome being an F:B ratio greater than one (which is indicative of obesity) and the other outcome being an F:B ratio less than or equal to one (which is indicative of an inconclusive result). Furthermore, when utilizing a logistic regression, I will be able to predict how the probability of the *firmicutes to bacteroidetes* ratio being greater than 1 (denoted as $P(\hat{y} > 1)$) is impacted by both the independent variables. The independent variables, in this case, are the Perceived Stress Index and the Healthy Eating Index (which is indicative of diet quality. Each index for each respondent can be extrapolated from the survey questionnaires. Since data will be collected for both unhoused and housed individuals of Skid Row, I would be able to capture two logistic curves per independent variable.

Additionally, my dependent variable of interest is the probability of the F:B ratio being greater than 1 since this hypothetical outcome is indicative of obesity and obesity has been proposed to be a pathophysiological mechanism to neuroinflammation and cognitive decline as stated in the introduction of this project.

Proposed Results

The proposed results include the following figures:

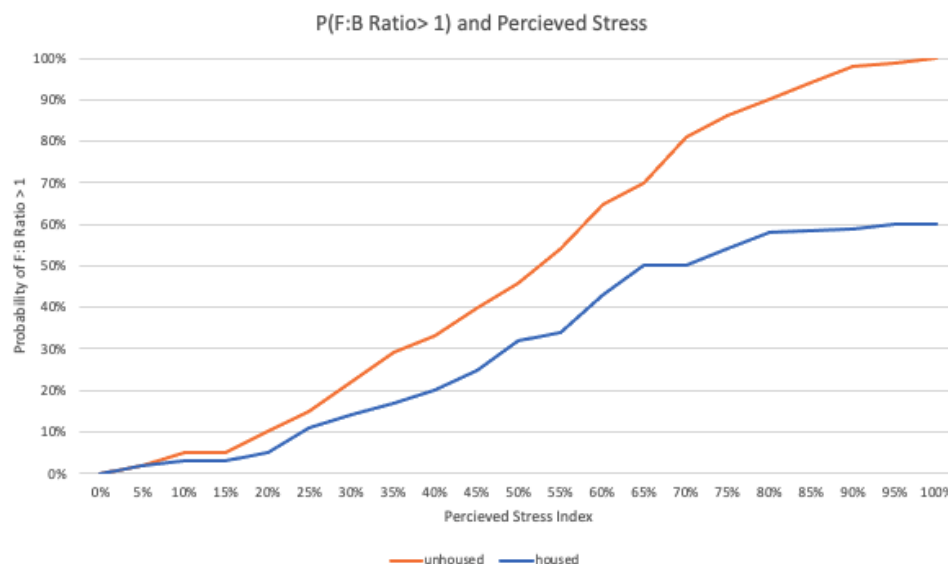


Figure 1: Displays the probability of the F:B Ratio >1 as the Perceived Stress Index increases for both unboxed and boxed individuals of Skid Row.

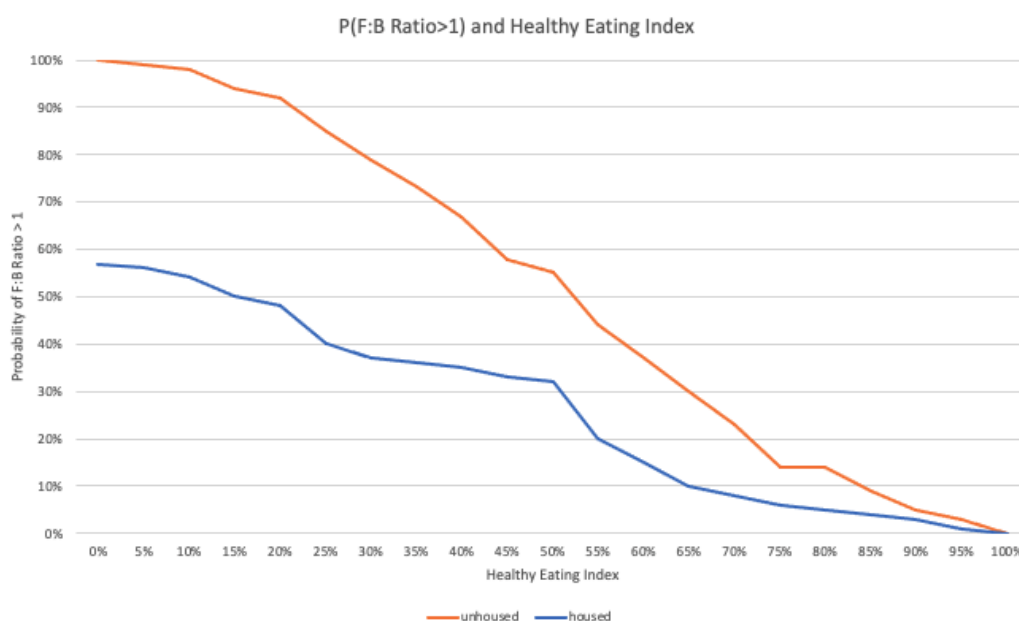


Figure 2: Displays the probability of the F:B Ratio >1 as the Healthy Eating Index (indicative of diet quality) increases for both unboxed and boxed individuals of Skid Row.

In Figure 1, I suspect that as the independent variable of Perceived Stress Index increases, the probability of the F:B ratio being greater than 1 will also increase for both groups. However, it is important to note that I expect to see a gap between the logistic regression curve for the unhoused group and the logistic regression curve for the housed group (Figure 1). This gap would represent a greater likelihood for unhoused individuals to have an F:B ratio greater than 1, while housed individuals will be less likely to have an F:B ratio greater than 1.

In Figure 2, I suspect that as the independent variable of the Health Eating Index (indicative of diet quality) increases, the probability of the F:B ratio being greater than 1 will decrease for both groups. Similarly, I expect to see a gap between both logistic regression curves between both groups (Figure 2). Both gaps in Figure 1 and 2 show that the unhoused group may be at a disproportionate disadvantage of negative dietary quality outcomes and may experience more chronic stress that shapes their self-perception of stress. Thus, the unhoused individuals of Skid Row will have a greater likelihood of having an F:B ratio that is greater than 1. The gaps may also illuminate the socio-structural disadvantages between both groups as the unhoused community of Skid Row are the vulnerable group of my study that are affected by social negligence and marginalization.

Discussion of Future Directions and Limitations

One of the most pertinent limitations to my study is the need for a more sophisticated statistical analysis. Since both of the independent variables (Perceived Stress Index and Healthy Eating Index) impact the dependent variable (F:B ratio), future development of this research might need to consider a multivariate analysis. In other words, one could consider the inclusion of interaction terms with adopting a new mathematical model. For example, if one of the

participants experiences high levels of chronic stress that may impact their self-perception of stress in addition to low-quality dietary intake, one should account for this interaction effect to see exactly how both experiences impact the probability of the F:B ratio being greater than 1. As such, I encourage others to adopt a multifaceted-approach to further the analysis of my study.

As literature evolves, I may also be able to include more relevant independent variables. For example, I can include variables such as age, antibiotic use, and exposure to environmental toxins, which may impact that F:B ratio. Including such variables will make the study more precise as I can exactly measure the effects of these variables on the probability outcome of the F:B ratio being greater than 1.

Discussion

For this study, I analyzed two exogenous variables (precisely, perceived stress and dietary quality) that impact the *firmicutes to bacteroidetes* ratio, a biomarker indicative of obesity. As stated in the introduction of this paper, obesity has been proposed to be a pathophysiological mechanism that invokes systemic inflammation within the body and later, neuroinflammation and brain atrophy. For the purposes of furthering human studies and to better understand the social determinants that impact the health of vulnerable populations, I proposed to logistically analyze the *firmicutes to bacteroidetes* ratio of an unhoused group of Skid Row and a housed group around the Skid Row area. This study tries to help identify the inconsistencies of such a ratio between the two groups in hopes of catalyzing conversations surrounding increased access to green spaces, healthy food, and education for vulnerable populations. Efficacious interventions and programming remain dire to ameliorate socio-economic and socio-structural injustices that affect multi-vulnerable communities. It is my hope that those who read this

proposed study take into account the social change that is needed and that science, sociology, and economics have an intricate interplay that can help prompt such change and spark equity.

Ethical Considerations by the Author

As part of this research proposal, I underwent some preliminary IRB training to further understand the ethics surrounding human studies. Preliminary IRB training requires academics to learn from about 30 training modules that provide ethical scenarios and ultimately test one's understanding surrounding the ethical aspects of researching subcultures. Once completed, one is granted a certificate that is submitted in conjunction with their study design to receive official IRB approval. Please find attached herewith a copy of my certificate for kind perusal:



Completion Date 01-May-2023
Expiration Date 01-May-2026
Record ID 55441103

This is to certify that:

Mauricio Guzman

Has completed the following CITI Program course:

Social & Behavioral Research - Basic/Refresher
(Curriculum Group)
Social & Behavioral Research - Basic/Refresher
(Course Learner Group)
1 - Basic Course
(Stage)

Under requirements set by:

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Appendix

Survey 1

Perceived Stress Questionnaire (PSQ)

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Purpose Consisting of 30 items, the PSQ was developed as an instrument for assessing the stressful life events and circumstances that tend to trigger or exacerbate disease symptoms. With stress bearing significantly on the quality and consistency of the sleep cycle [1], the PSQ is a potentially valuable tool for evaluating the underlying causes of sleep disturbances. The scale is specifically recommended for clinical settings, though it has been employed in research studies as well.

Population for Testing The PSQ has been validated with a population of in-patients, out-patients, students, and health care workers with a mean age of 31.8 ± 13.9 .

Administration The scale is a self-report, pencil-and-paper measure requiring between 10 and 15 min for completion.

Reliability and Validity Developers Levenstein and colleagues [2] conducted a psychometric evaluation of the scale and found an internal consistency ranging from .90 to .92 and a test-retest reliability of .82. Results of the PSQ correlated

highly with trait anxiety and with scores on Cohen's Perceived Stress Scale.

Obtaining a Copy A copy can be found in the original article published by developers [2].

Direct correspondence to:
Cesare Balbo 43
00184 Rome, Italy

Scoring In order to complete the PSQ, respondents receive one of two sets of scoring instructions: the general questionnaire queries stressful feelings and experiences over the course of the previous year or two, while the recent questionnaire concerns stress during the last month. Respondents indicate on a scale from 1 ("almost never") to 4 ("usually") how frequently they experience certain stress-related feelings. Higher scores indicate greater levels of stress. A total score is found by tallying each item (questions 1, 7, 10, 13, 17, 21, 25, and 29 are positive and are scored according to the directions accompanying the scale). A PSQ index can be found by subtracting 30 from the raw score and dividing the result by 90, yielding a score between 0 and 1.

The Perceived Stress Questionnaire*Instructions for the General questionnaire*

For each sentence, circle the number that describes how often it applies to you in general, during the last year or two. Work quickly, without bothering to check your answers, and be careful to describe your life in the long run.

	Almost	Sometimes	Often	Usually
1. You feel rested	1	2	3	4
2. You feel that too many demands are being made on you	1	2	3	4
3. You are irritable or grouchy	1	2	3	4
4. You have too many things to do	1	2	3	4
5. You feel lonely or isolated	1	2	3	4
6. You find yourself in situations of conflict	1	2	3	4
7. You feel you're doing things you really like	1	2	3	4
8. You feel tired	1	2	3	4
9. You fear you may not manage to attain your goals	1	2	3	4
10. You feel calm	1	2	3	4
11. You have too many decisions to make	1	2	3	4
12. You feel frustrated	1	2	3	4
13. You are full of energy	1	2	3	4
14. You feel tense	1	2	3	4
15. Your problems seem to be piling up	1	2	3	4
16. You feel you're in a hurry	1	2	3	4
17. You feel safe and protected	1	2	3	4
18. You have many worries	1	2	3	4
19. You are under pressure from other people	1	2	3	4
20. You feel discouraged	1	2	3	4
21. You enjoy yourself	1	2	3	4
22. You are afraid for the future	1	2	3	4
23. You feel you're doing things because you have to not because you want to	1	2	3	4
24. You feel criticized or judged	1	2	3	4
25. You are lighthearted	1	2	3	4
26. You feel mentally exhausted	1	2	3	4
27. You have trouble relaxing	1	2	3	4
28. You feel loaded down with responsibility	1	2	3	4
29. You have enough time for yourself	1	2	3	4
30. You feel under pressure from deadlines	1	2	3	4

Instructions for the Recent questionnaire

For each sentence, circle the number that describes how often it applied to you during the last month.

Work quickly, without bothering to check your answers, and be careful to consider only the last month.

Score 5-circled number for items 1, 7, 10, 13, 17, 21, 25, 29

Score circled number for all other items

PSQ Index = (raw score-30)/90.

Reprinted from Levenstein et al. [2]. Copyright © 1993, with permission from Elsevier.

Note: The 8 items listed above are inverted, i.e., 4=1, 3=2, 2=3, and 1=4.

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Representative Studies Using Scale

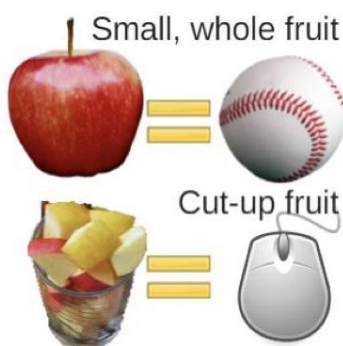
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Survey 2

The Short Healthy Eating Index Survey

Q1. On average, how many servings of **fruit** (not including juice) do eat **per day**?

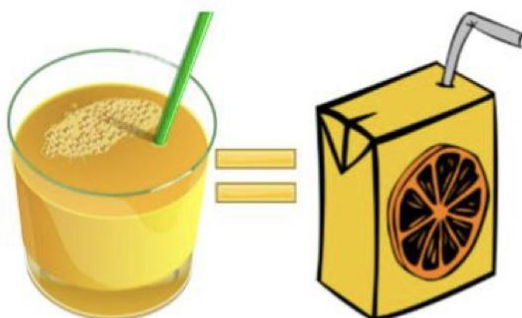
Example: 1 serving fruit = 1/2 cup cut-up fruit, 1/2 a banana, or one small piece of whole fruit (apple, orange, pear etc.) One small piece of whole fruit is the size of a baseball. 1/2 cup cut-up fruit is the size of a computer mouse.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q2. On average, how many servings of **100% fruit juice** do you drink **per day**? Note: **Do not include** fruit flavored drinks such as Hi-C, Tang, Sunny-D, etc.

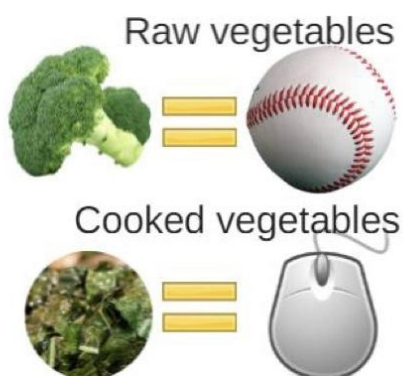
Example: 1 serving juice = 1/2 cup 100% fruit juice (apple, grape, orange, etc.), 1 cup of juice = juice box.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q3. Now, think about all the vegetables you eat in a day. On average, how many servings of **vegetables** do you eat **per day**? Note: Any vegetable or 100% vegetable juice counts as a member of the vegetable group.

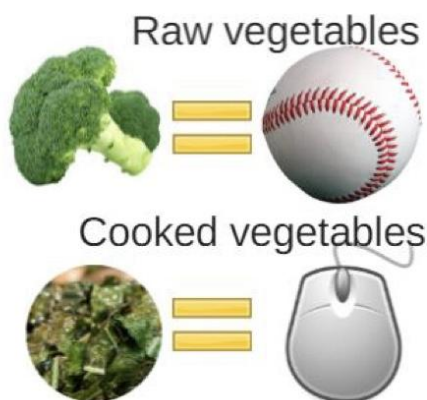
Example: 1 serving = 1 cup of raw vegetables, 1 cup of salad, 1/2 cup cooked vegetables, or 1/2 cup 100% vegetable juice. One cup of raw vegetables is the size of a baseball. 1/2 cup cooked vegetables is the size of a computer mouse.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q4. Now, think about just the green vegetables you eat in a day like spinach, green beans, kale, broccoli, zucchini, or other mostly green vegetables. On average, how many servings of **green vegetables** do you eat **per day**? NOTE: **Do not include** starchy vegetables like green peas.

Example: 1 serving=1 cup raw vegetables or ½ cup cooked vegetables. 1 cup raw vegetables is the size of a baseball. ½ cup cooked vegetables is the size of a computer mouse.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q5. Now, think about just the starchy vegetables you eat in a day like corn, green peas, or potatoes. On average, how many servings of **starchy vegetables** do you eat **per day**?

Examples: 1 serving= 1 cup raw vegetable or ½ cup cook vegetables. 1 cup raw vegetables is the size of a computer mouse.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q6. On average, how many servings of **grains** do you eat **per day**?

Examples: 1 serving= 1 slice of bread; ½ cup grits, 1 cup of ready-to-eat cereal, ½ cup oatmeal, 1 small tortilla, ½ cup cooked rice, or ½ cup pasta. 1 cup ready-to-eat cereal is the size of a baseball.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Please answer the next question ONLY if you selected “Less than 1” to the previous question.

Q7. On average, how often do you eat **grains**?

Examples: 1 serving = 1 slice of bread; ½ cup grits, 1 cup of ready-to-eat cereal, ½ cup oatmeal, 1 small tortilla, ½ cup cooked rice, or ½ cup pasta.

- ☐ A couple times per week
- ☐ A couple times per month
- ☐ A couple times per year
- ☐ Almost never
- ☐ Never
- ☐ Choose not to answer

Q8. Now, just think about whole grains you eat like whole wheat bread, whole grain crackers, brown rice, or oatmeal. On average, how many servings of **whole grains** do you eat **per day**?

Examples: 1 serving = 1 slice whole wheat bread, 5-6 whole grain crackers, 3 cups popcorn, ½ cup cooked brown rice, or ½ cup oatmeal.

- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Please answer the next question ONLY if you selected “Less than 1” to the previous question.

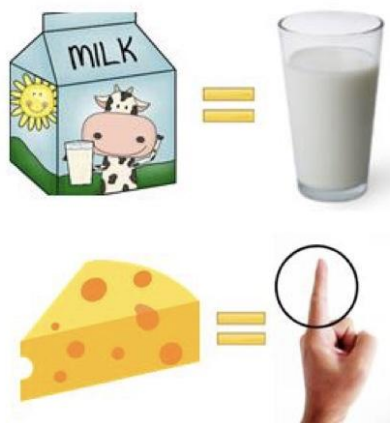
Q9. On average, how often do you eat whole grains?

Examples: 1 serving = 1 slice whole wheat bread, 5-6 whole grain crackers, 3 cups popcorn, ½ cup cooked brown rice, or ½ cup oatmeal.

- ☐ A couple times per week
- ☐ A couple times per month
- ☐ A couple times per year
- ☐ Almost never
- ☐ Never
- ☐ Choose not to answer

Q10. On average, how many servings of **milk** do you eat or drink **per day**?

Examples: 1 serving = 1 cup of milk, 1 cup of yogurt, 1.5 ounces of natural cheese, or 2 ounces of processed cheese. 1 cup of milk is the size of a carton of milk. 1 serving of cheese is the size of your index finger.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Please answer the next question ONLY if you selected “Less than 1” to the previous question.

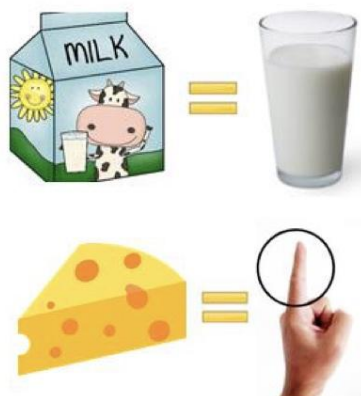
Q11. On average, how often do you drink or eat **milk** products?

Examples: 1 serving = 1 cup of milk, 1 cup of yogurt, 1.5 ounces of natural cheese, or 2 ounces of processed cheese.

- ☐ A couple times per week
- ☐ A couple times per month
- ☐ A couple times per year
- ☐ Almost never
- ☐ Never
- ☐ Choose not to answer

Q12. Now, just think about the milk products you eat per day. On average, how many servings of **low-fat milk** products do you eat **per day**?

Examples: 1 serving = 1 cup of skim milk, 1 cup of low-fat yogurt, or 1.5 ounces of low-fat cheese. 1 cup of milk is the size of a milk carton. 1 serving of cheese is the size of your index finger.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Please answer the next question ONLY if you selected “Less than 1” to the previous question.

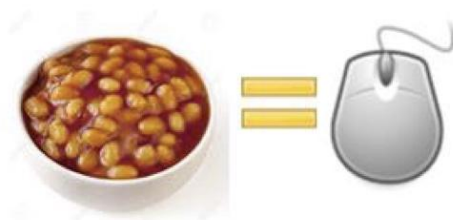
Q13. On average, how often do you drink or eat **low-fat milk** products?

Examples: 1 serving = 1 cup of skim milk, 1 cup of low-fat yogurt, or 1.5 ounces of low-fat cheese.

- ☐ A couple times per week
- ☐ A couple times per month
- ☐ A couple times per year
- ☐ Almost never
- ☐ Never
- ☐ Choose not to answer

Q14. On average, how many servings of **beans (legumes)** do you eat **per day**? Note: All foods made from dry beans, canned beans, peas, and lentils are considered part of this group.

Examples: 1 serving = $\frac{1}{2}$ cup cooked beans. $\frac{1}{2}$ cup cooked beans is the size of a computer mouse.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q15. On average, how many servings of **nuts or seeds** do you eat **per day**?

Examples: 1 serving = 1 tablespoon of peanut butter; $\frac{1}{2}$ ounces of nuts or seeds. 1 tablespoons of peanut butter is the size of the tip of your thumb.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Q16. On average, how many servings of **seafood** do you eat **per day**? Note: All foods made of fish, shrimp, crab, and shellfish are considered part of this group.

Examples: 1 serving = 3 ounces of fish. 3 ounces of fish is the size of a deck of cards.



- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Please answer the next question ONLY if you selected “Less than 1” to the previous question.

Q17. On average, how often do you eat **seafood**? Note: All foods made of fish, shrimp, crab, and shellfish are considered part of this group.

Examples: 1 serving= 3 ounce of fish.

- ☐ A couple times per week
- ☐ A couple times per month
- ☐ A couple times per year
- ☐ Almost never
- ☐ Never
- ☐ Choose not to answer

Q18. On average, how many **sugar-sweetened beverages** do you drink **per day**?

Examples: 12 ounces of soft drinks/soda, fruit flavored drinks, sweetened coffee, and sweet tea.

Do not include milk or 100% fruit juice. 12 ounces of soda is the size of one can.

- ☐ Less than 1
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more
- ☐ Choose not to answer

Please answer the next question ONLY if you selected “Less than 1” to the previous question.

Q19. On average, how often do you drink **sugar-sweetened beverages**?

Examples: 12 ounces of soft drinks/soda, fruit flavored drinks, sweetened coffee, and sweet tea.

Do not include milk or 100% fruit juice.

- ☐ A couple times per week
- ☐ A couple times per month
- ☐ A couple times per year
- ☐ Almost never
- ☐ Never
- ☐ Choose not to answer

Q20. On average, how much **added sugars** do you consume **per day**? Note: Added sugars are often in foods such as breads, cakes, candy, sweet tea, jam, ice cream, or sugar added to food at the table. **Do not include** naturally occurring sugars such as lactose in milk or fructose in fruits.

Examples: white sugar, brown sugar, raw sugar, corn syrup, corn-syrup solids, high-fructose corn syrup, malt syrup, maple syrup, pancake syrup, fructose sweetener, liquid fructose, honey, molasses, and dextrose.

- ☐ None/almost none
- ☐ Some
- ☐ A lot
- ☐ Choose not to answer

Q21. How many servings of **saturated fat** do you consume on average **per day**? Note: Saturated fats for these purposes should be considered to be solid fats. Solid fats are fats that are solid at room temperature.

Examples: butter, cakes, cookies, Crisco, coconut oil, beef fat (tallow, suet), chicken fat (lard), stick margarine, and shortening.

- ☐ None/almost none
- ☐ Some
- ☐ A lot
- ☐ Choose not to answer

Q22. On average, how much **water** do you drink **per day**?

- ☐ None/almost none
- ☐ Some
- ☐ A lot
- ☐ Choose not to answer

Q23. What is your gender?

- ☐ Female
- ☐ Male
- ☐ Choose not to answer

Short Healthy Eating Index Scoring Instructions

Prior to scoring the survey, please code each question response as indicated in the “Response Coding Rules” section of this document. The Short Healthy Index (sHEI) survey can provide an estimated total diet quality score and an estimation of food group consumption. These scoring rules are included in this document. Please note that code for R can be provided for both sets of scoring rules, upon request. Please email scolby1@utk.edu to request this code.

The following citation is recommended for the sHEI survey:

Colby S, Zhou W, Allison C, Mathews A, Olfert MD, Morrell J, Byrd-Bredbenner C, Greene G, Brown O, Kattelman K, Shelnutt K. Development and validation of the Short Healthy Eating Index (sHEI) survey with a college population to assess dietary quality and intake. *Nutrients*. 2020;12(9):2611.

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