CHANGES IN NLRP3 INFLAMMASOME AFTER MINDFULNESS TRAINING:  
AN ANCILLARY ANALYSIS OF DATA FROM THE SHINE  
RANDOMIZED CLINICAL TRIAL  

by  
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It is my hope that future research will result in the development of interventions to enhance the resilience and well-being of many who may benefit from mindfulness and other lifestyle enhancements.

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DEDICATION

I dedicate my dissertation to Elissa Epel, PhD, who has provided me with a most remarkable opportunity to be of service in such a meaningful way. There are innumerable and immeasurable ways that Dr. Epel has had such a profound impact on my RN, PhD path, including providing me with the opportunity to do an ancillary analysis of the SHINE randomized clinical trial, to facilitate genomic measurement of the very recently discovered NLRP3 inflammasome, and for her guidance as a committee member on my study. And most of all for the honor and pleasure of being acquainted with someone who has made such a profound difference in so very many lives, from bench to bedside, with such beauty and grace. I am truly grateful.
ABSTRACT

CHANGES IN NLRP3 INFLAMMASOME AFTER MINDFULNESS TRAINING:
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RANDOMIZED CLINICAL TRIAL

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This ancillary analysis of data from the SHINE randomized clinical trial investigated the relationships between perceived stress, trait mindfulness, and NLRP3 inflammasome activation.

Non-communicable diseases such as cardiovascular disease, type 2 diabetes, and some cancers are an emerging public health challenge. These diseases manifest in different ways physiologically, however, they have similar molecular response profiles from which they emerge. This includes the activation of the NLRP3 inflammasome, implicated in the inflammatory nature of psychological stress as a precursor non-communicable disease. This study investigated the change in the genomic expression of the NLRP3 inflammasome in subjects who received mindfulness training compared to those who have not and is significant as the first investigation to approach this question in this way.

Participants (n = 194) in the SHINE RCT were randomly assigned to a control or experimental group; the latter received mindfulness training around eating and stress
management. Data were collected from multiple physiologic and quantitative measures using instruments with previously established reliability and validity. Variables of interest in this study were stress, measured using the Perceived Stress Scale; trait mindfulness measured using the non-reactivity subscale of the Five Facet Mindfulness Questionnaire; and NLRP3 inflammasome measured in peripheral mononuclear blood cells samples collected at baseline and 6 months. Complete data on these three variables existed for 136 participants.

Data were analyzed with Pearson product moment correlations ($r$), $t$-tests and gain score analysis. There were no significant relationships among any of the study variables; computed effect size was 0. Analysis of the results revealed smaller than anticipated sample and effect sizes which likely influenced the findings rather than a lack of theoretical consistency.

This is an emerging area of inquiry in nursing research; further studies are warranted to understand the relationships between mindfulness and genomic variables at the molecular level.
LIST OF FIGURES

Figure 1.1. Diagram Model of Homeostasis.................................................................18
LIST OF TABLES

Table 3.1. Study Concepts with Definitions and Measurement........................................52
Table 4.1. Baseline characteristics of study participants (total sample, left panel); Group
differences across study variables at baseline (Control vs. Mindfulness, right panel)......65
Table 4.2. Bivariate correlations between inflammasome markers, total PSS score, and
FFMQ subscales at baseline ($n = 137$)........................................................................67
Table 4.3 Group comparisons in inflammasome marker change from baseline to 6-months
post-intervention...........................................................................................................68
# TABLE OF CONTENTS

ACKNOWLEDGMENTS ........................................................................................................ ii
DEDICATION ....................................................................................................................... iv
ABSTRACT ......................................................................................................................... vi
LIST OF FIGURES ............................................................................................................ viii
LIST OF TABLES ............................................................................................................... ix
CHAPTER ONE ................................................................................................................... 1
Introduction ....................................................................................................................... 1
Background and Significance ............................................................................................ 2

**Theoretical Framework: A Model of Homeostasis and Chronic Inflammation in Adults with Obesity** ................................................................. 5

Immune System ............................................................................................................... 6

Activation/Inhibition of Inflammasome ............................................................................. 10

Immune Dysfunction ...................................................................................................... 11

Visceral Adiposity ............................................................................................................ 12

Potential Treatment for IL-1β Associated Disease ......................................................... 15

Illustration of the Theoretical Framework .................................................................... 17

Purpose of the Study ........................................................................................................ 17

Research Questions and Hypotheses .............................................................................. 19

Hypotheses ...................................................................................................................... 19

Proposed Method ............................................................................................................. 20

Assumptions ..................................................................................................................... 21

Conclusion ....................................................................................................................... 21

CHAPTER TWO ................................................................................................................ 22
### Review of the Literature

#### Inflammation

- Inflammation Defined ................................................................. 22
- Inflammation and Its Relationship to Metabolism .............................. 23

#### The NLRP3 Inflammasome

- Definition and Function .................................................................. 25
- Pattern Recognition Receptors ...................................................... 25
- NLRP3 Inflammasome: Creation .................................................. 26
- Discovery of the NLRP3 Inflammasome ........................................ 28
- IL-1β: Considerations for Metabolic Disease and Associated NCDs .... 29
- NLRP3 Inflammasome-Related Pathologies ................................... 30

#### Mindfulness Meditation and Obesity Research

- Implications for Research .............................................................. 36

#### Conclusion

- CHAPTER THREE ........................................................................... 39

#### Methods and Procedures

- Identification of Research Design .................................................. 39
- Identification of the Sample .......................................................... 40
  - Sampling Criteria ....................................................................... 40
  - Sample Size ............................................................................... 42
- Description of the Setting .............................................................. 43
- Measurement Methods ................................................................. 44
  - NLRP3 Inflammasome ............................................................... 44
<table>
<thead>
<tr>
<th>Chapter Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Expression Analysis</td>
<td>44</td>
</tr>
<tr>
<td>Perceived Stress</td>
<td>46</td>
</tr>
<tr>
<td>Mindfulness</td>
<td>48</td>
</tr>
<tr>
<td>Data Collection Procedures</td>
<td>55</td>
</tr>
<tr>
<td>Randomization Procedure</td>
<td>55</td>
</tr>
<tr>
<td>Diet and Exercise Program</td>
<td>56</td>
</tr>
<tr>
<td>Ethical Considerations</td>
<td>58</td>
</tr>
<tr>
<td>Protection of Human Subjects</td>
<td>58</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>58</td>
</tr>
<tr>
<td>Potential Risks</td>
<td>58</td>
</tr>
<tr>
<td>Potential Benefits</td>
<td>59</td>
</tr>
<tr>
<td>Data Analyses</td>
<td>60</td>
</tr>
<tr>
<td>Assumptions and Data Cleaning</td>
<td>60</td>
</tr>
<tr>
<td>Description of the Sample</td>
<td>60</td>
</tr>
<tr>
<td>Primary Analyses</td>
<td>60</td>
</tr>
<tr>
<td>Assumptions</td>
<td>61</td>
</tr>
<tr>
<td>Delimitations</td>
<td>61</td>
</tr>
<tr>
<td>Conclusion</td>
<td>62</td>
</tr>
<tr>
<td>CHAPTER FOUR</td>
<td>64</td>
</tr>
<tr>
<td>Findings</td>
<td>64</td>
</tr>
<tr>
<td>Results</td>
<td>64</td>
</tr>
<tr>
<td>Description of the Sample</td>
<td>64</td>
</tr>
<tr>
<td>Primary Analyses</td>
<td>65</td>
</tr>
</tbody>
</table>
CHAPTER ONE

Introduction

This study was an ancillary analysis of data from the Supporting Health by Integrating Nutrition and Exercise (SHINE) randomized controlled clinical trial (RCT) by Hecht and Epel (2014; NCT00960414). Blood samples were obtained from subjects ($n = 194$) before and after a mindfulness-based weight loss intervention in adults with obesity. Specifically, in this study, changes in the NLRP3 inflammasome in peripheral blood mononuclear cell (PBMC) samples were measured. It was hypothesized that there would be a decrease in the NLRP3 inflammasome in the PBMC samples of the experimental group subjects who received the mindfulness training, compared to those in the control group who did not receive the training.

The SHINE RCT was funded and implemented from August 2009 through August 2013 (Hecht & Epel, 2014). Hecht and Epel (2014) compared two approaches to a diet and exercise-based weight loss program. Participants were 194 adults living in the San Francisco Bay Area with a body mass index (BMI) between 31-45 and a waist circumference of more than 120 cm (men) or 88 cm (women). Subjects were randomly assigned to a control or experimental group. Both groups received identical diet-exercise guidelines. Subjects in the experimental group received mindfulness training around eating and stress management practices (Daubenmier et al., 2016). The primary study outcome was 18-month weight change. Secondary outcome measures were fat distribution, insulin sensitivity, perceived stress, mood, stress hormones, autonomic nervous system function, adipocyte activity, and influenza vaccine response. Findings did not support mindfulness for weight loss as there was no significant difference between the intervention and control
groups. The researchers suggested that the intervention might promote long-term improvement in some aspects of metabolic health in obesity that requires further study (Daubenmier et al., 2016).

NLRP3 inflammasome was not measured as an outcome in the SHINE RCT (Hecht & Epel, 2014), thus this study was an ancillary analysis of the PBMC samples collected during the RCT. This study is relevant and unique, as it addresses an emerging health crisis, the incidence and prevalence of noncommunicable disease in the United States (Sagner et al., 2017).

**Background and Significance**

Of all the major health threats to emerge in the last ten years, the pervasive and unabated crisis of noninfectious, chronic non-communicable disease (NCD) has surpassed infectious disease as the greatest cause of death, challenging the very foundations of public health more profoundly than any other malady (Dietert, 2017; World Health Organization, 2017). Noncommunicable diseases have been on an epidemic increase for the past several decades, surpassing infectious diseases as the greatest cause of diminished quality of life and death, and exceeding the capacity of healthcare systems (Dietert, 2017). Unresolving or misregulated inflammation in tissues is the hallmark of virtually all NCDs. A diverse array of NCD manifestations arise in different physiological systems and organs including the microbiome-host defense barrier-immune complex, where dysfunction in one of these three biological units impacts gene expression and function in the other two (Dietert, 2017). Pathophysiological manifestations include allergic disease, cardiovascular disease, some cancers, respiratory disease, obesity, type-2-diabetes, non-alcoholic fatty liver
disease, and neurodegenerative disorders such as depression and Alzheimer’s disease (Sagner et al., 2017).

NCDs share a common cluster of environmental and lifestyle risk factors such as physical inactivity, standard American diet (SAD), chronic psychological stress, obesity, air and sound pollution, environmental endocrine disruptors, tobacco, and inadequate sleep (Allen, 2017; Arena, McNeil, Sagner, & Hills, 2017; Heindel et al., 2017; Sagner et al., 2017). In particular, obesity, considered one of the most important chronic diseases, has become a public health problem in the United States because of its high prevalence and its association with multiple comorbidities. The pathophysiology of obesity is complex due to several genetic, metabolic, endocrinologic, and environmental factors implicated in its development; however, an over-consumption of lipids and carbohydrates may explain most of the prevalence of this inflammatory state (Tapia et al., 2018). Inflammatory disease specifically associated with obesity is directly associated with visceral adiposity, which is highly inflammatory as compared to subcutaneous fat distribution (Foster, 2017). Along with these factors, socio-economic determinants, such as poverty, often associated with exposure to negative stressors and poor health behaviors that can begin in early life, compound the generation, severity, and management of NCDs (Acabchuk, Kamath, Salamone, & Johnson, 2017; Sagner et al., 2017).

NCDs occur via a dynamic cross-talk communication between two potent biological systems, the activated innate immune system, key for the fundamental integrity of an organism, and nutrient energy excess associated with a lack of metabolic homeostasis (Dietert, 2017; Emambokus, Granger, Mott, & Helenius, 2017). Associated with a lack of metabolic homeostasis specifically related to obesity-associated insulin resistance and
NCDs, this convergence is referred to as immunometabolism (McLaughlin, Ackerman, Shen, & Engleman, 2017; Saltiel & Olefsky, 2017). Immunometabolically-based NCD emergence is a 21st-century public health priority requiring a paradigm shift in thinking, away from consideration of a solely pathogenic immune response to one that is related to danger-associated sterile inflammation (Arena et al., 2015). A sterile innate immune response is a non-pathogenic, local, and systemic inflammation that occurs in response to tissue injury, trauma, or acute or chronic psychological stressor exposure in the absence of overt tissue damage (Boteanu et al., 2017; Carbone et al., 2016; Fleshner, Frank, & Maier, 2017).

Although these NCD pathologies appear in unique ways in different organs and are often studied and treated in isolation, they are intrinsically related and bear similar molecular profiles of immunometabolic dysregulation (Dietert, 2017; Frezza & Mauro, 2015; Liston & Masters, 2017; Logan & Jaka, 2014). This may include the activation of the Nod-like receptor pyrin containing 3 (NLRP3) inflammasome, a multiprotein signaling platform that is part of the innate immune response (Saltiel & Olefsky, 2017). The platform leads to the creation and activation of pro-inflammatory cytokines, Interleukin-1-beta (IL-1β) and IL-18, small innate immune proteins that signal further inflammation processes in cells surrounding them (Broz & Dixit, 2016).

The NLRP3 inflammasome, implicated in both immune and metabolic responses, is responsible for cellular processes related to numerous and diverse stimuli associated with both sterile and pathogenic inflammation (Abderrazak et al., 2015; Camell, Goldberg, & Dixit, 2015; Kaufmann et al., 2017). Within adipose tissue, NLRP3-dependent immunometabolic interactions specifically contribute to age-related inflammation and metabolic
dysfunction (Camell et al., 2017); therefore, proper maintenance of the NLRP3 inflammasome in immunometabolic homeostasis is crucial for health and well-being (Hotamisligil, 2017; Vigo, Thornicroft, & Atun, 2016).

Chronic psychological stress, which impairs optimal biological health through inflammatory mechanisms, has been shown to activate the NLRP3 inflammasome-mediated pro-inflammatory cytokine, IL-1β, and its response to energy metabolism (Acabchuk, Kamath, Salamone, & Johnson, 2017; Fleshner, Frank & Maier, 2017). Furthermore, this inflammatory effect is compounded with the synergistic combination of obesity and psychological stress (de Sousa Rodrigues et al., 2017). Exploring this relationship may facilitate new interventions to help mitigate this emerging NCD challenge (Hotamisligil, 2017; Reed & Raison, 2016).

One particular intervention that has been shown to dampen the inflammatory trajectory of chronic psychological stress is mindfulness (Black & Slavich, 2016; Fountain-Zaragoza & Prakash, 2017; Rosenkranz et al., 2016). Mindfulness, cultivated through evidence-based practices such as Mindfulness-based Stress Reduction, is defined as a state of being aware through the act of paying attention, on purpose, in the present moment, without judgment, and not getting caught and compounding suffering by judging the judging (Kabat-Zinn, 2017). Mindfulness interventions have been shown to be beneficial to both the psychological and physical health of adults who are overweight or obese (Daubenmier et al., 2016; Loucks et al., 2016; Rogers, Ferrari, Mosley, Lang, & Brennan, 2017). Daubenmier, Moran, and Kristeller (2016) suggested that mindfulness may promote long-term maintenance of weight loss through a focus on modifying health behaviors, rather than weight loss alone.
Functional genomic investigations into mind-body therapies such as mindfulness are beginning to clarify the role of these integrative modalities on human physiology (Niles, Mehta, Corrigan, Bhasin, & Denninger, 2014). With the potential to decrease the inflammatory nature of chronic psychological stress, mindfulness may be explored as an intervention to maintain or decrease the genomic expression of the NLRP3 inflammasome (Speaker & Fleshner, 2012).

The purpose of this study was to explore the relationship between mindfulness and the NLRP3 inflammasome. This study used PBMC blood samples obtained from subjects ($n = 194$) in the SHINE RCT (Hecht & Epel, 2014; Daubenmier et al., 2016), half of whom received mindfulness training around eating and stress management. To date, no study has investigated the change in the genetic expression of the NLRP3 inflammasome in subjects who have received mindfulness training compared to those who have not. This study was undertaken to address this gap in the literature.

**Theoretical Framework:**

**A Model of Homeostasis and Chronic Inflammation in Adults with Obesity**

The theoretical framework underlying this study includes many concepts; however, this study investigated only two: mindfulness and the NLRP3 inflammasome. The complete theory will be discussed to provide a context for understanding study that was undertaken.

Chronic psychological stress exposure increases the risk for poor clinical outcomes across a variety of major health conditions (Fleshner, Frank & Maier, 2017; Picard et al., 2015; Puterman et al., 2016), including the activation of a biological response that resembles inflammation caused by infection or trauma (Jope et al., 2016; Speaker & Fleshner, 2012). In conjunction with this, psychological stress-related inflammation is
frequently observed in obesity and related metabolic disease (Raghuraman, Donkin, Versteyhe, Barrès, & Simar, 2016). Scientists have only begun to develop genomic biomarkers that link the adverse effects of psychological stress with innate immune-related physiological disease pathogenesis as observed in obesity (Aschbacher et al., 2014; Slavich, 2016); therefore, it is timely to explore the evolutionarily conserved interactions between immune response and energy metabolism associated with chronic inflammatory disease, referred to as immunometabolism (Camell, Goldberg, & Dixit, 2015; Hotamisligil, 2017; O'Neill, Kishton, & Rathmell, 2016).

**Immune System**

**Homeostatic tissue function at the molecular level.** Homeostasis is the fundamental and conserved tendency of an organism to maintain stable equilibrium among its internal components while interacting with the external environment (Marks, 2015). Homeostasis may be viewed as a drive towards balance operating at the level of the entire organism, within tissue compartments, and within individual cells (Chovatiya & Medzhitov, 2014). Preserving stability is accomplished by maintaining key regulated variables within an acceptable range (Kotas & Medzhitov, 2015).

**Removal of danger without harm to host.** On all levels from systemic to tissue and cellular, the characteristic dynamic range of regulated biological variables is maintained by homeostatic control systems. When regulated variables change beyond the dynamic range, as a result of external insults, a stress response aims to restore homeostasis (Chovatiya & Medzhitov, 2014).

A major role of the innate immune system is to maintain this homeostatic tissue function. For example, when non-pathogenic sterile tissue damage occurs, the trauma
needs to be detected and repaired. Pathogens that invade and cause harm to tissues should be eliminated while commensal or friendly microbiomes must be tolerated, as they fulfill functions that are required for host survival (Latz, Xiao, & Stutz, 2013).

**Imbalance of homeostasis.** Protective innate inflammatory immune responses need to be temporally and spatially regulated to maintain homeostasis; otherwise, there will be sustained damage. Hence, broad or ongoing interferences targeting immune resolution may have unintended and adverse consequences for homeostasis and the metabolic health of the organism (Hotamisligil, 2017). This homeostatic imbalance can be seen with changes in human ecology over the past century, including over-nutrition, decreased physical activity, increasing population density, and lack of healthy microbial exposure. These lifestyle changes dramatically shifted the spectrum of human diseases from infectious to inflammatory in response to an interplay between genes, environment, culture and the microbiome (Dato et al., 2017; Kotas & Medzhitov, 2015). Genes that were selected to protect the organism from the challenges of starvation, infections, injury, and predation may now contribute to the increasing incidence of modern human diseases, including obesity, type 2 diabetes, atherosclerosis, autoimmunity, and certain psychiatric disorders (Gibbons, 2016; Raison & Miller, 2013). The two features common to these modern diseases are disruption of homeostasis and chronic inflammation (Kotas & Medzhitov, 2015).

To keep homeostasis in the organism, innate immune responses are elicited within the host against pathogenic microorganisms, harmful environmental factors, and host-derived stress or damage signals. These defenses depend on the tightly coordinated activation of a series of pattern recognition receptors (PRRs). The PRRs monitor the
extracellular and intracellular compartments of host cells for signs of infection or danger and activate a complex interplay of downstream inflammatory signaling pathways in response (Broz & Monack, 2013; Deswaerte, Ruwanpura, & Jenkins, 2017). The PRRs detect three different molecular patterns. The first are the pathogen-associated molecular patterns (PAMPs) arising from infectious pathogens such as microbes. The second, microbial-associated molecular patterns (MAMPs) are likely to be produced by enteric bacterial microbiota. The last, danger-associated molecular patterns (DAMPs) are endogenous host-derived signals that initiate a non-pathogenic or sterile inflammatory response (Deswaerte, Ruwanpura, & Jenkins, 2017; Fleshner, 2013).

Distinct from PRR inflammatory activation, Liston and Masters (2017) suggest that the nucleotide-binding oligomerization domain-containing (NOD)-like receptors (NLRs), including NLRP3, are a more sophisticated recognition system responding to homeostatic cellular balance rather than a simple and direct molecular pattern recognition of DAMPS and PAMPS. NLRs act as signal integrators capable of detecting perturbations, referred to as homeostasis-altering molecular processes (HAMPs), in cytoplasmic homeostasis. These HAMPs may detect evolutionarily novel infections that PRRs may not perceive; however, HAMP sensing may also underlie sterile inflammation that drives chronic inflammatory diseases (Liston & Masters, 2017). HAMP detection may escalate the risk of inappropriate inflammation, as even benign alterations of cellular homeostasis may theoretically degenerate into a proinflammatory state such as with chronic psychological stress (Davies, 2016; Liston & Masters, 2017).

Liston and Masters (2017) suggest that the simplest model for NLRP3 is as a signal integrator that detects any of a multitude of molecules and conditions that induce altered
homeostasis. NLRP3 initiates inflammatory signaling cascades through the NLRP3 inflammasome, an integral part of innate immunity. The NLRP3 inflammasome is a protein complex that enables maturation non-inflammatory pro-IL-1β and pro-IL-18 to the potent pro-inflammatory cytokines IL-1β and IL-18 (Hotamisligil, 2017; Latz, Xiao, & Stutz, 2013). These potent pro-inflammatory cytokines maintain tissue homeostasis in the short term; however, chronic NLRP3 inflammasome activation may drive uncontrolled inflammation with diverse pathological consequences of a wide spectrum of autoimmune and autoinflammatory disorders (Deswaerete, Ruwanpura, & Jenkins, 2017).

Pathogens. Invasion of a host by pathogenic infectious agents, such as bacteria, fungi, and microbes, triggers a battery of PAMP immune responses that are recognized by PRRs. These are expressed on innate immune cells such as dendritic cells, macrophages, and neutrophils (Deswaerete, Ruwanpura, & Jenkins, 2017; Kumar, Kawai, & Akira, 2011). The effective sensing of PAMPs rapidly induces host immune responses via the activation of complex signaling pathways, such as the NLRP3 inflammasome. These culminate in the induction of inflammatory responses (Kumar, Kawai, & Akira, 2011; Latz, Xiao, & Stutz, 2013). In the setting of infection, a successful immune action is potent but often short-lived, resulting in elimination of the pathogen followed by termination of the response. If the result is a failure, the organism perishes. In this evolutionary framework, there is no host survival advantage to ongoing inflammation or a low-grade response incapable of pathogen elimination (Hotamisligil, 2017).

Sterile inflammation stressors defined. Local non-pathogenic, DAMP-mediated sterile inflammation derived from self is a response to tissue injury and necrotic cell death (Fleshner, Frank, & Maier, 2017). Psychological stressor exposure in the absence of overt
tissue damage may also evoke a detectable local and systemic sterile inflammatory response through the NF-kB/NLRP3 pathway (Iwata et al., 2016). The NLRP3 inflammasome activation increases affective dysregulation and inflammatory proteins in tissues and blood in humans (Fleshner, Frank, & Maier, 2017). When prolonged, as with chronic psychological stress, sterile inflammation is found to play a role in several of the most burdensome and deadly diseases (Foster, 2017; Rider, Voronov, Dinarello, Apte & Cohen, 2017); therefore, sterile inflammation may be considered one of the most common pathways between psychological stress and disease (Slavich, 2016).

**Activation/Inhibition of Inflammasome**

**Definition of inflammation.** Inflammation, a host response defined by immune cells infiltrating affected tissues, has a central role in mediating host defense against pathogens, tissue repair, and restoration of homeostasis. Epithelial surface immune effectors and specialized stromal cells produce cytokines and antimicrobial defensins to orchestrate tissue repair and to minimize opportunistic infections, respectively (Rathinam & Chan, 2018). This protective response to defend and restore physiological functions can be thought of as the extreme conclusion of a distinct but overlapping spectrum that ranges from homeostasis to stress response to bona fide inflammatory response (Chovatiya & Medzhitov, 2014; Kotas & Medzhitov, 2015). The inflammatory response can only restore physiological functions by suppressing incompatible homeostatic controls. An ongoing situation may lead to homeostatic set point changes that are detrimental such as over-production of pro-inflammatory cytokines IL-1β and IL-18. This can be seen in chronic pathological states such as hyperglycemia leading to glucose toxicity and tissue damage (Kotas & Medzhitov, 2015). A vicious cycle may ensue that has the potential to become
locked in a state of chronic inflammation that fails to resolve. The non-resolving inflammation may, in turn, account for the persistence of chronic immunometabolic diseases (Kotas & Medzhitov, 2015).

**Immune Dysfunction**

The NLRP3 Inflammasome provides a pivotal platform for the host innate immune system to respond to exogenous infectious microbes such as viruses, bacteria, fungi; non-infectious environmental agents such as cigarette smoke and pollution; as well as endogenous danger signals of sterile inflammation (Deswaerte, Ruwanpura, & Jenkins, 2017). A key NLRP3 inflammasome effector function is to catalyze, via caspase-1, the maturation of the potent pro-inflammatory cytokines IL-1β and IL-18 (Próchnicki & Latz, 2017). Dysregulated expression and activation of the NLRP3 inflammasome is increasingly implicated in numerous peripheral chronic conditions including chronic autoinflammatory and autoimmune disorders, as well as central nervous system disorders, including Parkinson’s and Alzheimer’s diseases and depression (de Paula Martins et al., 2016; Deswaerte, Ruwanpura, & Jenkins, 2017; Guo, Callaway, & Ting, 2015; Saavedra, Demon, Van Gorp, & Lamkanfi, 2015).

**Autoinflammatory and autoimmune disease.** The NLRP3 inflammasome is the best characterized PRR associated with a multitude of autoinflammatory and autoimmune diseases. The initial relationship between the NLRP3 inflammasome and disease pathogenesis was reported just over a decade ago, with NLRP3 gene mutations associated with a group of rare autoinflammatory diseases. These pathologies are collectively referred to as Cryopyrin-Associated Periodic Syndromes (CAPS) and are associated with excessive IL-1β production as a causal factor (Deswaerte, Ruwanpura, & Jenkins, 2017). The
NLRP3 inflammasome has also been implicated as a factor in numerous autoimmune diseases such as systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease, comprising ulcerative colitis and Crohn’s disease (Deswaerte, Ruwanpura, & Jenkins, 2017; Freeman & Ting, 2016; Guo, Calloway, & Ting, 2015).

**Visceral Adiposity**

The homeostatic capacity of an organism determines its ability to adapt to varying environments. Homeostatic systems with fixed set points are inflexible but resistant to dysregulation. If their buffering capacity is overwhelmed, the consequences are likely to be catastrophic, acute, and transient, but rarely yield chronic disease. Comparatively, homeostatic systems with adjustable set points provide a greater degree of adaptability but are vulnerable to dysregulation and disease when the set points of the system are changed inappropriately, as often happens during chronic inflammation (Kotas & Medzhitov, 2015).

One such organ with a variable set point, visceral fat entails adipose tissue within the intra-abdominal cavity. As compared to lower body subcutaneous fat, located on the thigh, buttocks and lower stomach, visceral fat’s central adipose tissue distribution has a high pro-inflammatory capacity and is enhanced in response to a psychological stress-induced hypothalamic-pituitary-adrenal axis (HPA) activation and subsequent glucocorticoid receptor expression, furthering metabolic disease (Foster, 2017).

Unlike most other tissues, as visceral fat does not have a single set point value for size control as a function of body size, it is subject to homeostatic dysregulation. Adiposity, glucose, and lipid balance, are all characterized by adjustable set points as well, and vulnerable to homeostatic dysregulation (Kotas & Medzhitov, 2015).
Visceral adipose tissue is believed to be the first organ affected during the
development of obesity. The rapid expansion of adipose tissue occurs by increasing
adipocyte size and number to accommodate the excess of nutrients (Catrysse & van Loo,
2017). The swiftly enlarging adipose mass will experience endoplasmic reticulum stress,
oxidative stress, recruitment of inflammatory immune cells and NLRP3 inflammasome
activation. This sustained local and systemic inflammatory status along with adipocyte
production of adipokines, like leptin, adiponectin, and resistin, influence whole body
metabolism including insulin resistance and further development of obesity and its related
metabolic disorders (Catrysse, & van Loo, 2017).

**Metabolic disorders.** Nearly every process in human physiology relies on
homeostatic mechanisms for stability, but only some have demonstrated vulnerability to
dysregulation (Kotas & Medzhitov, 2015). Immunometabolic diseases such as obesity,
cardiovascular disease, hypertension, Alzheimer’s disease, and type 2 diabetes arise when
normal physiologic control goes awry and may thus be viewed as failures of homeostasis
(Kotas & Medzhitov, 2015). One of the distinguishing clinical phenotypes includes high
levels of inflammatory cytokine proteins (Bredesen, 2015; Camell, Goldberg, & Dixit,
2015; Dragsbæk et al., 2016). The convergence of lifestyle challenges of unmitigated
psychological stress and obesity, implicated in causing these diseases, has been attributed
to NLRP3 inflammasome-mediated IL-1β (Camell, Goldberg, & Dixit, 2015; Camell et al.,
2017).

In particular, obesity, a recently acquired human characteristic with an imbalance in
nutrient consumption and nutritional status, is accompanied by the critical role of innate
immune pathways in multiple immunometabolic processes (Cohen, Danzaki & MacIver,
2017; Henao-Mejia, Elinav, Thaiss, & Flavell, 2014). The evolutionary advantages of a powerful defense system are obvious in protecting against pathogens and a strong immune response is dependent on balanced energy sources; therefore, Hotamisligil (2017) suggests that the integration of these systems and their cooperation in responding to fluctuations in the energy and nutrient environment would be highly advantageous. From this perspective, immune mediators such as cytokines may be considered as metabolic hormones that serve both positive and negative functions, depending upon their inflammatory profiles and duration of inflammatory response (Hjorth & Febbraio, 2017; Hotamisligil, 2017).

An example of this, obesity-related excess energy will eventually overwhelm the storage capacity of the subcutaneous fat depots, spill over into visceral fat depots, and subsequently metabolic tissues such as skeletal muscle and liver (Foster, 2017; Speaker & Fleshner, 2012). Metabolic stress ensues with the activation of inflammatory signaling pathways and development of insulin resistance (Catrysse & van Loo, 2017). Glycolysis, saturated free fatty acids, cholesterol, or uric acids also serve as signaling molecules that promote the activation the NLRP3 inflammasome-mediated IL-1β as a critical sensor of nutrient overload in pancreatic islets, adipose tissues, blood vessels, or joints (Catrysse & van Loo, 2017; Pavillard, Marin-Aguilar, Bullon, & Cordero, 2018; Rathinam & Chan, 2018).

Dror and colleagues (2017) noted that insulin and IL-1β may promote each other after nutritional intake, which leads to increased glucose clearance and selective distribution of glucose to immune cells. This interaction may represent a mechanism for providing the immune system with enough energy to respond to pathogens, representing a link between metabolic homeostasis and healthy immune response (Dror et al., 2017);
however, Hotamisligil (2017) postulated that obesity may overwhelm mitochondrial activity, thereby promoting over-production of IL-1β induced immunometabolic disease.

While some obese patients show immunometabolic complications associated with their excess of body fat, other equally obese patients do not display expected metabolic abnormalities (Tcernof & Despres, 2013). Nearly one-third of obese individuals appear to be protected from acquiring further inflammatory disease, at least for a period (Medina-Urrutia et al., 2015). One factor that may help differentiate those who develop metabolic conditions is the level of psychological stress experienced (Foster, 2017; Lambert, Straznicky, Lambert, Dixon, & Schlaich, 2010; Peters & McEwen, 2015). The mechanistic link between stress, obesity, and metabolic disease has yet to be fully clarified (Raghuraman et al., 2016; Razzoli & Bartolomucci, 2016). A compelling theory by Speaker and Fleshner (2012) based on animal work, shows that psychological stress leads to non-pathogenic, sterile inflammation and visceral adiposity through the induction of the NLRP3 inflammasome.

**Potential Treatment for IL-1β Associated Disease**

The rising frequency of immunometabolic-related diseases makes them of utmost importance for development of novel therapeutic and preventative treatments. The NLRP3 inflammasome and its products, IL-1β and IL-18, are leading candidates for the development of novel therapeutics (Guo, Calloway, & Ting, 2013). These inflammatory by-products are generated in response to a diverse array of PAMPs, MAMPs, and DAMPs associated with immunometabolic disease, including chronic psychological stress (Fleshner, Frank, & Maier, 2017; Robbins, Wen, & Ting, 2014).
Improving health behaviors is fundamental to preventing and diminishing the adverse effects of the chronic diseases associated with the NLRP3 inflammasome. In regards to homeostasis, Taylor and colleagues (2010) have suggested that observed cortical, neuroendocrine, and molecular outcomes of regular mind-body practices such as mindfulness meditation practice may be connected through an executive homeostatic network that links calming mental processes to the maintenance of homeostasis in physiological systems; therefore, healthcare providers who utilize evidence-based mind-body behavioral change instruments, such as mindfulness meditation, may empower patients to engage in and sustain healthy behaviors (Sohl, Birdee, & Elam, 2016).

While the broad diversity of mind-body therapies such as mindfulness research designs presents a patchwork of results requiring further validation, clear immunomodulation themes are emerging, with effects on leukocyte transcription and function related to inflammatory and innate immune responses (Muehsam et al., 2017); therefore, mindfulness practices may be explored as anti-inflammatory interventions to decrease genomic expression of IL-1β, a known instigator of visceral obesity and metabolic disease (Black & Slavich, 2016; Rainone, et al., 2016). A further question to be asked is whether or not a mindfulness intervention is associated with decreasing stress-related eating (Mason et al., 2016a; Mason et al., 2016b) which would also help reduce the NLRP3 inflammasome, and thus immunometabolic disease.

Scientific understanding of how mindfulness affects health is only beginning to emerge, elucidating biological mechanisms that might link with metabolic disease (Prather et al., 2015). Psychological stress increases risk for a variety of immunometabolic diseases; however, individuals hold substantial power to reduce the mitigating effects of
stress and improve their personal and collective well-being via mindfulness and other mind-body practices (Slavich, 2016).

Illustration of the Theoretical Framework

The theoretical framework for the proposed study is illustrated in Figure 1. For this investigation, the relevant circle in the study is the one labeled “Activation/Inhibition of the Inflammasome.” This study sought to determine the change in gain scores of the NLRP3 inflammasome measured at baseline and 6 months later in subjects who received mindfulness training (experimental group) compared to those who did not. The larger illustration of the framework makes it explicit where the NLRP3 inflammasome sits in the model of homeostasis.

Purpose of the Study

The purpose of this ancillary analysis of data from the SHINE RCT (Hecht & Epel, 2014) was to determine if there is a change in NLRP3 inflammasome in PBMC samples from subjects who received mindfulness training compared to those who did not. This study is relevant as it addresses a potential genomic expression of psychological stress-related inflammatory disease, an emerging health challenge in the United States (Sagner et al., 2017).
Figure 1.1. Diagram Model of Homeostasis

Sources: Latz, Xiao, & Stutzenberg, 2013; Guo, Calloway, & Ting, 2015; Speaker & Fleshner, 2012
Research Questions and Hypotheses

1. Is there a relationship between perceived stress and the NLRP3 inflammasome?
2. Is there a relationship between trait mindfulness and the NLRP3 inflammasome?
3. What is the change in gain scores in the NLRP3 inflammasome, measured in PBMC blood samples, from baseline to six months post-intervention in subjects who received mindfulness training around eating and stress management practices (experimental group) compared to those who did not (control group)?

Hypotheses

$H_1$: There is a positive relationship between perceived stress and inflammation as measured by NLRP3 inflammasome levels.

$H_{01}$: There will be no relationship between perceived stress and inflammation as measured by NLRP3 inflammasome levels.

$H_2$: There will be a negative relationship between trait mindfulness and inflammation measured by NLRP3 inflammasome levels.

$H_{02}$: There will be no relationship between trait mindfulness and inflammation measured by NLRP3 inflammasome levels.

$H_3$: There will be decreased levels of NLRP3 inflammasome in PBMC samples drawn from subjects who received mindfulness training at six months from baseline, compared to NLRP3 inflammasome levels.

$H_{03}$: There will be no difference in the NLRP3 inflammasome levels at six months from baseline in the PBMC samples drawn from the experimental and control groups.
**Method**

The NLRP3 inflammasome was measured in pre-existing PBMC blood samples collected during the SHINE RCT (Hecht & Epel, 2014). Subjects were 194 men and women, aged 18 or older of any ethnicity, with a BMI of 31-45; waist circumference of 102 cm or more (men) or 88 cm or more (women). Potential subjects were excluded from participation if they had a diagnosis of type 1 or 2 diabetes mellitus; known history of coronary artery disease; untreated hypothyroidism; were pregnant or planning to be pregnant within a year; or were postpartum (less than 6 months) or breastfeeding. Additional exclusion criteria were a history or active condition of substance abuse, mental illness, or bulimia. Also excluded were those potential subjects who were currently on a weight loss diet or were taking medications, such as amphetamine-based salts, that could have an effect on their weight.

PBMC blood samples drawn at baseline were tested for NLRP3 inflammasome to ensure equivalence between the control and experimental groups by use of an independent samples $t$-test. The PBMC blood samples drawn six months from baseline were tested for NLRP3 inflammasome. To determine differences at six months post-baseline, gain scores were calculated for each subject by subtracting the post-test score from the pre-test score, with mean gain scores calculated for each of the two groups. Gain change analysis used an independent samples $t$-test to compare the two groups on the NLRP3 inflammasome measured 6 months from baseline (Huck, Cormier & Bounds, 1974). Level of significance was set at alpha = .05.
Assumptions

The following assumptions are relevant to this proposed study.

1. The PBMC blood samples that were used to measure the NLRP3 inflammasome were drawn in a sterile fashion, stored appropriately, and labeled correctly, clearly differentiating the sample as from a subject in the experimental or control group.

2. The NLRP3 inflammasome was present in the PBMC blood samples and had not degraded over time and therefore can be measured accurately.

Conclusion

This chapter identified the purpose and significance of this study and described the theoretical framework, with particular attention to the variables of this investigation. The research question and associated hypotheses were stated. Relevant assumptions were identified. The following chapter presents an in-depth review of literature related to the study purpose, variables, and relevant concepts. The synthesis of the literature provides a clear rationale for the ancillary analysis that was undertaken in this study.
CHAPTER TWO

Review of the Literature

This study was undertaken to determine if changes in NLRP3 inflammasome occurred in participants who received a mindfulness intervention compared to those assigned to an active control. NLRP3 inflammasome was derived from the blood samples of participants enrolled in a randomized controlled clinical trial (Daubenmier et al., 2016).

Chapter One described the current study’s significance and theoretical framework, relevant literature, identified three primary research questions and associated hypotheses, and stated assumptions. This chapter provides a critical review and synthesis of the literature that supports this research. Primary topics to be addressed include: inflammation and inflammatory markers, inflammasome-related metabolic disease, chronic stress, and mindfulness meditation.

Inflammation

Inflammation Defined

The first line of defense of the innate immune response, inflammation, is a protective response to extreme challenges to homeostasis, including infection, tissue stress, and injury (Colonna, 2017; Kotas & Medzhitov, 2015). Inflammatory signals such as cytokines and chemokines, biogenic amines, and eicosanoids, induce myriad changes in diverse biological processes. All of these changes, ranging from local vascular responses to alterations of body temperature, can be described in terms of their effects on homeostatic circuits (Kotas & Medzhitov, 2015).

While modernization has dramatically increased the lifespan, it has also been implicated in the increasing prevalence of non-pathogenic NCD such as obesity,
hypertension, and type 2 diabetes (Kotas & Medzhitov, 2015). Chronic inflammation, often the result of failures of homeostasis once normal physiologic control goes awry, is a commonly-cited accomplice of these chronic diseases (Kotas & Medzhitov, 2015; Reynaert, Gopal, Rutten, Wouters, & Schalkwijk, 2016).

**Inflammation and Its Relationship to Metabolism**

Life comprises a dynamic succession of homeostatic equilibria that entail physiological responses to ever-changing internal and external environmental exposures. In all of these physiological states, the immune and metabolic systems have co-evolved to communicate effectively with one another in order to maintain homeostasis (Zmora, Bashiardes, Levy, & Elinav, 2017). Both systems are vital for the organism. As energy management is required for every biological function, metabolism is an essential component of life. In addition, there has been a need for protection from both internal and external environmental insults, leading to the evolution of the immune system (Hotamisligil, 2017a). The convergence of these two systems, referred to as immunometabolism, forms a complex network of interactions to determine the host’s flexibility in response to shifting conditions in the environment (Zmora et al., 2017). The response is a coordinated pro- or anti-inflammatory action, depending on the situation, to ensure the organism's need for adaptation (Hotamisligil, 2017b; Zmora et al., 2017). Disruption of these interactions, leading to protective pro-inflammatory conditions, underlies the emergence of many pathologies, particularly NCDs (Hotamisligil, 2017a).

Acute inflammation, essential for repair, remodeling, and even renewal of tissues with critical metabolic function, needs to be temporally and spatially regulated to maintain homeostasis (Hotamisligil, 2017b). Inflammation that is more chronic in nature creates the
potential for broad, potent, or possibly permanent interferences targeting immune resolution or activation. Such interferences may have unintended and adverse consequences for metabolic tissue homeostasis and for the metabolic health of the organism overall (Hotamisligil, 2017b). Unmitigated inflammation is viewed as the driving factor in many diseases, including atherosclerosis, cancer, autoimmune disease, and infections, as well as a major contributor to age-related inflammatory processes (Hodes et al., 2016; Netea et al., 2017).

Inflammation is induced when host cells sense evolutionarily conserved structures on pathogens, referred to as pathogen-associated molecular patterns (PAMPS), or endogenous stress signals, known as danger-associated molecular patterns (DAMPS), through germline-encoded pattern-recognition receptors (PRRs), which sense perturbations in the cellular milieu (Netea et al., 2017). DAMP and PAMP cellular stimulation triggers inflammatory processes through the release of proinflammatory chemokines and cytokines such as the NLRP3 inflammasome-mediated IL-1β (Netea et al., 2017).

When inflammation occurs within the physiological parameters of a protective immune response, inflammation is essential for efficient immunity. Mechanisms that turn off the inflammatory response, referred to as resolution, have paramount importance in the return to homeostasis (Netea et al., 2017). Resolution is not simply the elimination of the stressing agent, but an active process involving functional reprogramming of cells through ad hoc production of mediators, including lipid mediator resolvins and anti-inflammatory cytokines, IL-10, IL-37 and TGF-β (Netea et al., 2017; Serhan, 2017).
The NLRP3 inflammasome

Definition and Function

One of the most important scientific discoveries in recent biomedical research is the role of immune-mediated inflammation in a multitude of chronic, nonpathogenic disease conditions related to substantial morbidity and early mortality (Serhan, 2017). A properly mounted acute, innate immune inflammatory response is indispensable for recognizing and eliminating danger arising from foreign invaders and tissue trauma (Afonina, Zhong, Karin, & Beyaert, 2017). Under normal conditions, acute inflammation is self-limiting such that following the elimination of a noxious stimuli, inflammation is attenuated with restoration of homeostasis and initiation of tissue repair (Afonina et al., 2017). When homeostasis falters, as seen in metabolic disease, the same innate immune inflammatory mechanism acts as a double-edged sword, paving the path for a broad array of chronic inflammatory pathologies including cancer, cardiovascular disease, autoimmune disease, and degenerative diseases (Patel, 2017). Understanding the mechanisms of this compromised immunometabolic pathway is a scientific and public health priority, as doing so will inform interventions to reduce related noncommunicable disease risk (Allen, 2017; Hotamisligil, 2017b; Slavich, 2015).

Pattern Recognition Receptors

Pattern recognition receptors (PRRs) provide the first line of defense in the innate immune system receptor-mediated surveillance system for pathogen or tissue damage (Patel et al., 2017). With infection, PRRs trigger inflammatory pathways to eradicate the pathogenic microbe and induce protective immunity. However, inflammation triggered by non-pathogen related sterile injury, such as seen with chronic psychological stress or tissue
damage, aims to limit the damage and facilitate tissue repair (Speaker & Fleshner, 2012). In both of these conditions, the duration and scale of the inflammatory response is a key determinant of outcome. The inflammatory response should be immediate and acute, with rapid recovery. There is no host survival advantage to chronic inflammation, which often occurs when the stimulus persists (Hotamisligil, 2017b).

PRRs are expressed in a variety of immune cells including macrophages, epithelial cells, dendritic cells, neutrophils, and adaptive immunity cells (Patel et al., 2017). Some PRRs, including toll-like receptors and C-type lectins, are activated directly on the cell surface via external PAMP pathological signals. PRRs are also triggered during sterile inflammatory diseases with a crucial role played by DAMPS as danger signals that host-derived rather than foreign (Patel et al., 2017). Both PAMPs and DAMPs may also trigger intracellular innate immune receptors directly, for example, via cytoplasmic recognition of DNA or RNA (Patel et al., 2017). Within cells, highly conserved cytosolic innate immune sensors such as NOD-like receptors (NLRs) perform critical functions in surveying the intracellular environment for the presence of infection, noxious substances, and metabolic perturbations to re-establish homeostasis (Patel et al., 2017; Zhong, Kinio, & Saleh, 2013).

**NLRP3 Inflammasome: Creation**

In response to harmful deviations from homeostasis, NLRs may assemble into a large macromolecular cytoplasmic protein scaffold referred to as an inflammasome (Patel et al., 2017; Prochnicki & Latz, 2017). The NLRP3 inflammasome is a molecular pathway activated by a wide range of cellular insults. This platform elicits innate immune defenses through the activation of caspase-1 and the maturation of an integral component of the innate immune system, IL-1β as well as IL-18 (Fusco et al., 2017; Parzych et al., 2017;
Patel et al., 2017). With certain stimuli, the NLRP3 inflammasome may also assist in the creation of pyrin, a highly inflammatory sensor for bacterial toxins such as botulinum toxin and Clostridium difficile, that inactivate RhoA, an enzyme on the inner cell membrane, facilitating cell death (Park, Wood, Kastner, & Chae, 2016).

The creation of the NLRP3 inflammasome arises in response to a variety of factors, including indirect sensing of PAMPS, such as bacterial products, mitochondrial DNA, or viruses, as well as DAMP factors including psychological stress, oxidized low-density lipoprotein, saturated fatty acids, amyloids, advanced glycation end-product, and cholesterol crystals, and endoplasmic reticulum stress (Patel et al., 2017; Speaker & Fleshner, 2012).

**NLRP3 inflammasome creation of IL-1β.** IL-1β, unlike the creation of other proinflammatory cytokines such as IL-6 and tumor necrosis factor-alpha (TNF-α), is not processed in the traditional manner through the Golgi apparatus. It is cleaved from its precursor molecule pro-IL-1β (Parzych et al., 2017). The creation and release of the mature IL-1β depends on two distinct regulated events. The first is the *de novo* induction of pro-IL-1β, a precursor to mature IL-1β. As IL-1β is a biologically inactive pro-protein, a cascade of epigenetic events is required to induce pro-IL-1β. The PRR family, Toll-like Receptors (TLRs), act as a priming transducer, linking to downstream signaling cascades in response to PAMPS and DAMPS (Lawlor & Vince, 2014). TLRs 2 and 4 are important regulators of metabolic inflammation, sensitive to free fatty acids as well as psychological stress (Frank, Watkins, & Maier, 2013; Jin & Flavell, 2013; Liu, Buisman-Pijlman, & Hutchinson, 2014). The TLRs ultimately activate a key proinflammatory transcription factor, NF-kB (Frank et al., 2013). This action is part of an essential ancestral-conserved
transcriptional response to adversity theme to upregulate activity of inflammatory transcription pathways with chronic stress as protection against imminent perceived threat to the body (Miller et al., 2014).

The first step, activation of pro-IL-1β, is generally via genomic factor, NF-kB-dependent transduction pathways (Frank et al., 2013; Parzych et al., 2017). The usual second step of the assembly and activation of the NLRP3 inflammasome, is generally reliant on active caspase-1, pannexin-1, and P2X7 receptor activation (Parzych et al., 2017). IL-1β release has several alternative pathways, such as when instigated by the TLR4 agonist, the highly inflammatory gut bacterial endotoxin, lipopolysaccharide (LPS), independent of both pannexin-1 and P2X7 activation (Gaidt et al., 2016; Parzych et al., 2017).

**Discovery of the NLRP3 Inflammasome**

Strong associations between dysregulated inflammasome activity and human heritable and acquired inflammatory diseases highlight the importance of exploring this pathway in tailoring immune responses (Schroder & Tschopp, 2010). In 2001, a rare autosomal recessive auto-inflammatory disease, Muckle–Wells Syndrome, was mapped to NLRP3. Martinon and colleagues (2002) were the first to characterize and biochemically define the inflammasome (Hoffman, Mueller, Broide, Wanderer, & Kolodner, 2001). In 2004, the discovery of the links between the NLRP3 mutations, NLRP3 inflammasome hyper-activation, and excessive production of IL-1β lead to the creation of IL-1 blockade strategies, including the recombinant IL-1 receptor antagonist, anakinra or anti-IL-1β antibodies, to cure patients inflicted with hereditary periodic fever syndromes (Hoffman & Wanderer, 2010). As more inflammasome-forming NLRs are being characterized and
studied, their importance in activating immune responses are becoming evident (Zhong et al., 2013). This emerging understanding may assist in driving down the ever-increasing presence of NCDs.

**IL-1β: Considerations for Metabolic Disease and Associated NCDs**

Improved characterizations of the immunological pathways are needed to explore effective therapeutics to treat IL-1β-based-metabolic syndrome (Garcia-Martinez, Shaker, & Mehal, 2015; Lukens & Kanneganti, 2014). Biomarkers in stress biology such as IL-1β hold great potential for assessing individual and population health, and for impacting NCDs (Iwata et al., 2016; McEwen, 2015).

In the exploration of molecular mechanisms of psychological stress-induced activation of the NLRP3 inflammasome, it is important to be aware of the vast range of stress exposures, ranging from acute to chronic and the differing stress responses (Alcocer-Gomez & Cordero, 2014). Further, it is important to consider the multiple neural determinants of resilience or vulnerability to stress, in addition to inflammatory profiles (Epel & Lithgow, 2014). For instance, circulating glucocorticoids, prior response to chronic social defeat, neurobiological substrates such as corticotropin-releasing factor, and neuropeptide Y may serve as important psychophysiological considerations when trying to understand mediators of the relationship between psychological stress and activation of the NLRP3 inflammasome (Wood & Bhatnagar, 2015).

Understanding the physiological and psychological mechanisms which may relate to metabolic health maintenance processes will aid in the design of successful health interventions (Kelder et al., 2015). More specifically, it will be important to better understand NLRP3 inflammasome IL-1β activation in different phenotype profiles (Esser,
Legrand-Poels, Piette, Scheen, & Paquot, 2014; Stienstra & Stefan, 2013). For example, Wood and colleagues (Wood et al., 2015) explored the association between gene expression and coping strategy in response to social stress in rats (e.g., active vs. passive coping). Passive coping was associated with proinflammatory processes, particularly IL-1β, whereas active coping and lower presence of stress-related pathology were associated with suppression of inflammatory processes (Wood et al., 2015). This research demonstrates the implications for the role of active coping via stress reduction in mitigating psychological stress implicated in inflammatory disease (Folkman, 2006).

**NLRP3 Inflammasome-Related Pathologies**

NLRP3 inflammasome activation is considered a major culprit of pathology and tissue damage involved in severe, life-threatening systemic infections, as well as in chronic inflammatory NCDs, including type 2 diabetes, cardiovascular and neurodegenerative diseases (Lamkanfi & Dixit, 2017). Activation of the NLRP3 inflammasome and subsequent secretion of IL-1β are critical determinants that precipitate inflammatory disease progression, (Buckner, Fan, Kim, Kim, & Chung, 2017). One example of this process is obesity.

Obesity, the second leading cause of preventable death, is a metabolic disorder where more energy is consumed than is expended, leading to excessive weight gain through the accumulation of adipose tissue (Avery et al., 2017). The disease state has risen in prevalence in the last 30 years and is implicated in the creation of other NCDs including cardiovascular disease, elevated peripheral inflammation, the development of type 2 diabetes as well as increased rates of depression, anxiety and reduced quality of life (Avery et al., 2017; Dunn et al., 2018). Obesity is a prime example of a chronic inflammatory state.
that primes the NLRP3 inflammasome, and contributes to inflammasome activation in sterile inflammatory disease (Patel et al., 2017). As obesity rates have reached epidemic proportions and significantly contributed to the growing prevalence of metabolic diseases, its association with NLRP3 inflammasome activation is particularly concerning (Ralston, Lyons, Kennedy, Kirwan, & Roche, 2017). Chronic adipose tissue inflammation associated with obesity, contributes to alterations in systemic glucose homeostasis (Hotamisligil, 2017a). In turn, obesity-associated inflammation, through immune cell infiltration into expanding adipose tissues, can lead to ectopic, metabolic complications in other tissues such as liver, skeletal muscle, and pancreas as well as in inflammatory signaling networks such as the NLRP3 inflammasome, a key regulator of metabolic inflammation. IL-1β, overproduced by leukocytes among individuals with obesity, is one of the most important biological mediators involved in adipose tissue inflammation and subsequent insulin resistance (Netea et al., 2017; Ralston et al., 2017; Rheinheimer, de Souza, Cardoso, Bauer, & Crispim, 2017). In a systematic review of this process, Rheinheimer and colleagues (Rheinheimer et al., 2017) found that nutrient excess generated DAMPS that subsequently activated NLRP3 inflammasome- maturation of IL-1β and IL-18. Contrary to the adverse impact of saturated fatty acids contributing to NLRP3 activation, monounsaturated fatty acids and polyunsaturated fatty acids have been shown to impede NLRP3 activity. Therefore, the NLRP3 inflammasome and associated metabolic inflammation have key roles in metabolic disease arising from obesity (Ralston et al., 2017).

In this context, mindfulness may be effective at limiting overconsumption of food and lack of physical activity (Loucks, Gilman et al., 2016). Mindfulness meditation
practices may serve as the missing piece of standard diet and exercise treatment by enhancing self-regulation and improving awareness of emotional and sensory cues that may be important for altering one’s relationship with food (Fung, Long, Hung, & Cheung, 2016). Thus, lack of mindfulness may be a novel determinant of obesity, as compared to being more mindful which may enhance self-regulation of consumption and cravings (Loucks et al., 2015). Individuals who exhibit greater mindfulness may be more resilient, more self-compassionate, more self-regulated, and better prepared to confront the challenges of weight management in an industrialized society with its overabundant availability of unhealthy food offerings and sedentary behavior (Dunn et al., 2018; Olson & Emery, 2015).

**Mindfulness Meditation and Obesity Research**

There is an urgent need for effective weight management techniques to address the more than one-third of United States adults who are obese or overweight (Dunn et al., 2018). There is strong support for inclusion of mindfulness, in the form of mindful eating as a component of weight management programs as a useful strategy to reduce food cravings, for portion control, and to decrease body mass index as well as body weight (Dunn et al., 2018). Mindful eating is thought to positively influence food consumption as practitioners become more aware of how their patient’s food choices and eating behavior impact their health; however, there is no standardized mindful eating behavior protocol (Fung et al., 2016). These interventions usually incorporate strategies that center on awareness of eating stimuli and regulating quantity of food intake through awareness of physiological needs.
The majority of the mindfulness meditation and obesity scientific literature has focused on weight management and eating disorders (Fung et al., 2016). Thus far, all interventions have incorporated mindfulness practices such as meditation, mindful eating, and understanding eating triggers; although, major differences exist among treatment protocols. Many interventions conducted among fairly homogenous populations of predominantly middle-aged, obese women have been variations of Mindfulness-Based Eating Awareness Training (MB-EAT) (Kristeller & Wolever, 2011), incorporating mindfulness practices to address eating-related processes, such as hunger triggers, food choice, and emotional regulation. Non MB-EAT studies used similar strategies that center in meditation. These included sensory input from foods such as taste, sight, smell, awareness of eating stimuli, and acceptance of one’s feelings.

Although mindfulness meditation is now becoming incorporated into more weight management programs, it remains unclear whether mindfulness actively influences weight loss (Olson & Emery, 2015). While many studies observed significant weight loss when mindfulness techniques were used, only a small number resulted in significantly more weight loss than the comparison group (Fung et al., 2016).

In a systematic review of 19 studies, including 13 randomized controlled trials and six observational studies, Olson and Emery (2015) reported significant weight loss across most of the studies; however, no solid evidence that changes in mindfulness were an active component of treatment when weight loss was observed. Olson and Emery (2015) suggested using more rigorous research designs such as a constructive research design, in which a control group receives all of the intervention components except mindfulness as compared to the treatment group, which receives all components of the intervention. In
this way, differential effects on outcome variables among mindfulness participants can be more readily attributed to the mindfulness variable (Olson & Emery, 2015).

O’Reilly and colleagues (2014) conducted a literature review to determine the effectiveness of mindfulness based interventions (MBIs) for treating obesity-related eating. The review consisted of 21 articles, including 38% randomized controlled trials and 62% pretest-posttest designs. In 86% of the reviewed studies, improvements in efficacy of MBIs for changing obesity-related eating behaviors such as binge eating, emotional eating and external eating were reported. O’Reilly and colleagues (2014) noted in the studies reviewed, mindfulness skills targeting the subject’s ability to cope with psychological distress in adaptive ways led to decreased binge eating, the most commonly reported problematic eating behavior among obese individuals.

Daubenmier and colleagues (2011) explored the effect of mindfulness on stress eating in a pilot study with 47 overweight/obese women (mean BMI = 31.2 kg/m²). The participants were randomly assigned to a 4-month intervention or waitlist group. Mindfulness, psychological distress, eating behavior, weight, cortisol awakening response (CAR), and abdominal fat (measured by dual-energy X-ray absorptiometry) were examined pre- and post-treatment. The treatment participants improved in mindfulness and had decreased anxiety and external-based eating compared to control group participants. Though the groups did not differ on weight or abdominal fat over time, obese treatment participants showed significant reductions in CAR and maintained their body weight as compared to the control group. There were significant improvements in mindfulness in the treatment group as measured by three Kentucky Inventory of Mindfulness Scale subscales: observe (0.17 [0.5]; d = 0.58), act aware (0.18 [0.5]; d = 0.56), and nonjudge (0.47 [0.7]; d
= 0.66). Improvements in these scores were associated with reductions in abdominal fat. This proof-of-concept study suggests that mindfulness training shows promise for improving eating patterns and decreasing CAR, which may reduce abdominal fat over time (Daubenmier et al., 2011).

One important aspect of mindfulness practice that may assist with obesity management is the cultivation of the personality trait of dispositional mindfulness, which is a process of attending to internal experiences with mindful awareness (Daubenmier, Hayden, Chang, & Epel, 2014). Dispositional mindfulness is the natural tendency to be aware of present-moment experiences in an accepting and non-judgmental manner (Tang, Holzel, & Posner, 2016). Both mindfulness practice and the trait of dispositional mindfulness have been associated with greater self-regulation and the ability to notice cravings without acting on them. Addressing emotions and physical sensations as passing events may assist people to tolerate cravings and overcome addictions, whether it is for cigarettes or consumption-related risks leading to obesity (Loucks, Britton, et al., 2016).

Loucks, Britton et al., (2016) explored the association between dispositional mindfulness, obesity, and central adiposity, using a prospective birth cohort of 394 obese subjects with a BMI of 30 kg/m² or greater. Participants were assessed for dispositional mindfulness using the Mindful Attention Awareness Scale (MAAS). Central adiposity was assessed using dual-energy X-ray absorptiometry scans to explore primary outcomes of android fat mass and android/gynoid ratio. Via multivariable-adjusted regression analyses, participants with low versus high MAAS scores were more likely to be obese (prevalence ratio for obesity = 1.00 (95% confidence limit (CL): 1.02, 1.77). This level was associated with a higher android fat mass and a greater android/gynoid fat mass ratio. The scores
were adjusted for age, gender, race/ethnicity, birth weight, childhood socioeconomic status, and childhood intelligence (Loucks, Britton et al., 2016). Participants who were not obese in childhood and became obese in adulthood \( (n = 154) \) had \(-0.21 \) (95 % CL \(-0.41, -0.01; p = 0.04)\) had lower MAAS scores than participants who were not obese in childhood or adulthood \( (n = 203) \). The authors concluded that dispositional mindfulness may be inversely associated with obesity and adiposity (Loucks, Britton et al., 2016). The authors suggested that replication studies are needed to adequately establish whether low dispositional mindfulness is a risk factor for obesity and adiposity.

Mind-body intervention approaches to obesity may prove effective in the long-term as they promote increasing awareness and long-term changes in automatic and habitual patterns of response (Fulwiler et al., 2015). Research findings need to be replicated by prospective studies to establish causality and to evaluate potential implications for mindfulness-based interventions to reduce risk of obesity, metabolic syndrome, and the incidence of NCDs such as type 2 diabetes (Loucks, Gilman, et al., 2016).

**Implications for Research**

O’Reilly and colleagues (2014) appreciated that MBIs were promising for the treatment of eating behaviors related to weight gain and obesity; however, they noted that this nascent field required future research to fill several gaps. There are various ways to operationalize mindfulness and numerous approaches have been used to measure it as a construct. Clarity is needed to establish reliable and consistent methods for measuring mindfulness and to empirically determine the degree to which increased mindfulness is a mechanism leading to weight loss (Olson & Emery, 2015).
Loucks, Britton et al. (2016) suggested that a major limitation of many of the published studies may be that MBIs were usually 8 weeks in duration; longer or higher dose mindfulness interventions may be needed for substantial weight loss (Loucks, Gilman, et al., 2016). Assessing longer-term effects of MBIs with follow-up periods of 6 months or more would help clarify maintenance effects of treatment as skills may continue to enhance weight loss efforts over time and have an impact on long-term weight management (Dunn et al., 2018).

O’Reilly and colleagues (2014) asserted that future studies should routinely measure changes in mindfulness to determine whether improved mindfulness is the mechanism for improved eating behavior outcomes. Studies comparing MBIs to other treatments may illuminate the comparative efficacy of mindfulness training to other approaches for treatment of obesity-related eating behaviors (O’Reilly et al., 2014).

Daubenmier and colleagues (2016) suggested that participant engagement is important. Specifically addressing whether the mindfulness aspect of an intervention impacted participants is important for treatment fidelity. Individuals with obesity who do not express interest in mindfulness approaches may respond less favorably to the inclusion of mindfulness training in weight loss programs.

Additional features of treatment fidelity such as provider training and treatment implementation are important areas for future study (Daubenmier et al., 2011; Olson & Emery, 2015). In a mindfulness/obesity study with a cohort of 194 adults, Daubenmier and colleagues (2016) discovered that efficacy of mindfulness training for weight loss may have been instructor-dependent. Groups led by mindfulness instructors rated as more helpful lost an estimated 4 kg more at 18 months compared to groups led by an instructor
rated as less helpful and 5 kg more than contemporaneous control groups. These findings suggest that greater weight loss benefit may be achieved with more effective instructors (Daubenmier et al., 2016).

White adult females are the most predominant cohort group in MBI interventions thus far (O’Reilly et al., 2014). It is critically important for future studies to include males as well as socioeconomically disadvantaged populations given that obesity rates are higher and existing weight loss interventions may be less effective in these groups (Fulwiler et al., 2015).

Dunn and colleagues (2018) suggest that future studies also address mindful eating as it relates to healthy eating as this combination was found to be more effective than mindfulness alone in a study by Mantzios and Wilson (2015). Addressing individuals’ beliefs regarding healthy eating behaviors in relation to mindfulness may facilitate identifying gaps and misconceptions regarding whether these behaviors result in any health benefits and may enhance outcomes (Vizireanu & Hruschka, 2018)

Conclusion

Optimal health is maintained by the interaction of multiple intrinsic and environmental factors at different levels of complexity—from molecular, to physiological, to social. Chronic stress alters reactivity to future stressors as gene expression patterns after recovery from stress do not reflect a return to the stress naive baseline (McEwen, Gray, & Nasca, 2015). Defining cellular and molecular mechanisms such as the NLRP3 inflammasome, underpinning the detrimental effects of chronic stress on metabolic health will assist in establishing the basis of mind–body interactions such as mindfulness meditation, expand the biomedical model, and hopefully lead to the design of higher-level
health-promoting interventions for decreasing NCDs (Hotamisligil, 2017a; Picard, Juster, & McEwen, 2014; Speaker & Fleshner, 2012).

A better understanding of how chronic stressors, inflammation, diet, and interventions such as mindfulness, work together to alter metabolic processes would benefit behavioral and nutritional research as well as the broader biomedical community (Kiecolt-Glaser et al., 2015; Mason et al., 2016a; Mason et al., 2016b). The ability to harness control of the interdependent immune and metabolic milieu, including the NLRP3 inflammasome, could serve to break the pro-inflammatory cycle that may be present in the obese and general population as well (Iwata et al., 2016). New psychosocial, nutritional, and pharmacologic strategies may arise from this understanding that can be used to improve human health (Johnson & Makowski, 2015; Slavich, 2015; Slavich & Irwin, 2014).

Although stressful experiences are often expressed as dysfunctions and disease, they may also serve as catalysts for positive adaptations such as incorporating mindfulness practices that protect against future trauma. Determining how to take advantage of this knowledge to make the most of adversity is the challenge (Valentino, Sheline, & McEwen, 2015).
CHAPTER THREE

Methods and Procedures

This chapter introduces the methods utilized for the current study, an ancillary analysis of an intervention study in which adults with obesity were randomized to a mindfulness plus diet-exercise program (intervention) or a diet-exercise only program (active control). The following outcome measures of interest for the current study were collected pre- and post-intervention: NLRP3 inflammasome from peripheral blood mononuclear cells (PBMC) to measure immunological inflammatory response; the Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983) to measure perception of stress; and the Five Factor Mindfulness Questionnaire (Baer, Smith, Hopkins, Krietemeyer, & Toney, 2006) to assess mindfulness.

Identification of Research Design

As randomized clinical trials (RCT) become more common in social science and healthcare research, new opportunities exist for the use of previously collected data to study new research questions. Thus, ancillary analyses of data from a completed RCT allow for the study of new variables and outcomes that were not part of the original research question(s). Ancillary analyses of previously collected data from subjects assigned to a treatment versus a control group continue to rely on the initial randomization to identify its effects on new outcomes. The method requires pairing randomized treatments the researchers did not oversee with outcomes that were not the focus of the original RCT. In this way, ancillary analyses provide many of the advantages of randomized interventions, but at lower cost, since the RCT has already been implemented (Baldwin & Bhavnani, 2015).
It is important to distinguish ancillary analyses from secondary analyses. The latter is defined by the examination of research questions from an existing dataset which were proposed in the original RCT, but not analyzed in the primary study (Castle, 2003; Dunn, Arslanian-Engoren, DeKoekkoek, Jadack, & Scott, 2015). By contrast, in ancillary analyses, a new research question is examined which was not part of the original research proposal (Baldwin & Bhavnani, 2015).

The data set used for the current study, the Supporting Health by Integrating Nutrition and Exercise (SHINE) trial (NCT00960414) included a list of proposed research questions involving eight outcome variables. The primary outcome variable was change in weight and other metabolic health indicators following randomization. Some of these variables have not yet been examined in primary study analyses and are addressed in studies by Daubenmier and colleagues (2016) as well as Mason and colleagues (2016a; 2016b). If one of these unreported research questions were chosen for the current study, this study would be classified as a secondary analysis. Rather, the current study examined changes in the NLRP3 inflammasome among adults with obesity in the SHINE trial who were randomized to a mindfulness plus diet-exercise program (intervention) or a diet-exercise only program (active control), which was not a part of the original research question. Thus, this research study represents an ancillary analysis.

Identification of the Sample

Sampling Criteria

The targeted study population included 194 non-diabetic men and women, 18 or more years of age, with a Body Mass Index (BMI) of 30-45.9 kg/m² who were enrolled in the SHINE RCT (NCT00960414; Daubenmier et al., 2016). They were randomized 1:1 to
a 5.5-month program with or without mindfulness training and identical diet-exercise guidelines. Missing data were addressed with the use of intention-to-treat analyses with multiple imputation. Participants were assessed at baseline, 3, 6, 12, and 18 months from intervention initiation for the original study. The primary outcome of the original study aims included 18-month weight change. The University of California, San Francisco (UCSF) Committee on Human Research approved the study and participants provided informed consent. Participants were compensated for their time during all assessment visits and the intervention was provided free of charge.

Other eligibility criteria included presence of abdominal obesity, defined as waist circumference greater than 102 cm in men or greater than 88 cm in women. Potential participants were excluded if they had known history of coronary artery disease; untreated hypothyroidism; were pregnant or planning to be pregnant within a year; or were postpartum (less than 6 months) or breastfeeding. Additional exclusion criteria included a history or active condition of substance abuse, mental illness, or bulimia nervosa. Also excluded were those potential participants who were currently participating in a weight loss program and/or a mindfulness-based stress reduction (MBSR) course, or were taking medications, such as amphetamine-based salts, that could have an effect on their weight.

This study was advertised as comparative weight loss intervention involving lifestyle changes in diet, exercise, and stress management. Recruitment efforts were targeted toward adults in the San Francisco Bay Area using fliers, newspaper advertisements, online postings, and referrals at UCSF medical clinics. Participants were enrolled in six cohorts beginning in July of 2009 and ending in February of 2012, with final assessments completed in October of 2013.
Sample Size

Sample size calculations and overall study enrollment for the main outcome study were based on a sample size of 200. In order to ensure this sample size was achieved and to account for an expected attrition of 15%, 230 participants were enrolled. The sample size was estimated to have sufficient power to detect small to medium relationships between intervention arms (mindfulness vs. active control) and metabolic outcomes at 18-month follow-up (e.g., trunk fat, weight). With regard to one of the primary outcome measures, change in trunk fat, it was estimated that total trunk fat would be approximately 9 kg, with a standard deviation (SD) of 2.0 kg across all participants at baseline (Kelley, Thaete, Troost, Huwe, & Goodpaster, 2000). It was also anticipated that the standard deviation of within person changes would be substantially less than that of between person differences at one time point, thus it was anticipated that there would be an SD of 1 kg or less. With 200 participants, the main outcome study had approximately 80% power to detect a statistically significant ($p < 0.05$) difference in the mean change in trunk fat between groups if the true difference in the mean change in trunk fat between groups was 0.4 kg or greater. With regard to the other primary outcome of weight loss, a sample size of 200 (100 participants in each intervention arm) was estimated to have greater than 85% power to detect a statistically significant ($p < 0.05$) difference in weight change if the true difference between the intervention groups was 1.1 kg or greater with the same SD’s as observed in a pilot study. In the pilot study, obese participants in the mindfulness group lost 0.3 kg compared to a gain of 1.7 kg in the control group.

With regard to the current study’s proposed analyses, and due to a lack of studies that have examined a similar research question, it was determined that the current sample
size of 194 participants was estimated to have sufficient power to detect small relationships between intervention arm (mindfulness vs. active control) and change in NLRP3 inflammasome from baseline to 6-months post-intervention. Specifically, with \( n = 194 \), and with power set to 80\% to detect a statistically significant difference \((p < 0.05)\), the detectable mean difference between groups with regard to the NLRP3 inflammasome was 0.40.

**Description of the Setting**

All aspects of the SHINE RCT (NCT00960414) were conducted at the University of California, San Francisco (UCSF) within the Osher Center for Integrative Medicine (OCIM). UCSF is one of the leading academic medical centers in the nation. The Osher Center is a campus-wide multidisciplinary program established in 1997 with support from the School of Medicine, and an $8 million endowment from the Bernard Osher Foundation. The missions of the Osher Center are: 1) to scientifically research complementary and alternative approaches to health care; 2) to bring to the patient approaches of proven value; 3) to bring to students and practitioners in the health professions a new paradigm of relationship centered medicine; and 4) to disseminate information on the efficacy of integrative medicine to the public and the medical community. The research faculty of the Osher Center represent anthropology, psychology, epidemiology, nursing, and medicine. PBMC samples collected at UCSF were subsequently sent to the University of California, Los Angeles (UCLA), for analysis within the Social Genomics Core Laboratory of Steven Cole, PhD, Professor of Medicine and Psychiatry and Biobehavioral Sciences.
Measurement Methods

NLRP3 Inflammasome

Using existing blood samples to analyze supplemental information of the NLRP3 inflammasome, blood samples were obtained from subjects \((n = 194)\) before and 6-months after a mindfulness-based weight loss intervention in adults with obesity. Specifically, in this study, changes in the NLRP3 inflammasome in peripheral blood mononuclear cell (PBMC) samples will be measured from baseline to 6-months post-intervention. At least 40 mL of peripheral blood was obtained by venipuncture following 15 minutes of seated rest while participants fasted, at the baseline and 6-month follow-up visits, using 10 ml sodium heparin Vacutainers (Becton–Dickinson, San Jose CA). All subsequent gene expression arrays for NLRP3 inflammasome were conducted at the University of California, Los Angeles (UCLA) Neuroscience Genomics Core under the direction of Dr. Steven Cole.

Gene Expression Analysis

Social environments are able to influence human gene expression via physicochemical stimuli and psychological processes such as perceived threat and uncertainty. This triggers neural and endocrine responses such as activation of the sympathetic nervous system. In both situations, biochemical mediators engage cellular receptor systems that activate intra-cellular signal transduction pathways (Cole, 2017). This action culminates in the activation or repression of protein transcription factors such as NF-κB that proximally regulate the transcription of genes bearing response elements for that particular factor, including the NLRP3 inflammasome. Although this action is
presumably adaptive under ancestral conditions, it may have distinct and maladaptive effects in the qualitatively different conditions of contemporary human life (Cole, 2017).

Expression of immune response genes will be evaluated, specifically the activity of the gene for the NLRP3 inflammasome, in total PBMC. Genome-wide transcriptional profiling will be conducted on quality-assured cell samples using Illumina HT-12 bead arrays. Dr. Cole’s laboratory developed the Transcription Element Listening System (TELiS) to identify intracellular transcription control pathways that mediate large-scale alterations in gene expression based on the differential representation of transcription factor binding motifs in the promoters of differentially expressed genes. This approach accurately detects experimental activation of hormone response pathways, including glucocorticoids (Cole et al., 2005). It has been used to identify transcription control pathways that drive global alterations in leukocyte gene expression as a function of biobehavioral risk factors. For example, it was used with pro-inflammatory NF-κB signaling and glucocorticoid resistance linked to chronic social isolation (Cole et al., 2007) as well as with pro-inflammatory MAPK and NF-κB signaling in relationship to CREB activation during experimental sleep deprivation (Irwin, Wang, Campomayor, Collado-Hidalgo, & Cole, 2006). Affymetrix U133A high-density oligonucleotide arrays will be used to assay genome-wide expression profiles, as previously described (Cole et al., 2007; Cole et al., 2005). Briefly, total RNA will be extracted from \(10^7\) ficoll-separated PBMC samples (Qiagen RNeasy), checked for purity and integrity (Agilent 2100 bioanalyzer), and subject to probe synthesis and array hybridization in the UCLA DNA Microarray Core.

For the pre-specified target NLRP3 inflammasome gene, 30 gene transcripts identified as differentially expressed in microarray analyses will be confirmed by real-time
RT-PCR analyses carried out in triplicate and normalized to beta-actin mRNA (Applied Biosystems TaqMan Gene Expression Assays), as previously described (Cole et al., 2007). Bioinformatic assessment of transcription control pathway activity will be carried out using the TELiS bioinformatics tool as previously described (Cole et al., 2005).

NLRP3 inflammasome gene expression measures reflect relative RNA abundance. They are fluorescence-intensity measures for microarray hybridization; therefore, they have no absolute intrinsic metric measure regarding RNA molecules. Rather, the values are relative continuous metric, ranging from 1-100,000-fold above lower limit of detection of NLRP3 inflammasome RNA (Cole, 2017; see Table 1).

The coefficient of variance is used to predict the level of error with laboratory assays. The inter-assay coefficient of variance for microarray gene abundance determinations is anticipated to fall below 5% and average around 1% (Mehl, Raison, Pace, Arevalo, & Cole, 2017).

**Perceived Stress**

Perceived stress was measured using the Perceived Stress Scale (PSS) developed by Cohen and colleagues in 1983 (see Appendix A). In the SHINE RCT (NCT00960414), subjects completed the PSS multiple times; for this study data from completion at baseline and at 6 months post-intervention will be used for analysis.

Stress is not a unitary construct, but rather comprises exposure to stressors, perception of stress, and the physiological stress response (Mathur et al., 2016). Mathur and colleagues (2016) note that with a high incidence of reported stress as well as the complex interplay between life events, perceptions of their importance through the
construct of perceived stress, and the development of disease, there is a need for further research exploring relationships between fundamental cellular physiology and psychology.

With respect to obesity, Groez and colleagues (2012) found that greater reported stress, both exposure and perception, was associated with indices of a greater drive to eat, suggesting that stress exposure may contribute to non-homeostatic eating behaviors, which may in turn promote excessive weight gain. Mason and colleagues (2016a; 2016b) noted that psychological stress, paired with reward-based eating were factors that may compromise weight loss; furthermore, when weight loss does occur due to a diet, psychological stress neurocircuitries overlapping those of eating behavior, and energy homeostasis, may lead to an increase risk for weight regain (Mason et al., 2016a; Mason et al., 2016b). Exploring the relationships between stresses and eating behavior are of importance to public health given the ongoing increase in reported stress and obesity rates (Groesz et al., 2012).

One commonly used measurement to assess perceived psychological stress is Cohen’s Perceived Stress Scale (PSS), a standard 10-item questionnaire that assesses individual's subjective perceptions of stress over the previous month. The scale has been normed in several large national studies, between the years of 2006 and 2009 among women; the mean total PSS score was determined to be 16 (Cohen & Janicki-Deverts, 2012). Items from the PSS reflect uncontrollability, unpredictability, and the feeling that demands outweigh one’s coping resources. Response options are in the form of a 5-point Likert scale ranging from 0 = never to 4 = very often. Higher scores reflect higher levels of perceived psychological stress. Cronbach's alpha measurement was 0.91 in two large samples from 2006 and 2009 (Cohen & Janicki-Deverts, 2012). In a study by Mason and
colleagues (2016b), utilizing data from the SHINE RCT (NCT00960414), at baseline, PSS internal consistency was good ($\alpha = 0.86$), and the mean score was 14.41 (SD= 5.76, range: 1 to 30).

Addressing threats to construct validity, Cohen (1986) found that the scale principally assessed what it was designed to measure, the cognitive evaluation of stress (i.e., appraisal). The instrument was originally defined as a single construct, with a distinction between the two different dimensions of positively and negatively scored items. A one factor model with all the items as indicators and a two-factor model with items corresponding to the positive and negative factors were fitted to the covariance matrix of the corresponding PSS items (Andreou et al., 2011). Andreou and colleagues (2011) further confirmed by confirmatory factor analysis (CFA) that the one-dimensional model did not provide acceptable fit, while the two-dimensional model tended to show a better fit both in the PSS-10. Cronbach’s alphas, correlations, $t$-tests, Mann-Whitney, Kruskal-Wallis tests as appropriate were computed using IBM SPSS Statistics version 16 (IBM Inc., Armonk, NY, USA). All statistical tests were two-tailed and results were considered significant at $p < 0.05$ (Andreou et al., 2011).

**Mindfulness**

Mindfulness was assessed by the Five Facet Mindfulness Questionnaire (FFMQ) which was created by Bauer and colleagues (2006; See Appendix B). In the SHINE RCT (NCT00960414), subjects completed the FFMQ multiple times; for this study data from completion at baseline and at 6 months post-intervention were used for analysis. Further for this study, the Non-Reactivity Scale was used for the mindfulness score.
Mindfulness is emerging as an ever-more important contributor to health and well-being, although its accurate assessment represents an ongoing challenge (Medvedev, Siegert, Kersten, & Krägeloh, 2017). Mindfulness as a form of stress management has been shown to be effective in reducing symptoms related to stress as well as improving quality of life; however, mindfulness' precise biological mechanisms, including markers of inflammation, are only now beginning to be explored (Bower & Irwin, 2016; Pascoe, Thompson, Jenkins, & Ski, 2017). Given the frequency with which people are choosing meditation as a form of self-management and stress-reduction, it is important to validate if the practice is effective in mediating physiological stress-reactivity using well-controlled studies (Pascoe et al., 2017). Mindfulness-based interventions may be both physiologically and psychologically beneficial for adults who are overweight or obese, but further high quality research examining the mechanisms of action are needed (Rogers, Ferrari, Mosely, Lang, & Brennan, 2017).

For the current study, the Non-Reactivity Scale of the 39-item FFMQ was used to assess trait mindfulness. The Non-Reactivity Scale consists of items 4, 9, 19, 21, 24, 29, and 33 from the total scale. Baer and colleagues (2006) explored common dimensions among the item pools from five separate mindfulness questionnaires, each based on a different conception of mindfulness meditation. These included the Freiburg Mindfulness Inventory (Buchheld, Grossman, & Walach, 2001), the Mindful Attention Awareness Scale (Brown & Ryan, 2003), the Kentucky Inventory of Mindfulness Skills (Baer, Smith, & Allen, 2004), the Cognitive and Affective Mindfulness Scale—Revised (Feldman, Hayes, Kumar, Greeson, & Laurenceau, 2006), and the Southampton Mindfulness Questionnaire (Chadwick et al., 2008). The psychometrically strongest items of each of the five
questionnaires were selected to create the FFMQ. These items were incorporated into five subscales: 1) observing of internal and external present-moment stimuli (sensations, thoughts, emotions, sights, sounds, scents); 2) describing or labeling these experiences with words; 3) acting with awareness (rather than behaving automatically with attention focused elsewhere); 4) nonjudging of internal experiences; and 5) nonreactivity to inner experiences (Baer et al., 2006).

“Observing” refers to attending or noticing internal and external experiences (e.g., sounds, emotions, thoughts, bodily sensations, smells), whereas “describing” includes the ability to express in words one’s experiences (Baer et al., 2006). “Acting with awareness” involves attending to one’s present moment activity, rather than acting automatically, while attention is focused elsewhere. “Nonjudging of inner experiences” involves acceptance as well as not evaluating thoughts and emotions as either positive or negative. “Nonreactivity to inner experiences” refers to the ability to detach from thoughts and emotions, allowing them to arise and depart without getting involved or carried away by them (Baer et al., 2006). The FFMQ’s 39 items are rated on a 5-point Likert scale, ranging from 1 (never or very rarely true) to 5 (very often or always true). Examples of items from different factors are: “I pay attention to how my emotions affect my thoughts and behavior” (observing), “I think some of my emotions are bad or inappropriate and I shouldn’t feel them” (non-judging of inner experience, reverse scored).

The FFMQ has generally demonstrated sound psychometric properties, including satisfactory convergent and discriminant validity, internal consistency, incremental validity in predicting psychological symptoms and well-being across samples of regular meditators and nonmeditators, as well as interpretability in distinguishing between participant
The FFMQ has been shown to have adequate to good internal consistency (subscale alphas range from 0.75 to 0.91), and convergent and discriminant validity in relation to other psychological constructs in meditating and non-meditating samples (Baer et al., 2006).

Studies that consist of individuals with meditation experience have consistently supported this five-factor hierarchical model, in which all five subscales can be understood as elements of an overarching mindfulness construct; however, in samples lacking meditation experience, the “observing” subscale often shows inconsistent relationships with the other subscales and does not load significantly onto the overarching mindfulness construct (Gu et al., 2016). Gu and colleagues (2016) speculate this pattern suggests attention to present-moment experience can be either reactive and judgmental in those who have not been trained in mindfulness, or open, curious, and accepting in those who practice mindfulness meditation.

Judgmental, reactive observation tends to be correlated with maladaptive psychological functioning, in sharp contrast to mindful observation that is developed with meditation experience (Baer et al., 2006; Baer et al., 2008). The FFMQ structure may change over the course of mindfulness-based treatment, as participants learn to bring more mindful qualities to their present-moment observation (Gu et al., 2016). Gu and colleagues (2016) demonstrated this by omitting the observing subscale factor hierarchical structure to a pre-intervention FFMQ to depressed patients prior to training with Mindfulness-based Cognitive Therapy (MBCT), which incorporates mindfulness along with basic cognitive therapy (Segal, Williams, & Teasdale, 2013). After the MBCT intervention, the five-factor hierarchical model was superior, supporting the conceptualization of mindfulness as a
multifaceted construct while suggesting that the observing subscale is not a valid indicator of mindfulness in nonmeditating samples (Gu et al., 2016). Gu and colleagues (2016) suggest that when using the FFMQ, the observing subscale should be omitted when computing a total mindfulness score for non-meditating samples, and the other subscales summed. Further research is needed to validate this approach to a total score for the FFMQ. Baer (2016), one of the creators of the FFMQ acknowledges that though this finding is significant, there is a continue need for refinements in self-report methods and the development of more-objective performance-based methods is widely recognized (see Table 3.1).

Table 3.1. Study Concepts with Definitions and Measurement

<table>
<thead>
<tr>
<th>Concept Name</th>
<th>Mindfulness</th>
<th>Perceived Stress</th>
<th>NLRP3 Inflammasome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conceptual Definition</strong></td>
<td>A state of being aware through the act of paying attention, on purpose, in the present moment, without judgment, and not getting caught and compounding suffering by judging the judging (Kabat-Zinn, 2017).</td>
<td>The impact of a stressful event is determined by one’s perception of the stressfulness of the event (Cohen et al., 1983)</td>
<td>A multiprotein signaling platform that is part of the innate immune response (Saltiel &amp; Olefsky, 2017)</td>
</tr>
<tr>
<td><strong>Operational Definition</strong></td>
<td>Mindfulness is a two component model with the first component involving self-regulation of intention so that it is maintained on immediate experience, allowing for increased recognition of events in the present moment. The second component is</td>
<td>Measure of perception of stress, or the degree to which situations in one’s life are appraised as stressful. Items were designed to tap how unpredictable, uncontrollable, and overloaded respondents find their lives.</td>
<td>Changes (increase or decrease) in the NLRP3 inflammasome indicate levels of the pro-inflammatory cytokine, IL-1β in the circulatory system.</td>
</tr>
<tr>
<td>Concept Name</td>
<td>Mindfulness</td>
<td>Perceived Stress</td>
<td>NLRP3 Inflammasome</td>
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<tr>
<td>Concerned with adopting a particular orientation to one's experience in the present moment, accompanied by curiosity, openness, and acceptance (Bishop et al., 2004)</td>
<td></td>
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</tr>
<tr>
<td>Measurement of the Concept</td>
<td>Five Facet Mindfulness Questionnaire-Factor analytic study of five independently developed mindfulness questionnaires. The analysis yielded five factors that appear to represent elements of mindfulness as conceptualized in the psychological literature. The five facets are observing, describing, acting with awareness, non-judging of inner experience, and non-reactivity to inner experience (Baer et al., 2006)</td>
<td>Perceived Stress Scale (PSS) The questions in the PSS ask about feelings and thoughts during the last month. In each case, respondents are asked how often they felt a certain way.</td>
<td>NLRP3 inflammasome gene expression measures reflect relative RNA abundance. They are fluorescence-intensity measures for microarray hybridization; therefore they have no absolute intrinsic metric measure regarding RNA molecules. Rather, the values are a relative continuous metric, ranging from 1-100,000-fold above the lower limit of detection of the NLRP3 inflammasome RNA (Cole, 2017)</td>
</tr>
<tr>
<td>Type of measure</td>
<td>Questionnaire, Likert-type scale</td>
<td>Questionnaire, Likert-type scale</td>
<td>Physiological</td>
</tr>
<tr>
<td>Level of measurement</td>
<td>Ordinal</td>
<td>Ordinal</td>
<td>Continuous</td>
</tr>
<tr>
<td>If a paper measure:</td>
<td></td>
<td></td>
<td>n/a</td>
</tr>
<tr>
<td>Number of items</td>
<td>39</td>
<td>10</td>
<td>n/a</td>
</tr>
<tr>
<td>Concept Name</td>
<td>Mindfulness</td>
<td>Perceived Stress</td>
<td>NLRP3 Inflammasome</td>
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<tr>
<td>Response format</td>
<td>Fixed choice response format scored along a range with 1= never or very rarely true; 2 = rarely true; 3 = sometimes true; 4 = often true; 5 = always true</td>
<td>Fixed choice response format scored along a range 0 = never 1 = almost never 2 = sometimes 3 = fairly often 4 = very often</td>
<td>n/a</td>
</tr>
<tr>
<td>Scoring process</td>
<td>Score 5 facets: 1: Non reactivity to Inner Experience: Items 4, 9, 19, 21, 24, 29, 33 2: Observing/noticing/attending to sensations/perceptions/thoughts/feelings: Items1, 6, 11, 15, 20, 26, 31, 36 3: Acting with awareness/automatic pilot/concentration/non-distraction: Items 5, 8, 13, 18, 23, 28, 34, 38 4: Describing/labeling with words: Items 2, 7, 12 (reverse coding), 16 (reverse coding), 22 (reverse coding), 27, 32, 37 5: Non-judging of experience: Items 3, 10, 14, 17, 25, 30, 35, 39 (reverse all) Each facet is summed separately; there is not one summed score.</td>
<td>PSS scores are obtained by reverse coding items 4, 5, 7 and 8, then summing all items. Scores can range from 0 to 40. Higher scores indicate higher perceived stress. Scores ranging from 0-13 would be considered low stress. Scores ranging from 14-26 would be considered moderate stress. Scores ranging from 27-40 would be considered high perceived stress.</td>
<td>n/a</td>
</tr>
<tr>
<td>Time points</td>
<td>Baseline, 6 months</td>
<td>Baseline, 6 months</td>
<td>Baseline, 6 months</td>
</tr>
</tbody>
</table>
Data Collection Procedures

Randomization Procedure

In the SHINE RCT (NCT00960414) eligible participants were randomized in a 1:1 ratio to either the mindfulness or active control arm using a computer-generated random allocation sequence. Block randomization with randomly varying block sizes of 4 to 8 were used. Assignment to appropriate treatment arms was performed by a database manager not involved in enrollment, using a programmed computer database. No other staff had access to the randomization sequence. When a participant ID number was entered into the database by study staff, a group assignment was revealed. The project director accessed the allocation sequence using a programmed database that could not be altered once randomized condition was revealed.

Intervention arms. Both intervention arms (mindfulness intervention and active control intervention) lasted 5.5 months and included 16 sessions lasting 2 to 2.5 hours each, including 12 weekly, 3 biweekly, and 1 follow-up session 4 weeks later, as well as one all-day weekend session near the 8th week of the program. The all-day session for the mindfulness arm lasted 6.5 hours and the all-day session for the control arm was 5 hours in duration. The additional control intervention diet, exercise, and stress management content was shortened by 30 min in sessions 9–16, to increase participant acceptability. The primary goal was to match groups on expectations of benefit (Daubemier et al., 2016). Based on pilot testing, the study investigators believed that longer sessions might decrease perceived benefit.

Both programs contained the same diet and exercise components. The active control arm included additional educational content to ensure equivalence of time and
attention across intervention arms, including information about nutrition and physical activity, and instruction in progressive muscle relaxation. The mindfulness intervention was led by one of three mindfulness meditation instructors and co-led by the same registered dietitian, with the exception of one cohort. The control intervention was led by one of three registered dietitians (Daubenmier et al., 2016). Each of the participants had three individual consultations with instructors.

**Diet and Exercise Program**

This program, which was a component of both the active control and mindfulness intervention arms, included diet-exercise guidelines that were presented in 45-minute segments per session. Healthy food choices were recommended that emphasized modest calorie reduction of roughly 500 kcal/day, including decreasing calorie-dense, nutrient-poor foods, decreasing simple carbohydrates with the replacement of whole grains, and increasing consumption of fresh fruits and vegetables, healthy oils, and proteins. The exercise component emphasized increasing daily activity with moderate-intensity exercise, primarily through walking and strength training (see Daubenmier et al., 2016 for detailed description).

**Mindfulness intervention.** The Mindfulness arm incorporated elements of the Mindfulness-Based Eating Awareness Training program (MB-EAT), including training to improve eating awareness and mindfulness-based programs that incorporate training for stress management and emotion regulation, including sitting meditation and mindful yoga. Participants were encouraged to eat preferred foods in smaller portions rather than avoiding them altogether, to practice mindful eating, and to practice mediation at home. Mindfulness training for stress management, eating, and exercise incorporating meditation
practices was modeled on the mindfulness-based stress reduction program, included sitting meditation, loving kindness, and yoga postures (Kabat-Zinn, 1990). Mindful eating practices were modeled on Kristeller and colleagues' Mindfulness-Based Eating Awareness Training program, to enhance awareness and self-regulation of physical hunger, stomach fullness, taste satisfaction, food cravings, emotions, and other eating triggers along with mindful walking (Kristeller, Wolever, & Sheets, 2014). The participants' home practice guidelines included meditation practice for up to 30 minutes each day, 6 days a week, eating meals mindfully, and use of mini-meditations (Daubenmier et al., 2016).

**Active control arm.** To control for attention, social support, expectations of benefit, food provided during the mindful eating exercises, and home practice time in the mindfulness intervention, the active control arm consisted of additional nutrition and physical activity information, strength training with exercise bands, discussion of societal issues concerning weight loss, snacks, and home activities. A mindfulness approach to stress management was addressed with progressive muscle relaxation and cognitive-behavioral training in the control arm, although at a lower dose than in the mindfulness intervention arm.
Ethical Considerations

Protection of Human Subjects

The current research, which represents an ancillary analysis from the recently completed SHINE clinical trial (NCT00960414), involves human participants and meets the NIH definition of clinical research. The study does not meet criteria for exempt status under HHS regulations. All procedures described in the proposed study have been previously conducted in similar samples by the investigators at UCSF. The data analyzed will be de-identified and anonymous, with no Protected Health Information (PHI).

Informed Consent

Informed consent was obtained from all study participants during enrollment. Study staff personnel verbally outlined the important points of the consent form, including that the study was voluntary and the participant could drop out at any time, that participation or lack of participation would not affect their medical care, that all information was kept confidential, as well as the main requirements and any inconveniences involved in participation in the study. They were also informed about physical risks involved. Interested subjects then signed the consent form, the interviewer cosigned it, and a photocopy was given to the subjects to keep. The consent forms were kept in a locked filing cabinet and that did not contain the subject ID numbers on them.

Potential Risks

It was not anticipated that the SHINE RCT (NCT00960414) would pose serious risks to participants’ physical or psychological health. Potential mild risks and inconveniences were considered in the initial study protocol.
1. The psychological questionnaires (PSS and FFMQ) carried some risk of emotional discomfort when reporting about possibly distressing emotional states.

2. There was a small risk that the venipuncture associated with the blood draw could cause bruising or infection at the site of the needle insertion.

3. **Intervention:** Similar meditation interventions have been used with thousands of persons including many with serious illness, and there have not been important adverse events reported. The meditation intervention was thus considered very safe, but did carry some risk of having emotionally distressing material emerge at some point during the meditation sessions. Participants could also experience restlessness. There were minor risks of injury or coronary artery events during exercise, but these risks were minor and far out-weighed by the potential benefits of exercise. Reducing caloric intake on diet could have led to the experience of hunger at different times, but the caloric restriction was designed to be modest.

4. **Body measurements:** There were no physical risks involved in measuring body size (weight, height, waist and him circumferences). There was a risk of embarrassment or discomfort at being measured in one’s underclothes.

Overall, the study presented very limited risks to participants.

**Potential Benefits**

All participants were offered either the mindfulness weight loss intervention or diet and exercise-based weight loss program at no cost, which may have been of benefit to them. Participants had the potential to lose weight and reduce their stress levels.

Participants received copies of standard test results upon request. Participants were
compensated $30 for travel expenses and time for each interview and blood draw. Participants also received free influenza vaccinations.

Data Analyses

Assumptions and Data Cleaning

All analyses were performed with IBM SPSS Statistics version 24.0 (IBM Inc., Armonk, NY, USA). In all analyses, differences between groups were considered significant when p values were less than 0.05. All tests were two-tailed. Procedures advocated by Behrens (1997) were used to examine study variables to determine whether the assumptions of univariate and multivariate analyses were met. All data were screened for problems of outliers, skew, and kurtosis.

Description of Sample

Independent sample t-tests were conducted to describe participants randomized to the mindfulness intervention versus the control group along baseline NLRP3 inflammasome values.

Primary Analyses

Hypothesis 1. In order to assess whether there is a relationship between perceived stress and NLRP3 inflammasome at baseline, a bivariate correlation between total PSS score and NLRP3 inflammasome in PBMC samples for all subjects was conducted.

Hypothesis 2. In order to assess whether there is a relationship between trait mindfulness and NLRP3 inflammasome at baseline, a bivariate correlation was conducted between the Non-Reactivity subscale score of the FFMQ and NLRP3 inflammasome in PBMC samples for all subjects.
Hypothesis 3. In order to assess the change in gain scores in the NLRP3 inflammasome, measured in PBMC blood samples, from baseline to six months post-intervention in subjects who received a mindfulness training around eating and stress management practices (experimental group) compared to those who did not (control group) an independent sample t-test was conducted to compare the gain score in NLRP3 inflammasome.

Assumptions

The following assumptions are relevant to this proposed study.

1. The PBMC blood samples that were used to measure the NLRP3 inflammasome were drawn in a sterile fashion, stored appropriately, and labeled correctly, clearly differentiating the sample as from a subject in the experimental or control group.

2. The NLRP3 inflammasome was present in the PBMC blood samples and had not degraded over time and therefore was measured accurately.

Delimitations

The following delimitations are relevant to the proposed study.

1. Although data were collected at multiple times in the SHINE RCT (NCT00960414), for this study, only data collected at baseline (0) and 6 months post-intervention were used for analysis. This allowed for precise targeting of relationships among the key study variables (NLRP3 inflammasome, stress, and mindfulness).

2. Mindfulness was measured using only one scale (non-reactivity) of the FFMQ. Psychometric testing has shown the five scales of the FFMQ to be independent of each other and recommend not summing the scales to achieve an overall score
(Baer et al., 2008). Recognizing this limitation, it was decided to use only one scale to measure mindfulness for this study.

3. Gain score analysis was used to test the change in NLRP3 inflammasome in Hypothesis 3. While some argue that gain score analysis is unreliable (Gupta, Srivastava, & Sharma, 1988), first Rogosa (1988) then Knapp and Schafer (2009) demonstrated that analysis of gain scores are an unbiased estimate of true change. Further, with only two measurements from each subject (baseline and 6 months), gain score analysis is mathematically equivalent to a repeated measures ANOVA (Anderson et al., 1980).

**Conclusion**

This chapter has identified the methods that will be used in this study. The research design has been identified and sampling criteria have been discussed. The three major study variables: NLRP3 inflammasome, perceived stress, and mindfulness have been identified, defined both conceptually and operationally, and discussed in relation to the theoretical framework. Measurement methods for all three variables have also been delineated and referenced to prior research that has been undertaken to document that they are reliable and valid measures that are relevant to the proposed study. Ethical issues to protect the rights of the subjects who participated in the original SHINE RCT (NCT00960414) were reviewed. Data collection for the SHINE RCT (NCT00960414) was described in detail and the sources of data from the RCT that will be used in this ancillary analysis were discussed. Procedures for data analyses, in relation to the three study hypotheses, were described. The chapter concluded with the identification of study
assumptions and delimitations. The following chapter will describe the results of the data analysis and the final chapter will be a thorough discussion of the findings.
CHAPTER FOUR
Findings

In this chapter, the findings of the ancillary analysis of data from the Supporting Health by Integrating Nutrition and Exercise (SHINE) randomized controlled clinical trial (RCT) by Hecht and Epel (2014; NCT00960414) are reported. The research findings are organized in response to the three questions that guided the study, which were:

1. Is there a relationship between perceived stress and the NLRP3 inflammasome?
2. Is there a relationship between trait mindfulness and the NLRP3 inflammasome?
3. What is the change in gain scores in the NLRP3 inflammasome, measured in PBMC blood samples, from baseline to six months post-intervention in subjects who received mindfulness training around eating and stress management practices (experimental group) compared to those who did not (control group)?

Results

Description of the Sample

Most participants ($n = 138$, mean age 49.14 ± 12.45) were female (78.4%), identified as non-Hispanic White (65.2%) and had at least a bachelor’s degree for highest level of education (73%). By study design, all participants were obese (mean BMI 35.35 ± 3.63 kg/m$^2$). Their average NLRP3 value was 10.25 ± 0.86 (range: 7.61-12.65); their average PSS score was 14.38 ± 5.87 (range: 3-30) and their average non-reactivity FFMQ score was .26 ± 0.64 (range: 1.14- 4.86). Participants randomized to the control vs. mindfulness program did not differ at baseline with regard to age, sex, BMI, race/ethnicity, education, waist circumference, PSS, any FFMQ subscale, or NLRP3 (all $p > .05$, see Table 4.1).
Table 4.1. Baseline characteristics of study participants (total sample, left panel); Group differences across study variables at baseline (Control vs. Mindfulness, right panel)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Sample (n=138)</th>
<th>Control (n=62)</th>
<th>Mindfulness (n=76)</th>
<th>t or χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.14 ± 12.45 (18.47–68.57)</td>
<td>50.04 ± 11.75</td>
<td>48.68 ± 12.90</td>
<td>t = 0.64</td>
<td>.52</td>
</tr>
<tr>
<td>Sex, % female (n)</td>
<td>78.4% (n = 109)</td>
<td>82.6% (n = 51)</td>
<td>76.32% (n = 58)</td>
<td>χ² = 0.73</td>
<td>.39</td>
</tr>
<tr>
<td>Ethnic Origin, % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>65.2% (n = 90)</td>
<td>59.68% (n = 37)</td>
<td>69.74% (n = 53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>13.0% (n = 18)</td>
<td>14.52% (n = 9)</td>
<td>11.84% (n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>8.7% (n = 12)</td>
<td>12.90% (n = 8)</td>
<td>5.26% (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latina/Latino</td>
<td>9.4% (n = 13)</td>
<td>11.29% (n = 7)</td>
<td>7.89% (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native American</td>
<td>0% (n = 0)</td>
<td>0% (n = 0)</td>
<td>0% (n = 0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3.6% (n = 5)</td>
<td>1.6% (n = 1)</td>
<td>5.26% (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education, % (n)</td>
<td>73% (n = 100)</td>
<td>69.35% (n = 43)</td>
<td>76% (n = 57)</td>
<td>χ² = 0.76</td>
<td>.38</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>35.35 ± 3.63 (29.98–45.65)</td>
<td>35.15 ± 3.87</td>
<td>35.51 ± 3.43</td>
<td>t = 0.59</td>
<td>.56</td>
</tr>
</tbody>
</table>
Primary Analyses

Research Question 1. There was a positive relationship between baseline PSS total score and baseline NLRP3 value, as predicted, but it was a very small effect and not statistically significant ($r = 0.10, p = .25$; see Table 4.2). Based on this finding, the conclusion for the first research question is that there is no meaningful relationship between perceived stress and NLRP3 inflammasome at baseline.

Table 4.2. Bivariate correlations between the inflammasome marker NLRP3, total PSS score, and FFMQ subscales at baseline ($n = 137$).

<table>
<thead>
<tr>
<th></th>
<th>Pearson Correlation Coefficient</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>0.10</td>
<td>.25</td>
</tr>
<tr>
<td>FFMQ non-reactivity</td>
<td>0.08</td>
<td>.35</td>
</tr>
</tbody>
</table>

Note. $p \leq .05$
**Research Question 2.** There was no significant correlation between baseline trait mindfulness (FFMQ: non-reactivity subscale score) and baseline NLRP3 values ($r = .08, p = .35$). Similar to the first research question, the non-negative Pearson product moment correlation indicates a positive relationship although it was not significant. It is in the opposite direction as predicted. Higher levels of mindfulness were related, although nonsignificantly, to higher levels of NLRP3. Based on this finding, the conclusion for the second research question was that there is not a relationship between baseline trait mindfulness and NLRP3 inflammasome at baseline (see Table 2).

**Research Question 3.** The two groups did not differ with regard to change in NLRP3 from baseline to 6-months post-intervention ($t = 0.02, p = .99$; See Table 4.3). Participants randomized to the control group increased in NLRP3 by 0.32. Similarly, participants randomized to the mindfulness group increased in NLRP3 by 0.32. The calculated gain score for the two groups was zero.

Table 4.3. Group comparisons in inflammasome marker change from baseline to 6-months post-intervention.

<table>
<thead>
<tr>
<th></th>
<th>t</th>
<th>p value</th>
<th>M ± SD</th>
<th>M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control (n = 62)</td>
<td>Mindfulness (n = 76)</td>
</tr>
<tr>
<td>NLRP3 change</td>
<td>0.02</td>
<td>.99</td>
<td>+0.32 ± 0.89</td>
<td>+0.32 ± 0.91</td>
</tr>
</tbody>
</table>

**Summary**

The findings did not provide statistical evidence to support any of the three research hypotheses derived from the research questions, thus the null hypothesis was retained in all three cases. Neither the PSS nor the FFMQ were significantly correlated with NLRP3 inflammasome activation. There was also no change from baseline to 6 months in NLRP3.
inflammasome activation between the two groups. These findings may be a result of a smaller than anticipated sample as well as a smaller than anticipated effect size.

Assumptions made and presented in Chapter Three were an $n = 194$, and power of 80% to detect a statistically significant difference ($p < 0.05$), the detectable mean difference between groups with regard to the NLRP3 inflammasome is 0.40. However, the number of subjects with data for PSS, FFMQ, NLRP3 inflammasome was $n = 138$. The gain score of 0 with a standard deviation of .89 (PSS) and .91 (FFMQ) indicates no effect (no group differences at all), not reaching the threshold of 0.40. The small sample and lack of effect size for group differences are likely the major contributors to the lack of significant findings. This will be discussed in detail in Chapter Five.
CHAPTER FIVE

Discussion

In this chapter, the findings of the ancillary analysis of data from the Supporting Health by Integrating Nutrition and Exercise (SHINE) randomized controlled clinical trial (RCT) by Hecht and Epel (2014; NCT00960414) are discussed. The topics are organized in response to the three questions that guided the study:

1. Is there a relationship between perceived stress and the NLRP3 inflammasome? Is there a relationship between trait mindfulness and the NLRP3 inflammasome?

2. What is the change in gain scores in the NLRP3 inflammasome, measured in PBMC blood samples, from baseline to six months post-intervention in subjects who received mindfulness training around eating and stress management practices (experimental group) compared to those who did not (control group)?

3. What is the change in gain scores in the NLRP3 inflammasome, measured in PBMC blood samples, from baseline to six months post-intervention in subjects who received mindfulness training around eating and stress management practices (experimental group) compared to those who did not (control group)?

Non-Significant Findings: Consideration of These Results

There were no significant findings for any of the three research questions. Neither the PSS nor the FFMQ were correlated with NLRP3 inflammasome activation. There was also no change from baseline to 6 months in NLRP3 inflammasome activation between the two study groups: experimental, who received the mindfulness training intervention and control, who received education on nutrition and exercise, but not mindfulness.

There are several considerations of why these findings were not as expected. There was a smaller than anticipated sample size as well as a smaller than anticipated effect size.
which very well may have affected the results. Assumptions made and presented in Chapter Three based on 194 participants in the study, estimated an 80% to detect a statistically significant difference (p < 0.05), with a detectable mean difference between groups in regard to the NLRP3 inflammasome being 0.40; however, the number of subjects with complete data available for analysis on the PSS, FFMQ, NLRP3 inflammasome was 138. This provides lower statistical power to detect significant relationships. The gain score of 0 with a standard deviation of .89 (PSS) and .91 (FFMQ) indicates no effect (no group difference), not reaching the threshold of 0.40. The small sample and small effect size are likely the major contributors to the lack of significant findings.

**Mindfulness interventions and social genomics**

Although there was no difference between the mindfulness (experimental) group and the control group in this ancillary analysis, substantial evidence has emerged over the past several years supporting the conclusion that mind-body interventions facilitate health outcomes; this includes mindfulness (Daubenmier et al., 2016; Bower & Irwin, 2016; Garcia-Campano et al., 2017). These therapies may act as effective adjuncts to conventional medical treatment and can be effectively integrated into the evolution of the mainstream medical paradigm (Davidson & Kraszniak, 2015; Muehsam et al., 2017). Genomic expression impacted by environmental and behavioral influences, such as that being explored in this study, may underlie some of these changes (Black & Slavich 2016; Garcia-Campayo et al., 2017).

Studies in psychosocial genomics that implement gene expression analysis in mindfulness-based interventions (MBI) research have begun to appear, demonstrating activation of gene transcription factor nuclear factor-kappa B (Bulger & Groudine, 2011;
This is relevant to inflammation as nuclear factor kappa B (NF-κB), produced when stress activates the sympathetic nervous system, triggers the expression of genes coded for inflammatory cytokines, including the NLRP3 inflammasome-activated IL-1β (García-Campayo et al., 2017) Muehsam et al., 2017; Hughes & O'Neill, 2018). NF-κB activation was explored in a study of 17 meditators who had been practicing for at least 10 years compared to 17 meditation-naïve participants by García-Campayo and colleagues (2017). The researchers also found 64 differentially methylated regions (DMRs) signaling epigenetic difference in the 17 meditators compared to the 17 controls, corresponding to 43 genes. Notably, almost 50% of the mindfulness-related DMRs involved genes linked to common human diseases, such as neurological and psychiatric disorders, cardiovascular disease, and cancer with the associated downregulation of crucial transcriptional regulators NF-κB and Tissue Necrosis Factor (TNF), a potent inductor of the NF-κB signaling pathway as a response to mindfulness practice (Garcia-Campayo et al., 2017). Garcia-Campayo and colleagues' (2017) study as well as others (Creswell et al., 2012; Kaliman et al., 2014) have significantly advanced the understanding of transcriptional pathways relevant to mindfulness and inflammatory processes which may be implicated in the activation of the NLRP3 inflammasome. Their research demonstrates the potential mechanisms of action by which these interventions influence stress and concomitant reductions in inflammatory activity and enhanced innate immune response (Muehsam et al., 2017).
Psychological Stress

The present study was the first to explore psychological stress and the NLRP3 inflammasome in humans. Animal studies addressing this topic induced chronic or acute intense stressor exposure in the absence of overt tissue damage (Iwata et al., 2016; Maslanik et al., 2013; Speaker & Fleshner, 2012). It is important to note that the scores on the PSS level were not elevated in this cohort. The average stress levels of this sample were 14, below the national average of 16 (Janiki & Cohen, 2006). Stress often leads to overeating of unhealthy food which can affect weight and metabolism (Adam & Epel, 2005; Masih, Dimmock, Epel, & Guelfi, 2017.). It may be that stress eating would have a bigger impact on the inflammasome than perceived stress alone.

Stress-related eating is associated with maladaptive emotional regulation, a transdiagnostic risk link and a psychological characteristic of all eating disorders (Lattimore et al., 2017). Mindfulness has been associated with decreases in emotional or stress-related eating by promoting more effective emotion regulation and greater flexibility responding to mental and external events (Daubenmier et al. 2011; Lattimore et al., 2017).

Limitations of the Study

First and foremost, ancillary studies rely on interventions that have already been undertaken and can therefore provide a low-cost method with which to identify new effects on a wide variety of outcomes; however, they are also associated with limitations (Baldwin & Bhavnani, 2015). Along with the small sample size which was limited due to the number of subjects from the prior study, effect sizes are likely to be small in new areas of research inquiry (Cohen, 1988). This is thought to be directly related to the influence of uncontrollable extraneous variables of measurement, such as inadequate sample size, that
make the signal of the effect size difficult to detect rather than the theoretical framework itself; therefore, the theory remains to have relevance in light of other related mindfulness genomic studies with statistical significance (Black & Slavich, 2016; Buric, Farias, Jong, Mee, & Brazil, 2017; Cohen, 1988; Muehsam et al., 2017). NICE!!!

Another factor to be considered is the mindfulness intervention. Rather than all dimensions of the FFMQ, only one factor, nonreactivity was utilized. Lattimore and colleagues (2017) note that only three facets of the FFMQ are related to mindfulness and eating behavior. The facets of acting with awareness addressing attending to present moment experiences, non-reactivity including letting thoughts and feelings come and go without getting caught up in them and observing of sensory experiencing may be particularly relevant to disinhibited eating behaviors and symptoms (Lattimore et al., 2017).

Furthering the importance of addressing more than one FFMQ facet, Sala and Levinson (2017), explored the role mindfulness played in disinhibited eating in young adult women (N = 300), via the FFMQ at baseline and 6 months later. Non-reactivity inversely predicted binge eating and bulimic symptoms across 6 months. Observing predicted higher external and emotional eating across 6 months, demonstrating the importance of addressing more than one FFMQ facet (Sala & Levinson, 2017).

In regard to mindfulness teacher qualifications and abilities, as Daubemier and colleagues (2016) note in their prior study utilizing this same cohort, there were concerns that mindfulness teaching by instructor C might have been less effective than that of other instructors. It was surmised that efficacy of mindfulness training for weight loss may be instructor-dependent (Daubemier et al., 2016).
Davidson and Kaszniak (2015) suggest that indices of teacher training, certification, and experience should be provided not only for the meditation teacher, but for the comparison intervention teachers as well. This would include relevant teaching measures such as participant perception of the teachers and the credibility and expectancy of the intervention (Davidson & Kaszniak, 2015). Intervention credibility can be assessed using a slightly modified version of the Credibility and Expectancy Questionnaire, a clinical measure designed to assess the expectancy and credibility of interventions perceived by patients in therapy for the treatment of anxiety (Devilly & Borkovec, 2000). There was no clinical measure of assessment done for this study.

The 6 month duration of the mindfulness intervention may also be a limitation. Even with the consideration of a larger study sample, the NLRP3 inflammasome may require longer duration of mindfulness practice to show changes. It may take longer than 6 months to reduce the level of inflammation in the blood (Sanada et al., 2017).

Davidson and Kaszniak (2015) noted that the measure of mindfulness in those who have not been trained in this intervention will be compromised with respect to both reliability and validity as reports of conscious experience derived from minds that have not had this form of training could be tainted by distraction. Self-reports on mindfulness questionnaires may reflect very different processes at different levels of training. There is the expectation that among individuals with no or little mindfulness practice, the quality of the data from such reports will be different from that obtained from longer-term practitioners who have developed considerably more familiarity with the nature of their own experience (Davidson & Kraszniak, 2015).
Addressing the PSS and a lack of an association with the NLRP3 inflammasome, prior studies were only done in animal models exhibiting extreme stress and the present clinical sample of this observational study presented with a general low level of stress (Speaker & Fleshner, 2012; Iwata et al., 2016). Furthermore, social genomics research by Mehl and colleagues (2017) has identified genomically expressed language style markers that more clearly link inflammatory processes with implicit, unconscious manifestations of psychological states rather than explicit conscious ones such as perceived stress. These implicit markers such as conserved transcriptional response to adversity, which includes chronic stress, may provide a more evidenced-based measurable behavioral indicator of the neurobiological processes that mediate social influences on gene expression in immune cells (Cole, 2017; Mehl et al., 2017).

The consideration of more than one stress-related scale along with combining interventions has the potential to lead to greater dimensionality of outcomes. Corsica and associates (2014) compared three groups regarding both stress-eating and perceived stress. The groups consisted of an MBSR intervention, a cognitive behavioral stress-eating intervention (SEI), and a combined intervention that included b MBSR and SEI components. Separately, the three interventions significantly reduced perceived stress and stress-eating; however, the combination intervention resulted in greater reductions and also produced a moderate effect on short term weight loss benefits that persisted at a 6 week follow-up (Corsica, Hood, Katterman, Kleinman, & Ivan, 2014).

Regarding the blood drawn for evaluation, studies of cytokine gene mRNA expression, as examined in the present study, need to account for tissue specificity that guides the selection of the study design (Cole, 2017; Gilbertson-White, Aouizerat, &
Miaskowski, 2011). In the present study, mRNA was measured in PBMC only; however, consideration might be given to mRNA measurement in adipose tissue as well. Kursawe and colleagues (2016) note that NLRP3 inflammasome activation is present in subcutaneous adipose tissue (SAT). The SAT NLRP3 inflammasome is linked to the downregulation of SAT adipogenesis/lipogenesis in obesity, in turn leading to altered abdominal fat partitioning with an increase in visceral adiposity and insulin resistance. In addition, factors that may not be cyclical such demographic variables, comorbidities, and health behaviors are also known to effect cytokine expression (Gilbertson-White, Aouizerat, & Miaskowski, 2011). The limitation in this design was that these covariates were not incorporated in the study; therefore, the results may have been affected by this omission.

Cole (2017) also suggested that it is advisable to conduct pilot studies to assess the empirical kinetics of RNA response for a small number of target genes prior to finalizing the sampling protocol for a full-scale study. This was not able to be done to indicate the null response. Most importantly, individual gene discovery studies generally test an unrealistic statistical hypothesis that each single gene shows a fixed dependent relationship to the phenomena being studied (Cole, 2017). Statistical tests that focus on sets of genes that have substantially greater statistical power; therefore, it is more advantageous to identify pathways of a cluster of genes such as a group of inflammasomes instead of the one singular NLRP3 inflammasome gene, to identify molecular pathways that mediate psychological and social influences on physiologic function (Cole, 2017). Hyper-stringent gene specific statistical significance implies that either very large effect sizes must be anticipated or large samples need to be engaged to adjust for a false positive error rate.
(Cole, 2017). Cole (2017) also pointed out that it is wise to pilot test the kinetics of the basal transcriptome response to chronic environmental exposures to select optimal time points for assessment; this was not able to be done in the present study.

**Considerations and Implications**

Work in psychosomatic medicine is driven by the collective vision to integrate biological, psychological and social factors in healthcare. This endeavor is facilitated by the identification of biological intersection points where psychosocial and biological factors converge, interact, and trigger measurable cellular and health effects. The immune system represents such an intersection point (Picard & McEwen, 2018). This NLRP3 inflammasome study which addressed such a convergence, did not find that the mind-body intervention of mindfulness meditation lead to changes in the inflammasome; however, there were many limitations as discussed earlier, making this a non-definitive test of the hypothesis. Other studies have shown mind-body influencing other indices of inflammation including gene expression studies.

**Implications for Stress and Mindfulness Research**

Specifically addressing the mechanistic link between stress and the variables of mindfulness, obesity, and inflammation has not been fully clarified, partly due to the inherent complexity exemplified by the bidirectional effect of stress on eating and body weight (de Sousa Rodrigues et al., 2017; Razzoli & Bartolomucci, 2016). The rationale for cultivating mindfulness skills as a component of therapy for eating disorders such as stress-eating rests on the proposition that by cultivating mindful awareness of internal experiences such as emotions and physical sensations, the opportunity exists for self-acceptance, cognitive flexibility, compassion and the ability to respond adaptively to
disturbing emotions is facilitated (Katterman, Klienman, Hood, Nackers, & Corsica, 2014).

Addressing this topic, O’Reilly and colleagues (2014) found that 86% of the 21 reviewed studies of mindfulness and problem eating behaviors reported improvements in the targeted eating behaviors, supporting the efficacy of mindfulness-based interventions for changing obesity-related eating behaviors, including stress-related eating; therefore, future research may address the multifactorial stress-related eating instead of just the variable of perceived stress, in relation to the presence of NLRP3 inflammasome-mediated IL-1β implicated in obesity-related adipose tissue inflammation (Grant & Dixit, 2015).

Exploring the effect of mindfulness meditation on stress-eating and the NLRP3 inflammasome might be a timely pursuit in light of the rise in rates of obesity in the United States, implicated in the recent attenuation in declining death rates for heart disease, stroke, and diabetes between 1988 and 2011 by more than half a percentage point—equivalent to a 23% relative reduction in the rate of mortality decline (Preston, Vierboom, & Stokes, 2018).

One other important consideration in future studies in this area of study, activation of the NLRP3 inflammasome with a higher stress cohort reflected in the prior animal studies (Speaker & Fleshner, 2012; Iwata et al., 2016). Including a more experimental paradigm where participants of high stress vs. low stress are recruited for comparison of the activation of the NLRP3 inflammasome might yield more statistical relevance.

More generally, stress as a variable is important to assess in the study of health, yet a lack of consistency and thoroughness in its measurement are critical barriers that prevent scientific progress (Epel et al, 2018). Measurement of stress is inherently complex because
stress is experienced on multiple levels – social, psychological, and physiological. Stress measurement also depends on the context of an individual’s biographical context such as age and genetic make-up, socio-cultural context such as socio-economic status and cultural norms, and their history of and current exposure to stress (Epel et al., 2018).

Across studies, measurement is inconsistent, often superficial, and heterogeneous constructs are conflated; therefore, Epel and colleagues (2018) call for more articulate measurement approaches using a common language of stress, as well as more complex and precise stress models that take into account the multi-level nature of stress.

Stress is important to assess in the study of health, yet a lack of consistency and thoroughness in its measurement is a critical barrier that prevents scientific progress (Epel et al, 2018). Measurement of stress is inherently complex because stress is experienced on multiple levels – social, psychological, and physiological, with few agreed upon gold standard measures. Across studies, measurement is inconsistent, often superficial, and heterogeneous constructs are conflated; therefore, Epel and colleagues (2018) call for more articulate measurement approaches using a common language of stress, as well as more complex and precise stress models that take into account the multi-level nature of stress.

Consciously perceived and self-reported ratings of stress using standard scales such as Perceived Stress Scale, explain only a limited amount of variance in physiological stress reactivity and biological outcomes. The challenge is that the mechanistic pathway linking psychological stress to worsening health is hypothesized to be through dysregulated stress reactivity profiles. Events are not exclusively experienced through conscious perception as assumed in basic stress models (Epel et al., 2018; Mehl et al., 2017). Secondly, subjective reports of being stressed are potentially limited by individuals’ unwillingness or inability to
report their veridical stress state. Some cultural groups' self-reports of stressor-related bodily experiences such as pain, sleep disturbances, or somatic health symptoms such as headaches or stomachaches, may serve as a better indicator of stressor exposure and high perceived stress than asking about feelings or thoughts directly (Epel et al., 2018). There may also be an inability to report due to a relative comparison process where one’s subjective understanding of stress is calibrated relative to other adverse aspects of their lives (Epel et al., 2018).

Stress models can be improved by taking into account the reciprocal relationship between individual-level factors as illustrated in a transdisciplinary model by Epel and colleagues (2018) that describes stress as a set of interactive and emergent processes. These processes would include age and personality, as well as the context of the person’s life, including socio-economic status and historical stressor exposures, habitual responses such as baseline allostatic physiological states and mental filters, and the neural and peripheral physiological responses to stressors (Epel et al., 2018).

In order to advance conceptualization of how stress influences trajectories health, stress must be measured in context. Examining the impact of a single stressor exposure without measuring the context in which a person is experiencing the stressor limits the predictive ability as the historical context influences the habitual responses to stress and cumulative effects that may lead to early disease (Epel et al., 2018). To further advance health research, it is vital to understand a person’s risk for stress-related disease with a life history self-report as well as reactivity measures. Looking at these variables in tandem may help uncover who is at highest risk for stress related disease with greater predictive models;
thereby, laying the foundation for individual and social interventions as well as policies geared toward promoting the wellbeing and healthspan of many (Epel et al., 2018).

Moving forward, a number of key issues and concerns need to be addressed within the domain of mindfulness research. These include the problematic meaning of the term itself, the differing measures of mindfulness and challenges to construct validity (Van Dam et al., 2018a). Scientists must be called to a more rigorous standard, specifying with greater precision how the constructs are being measured (Davidson & Dahl, 2018).

Challenges for clinical intervention methodology will need to be considered. These include the variations in the types and content of various MBIs incorporated as well as key issues of duration, intensity, the spacing of the practice, the outcome desired, and the extent to which formal meditation practice is required (Davidson & Dahl, 2018; Van Dam et al., 2018a).

Ascertaining appropriate dosages of mindfulness practice, specifying the desired outcome of the practice, as well as gathering sufficient information obtained about potential negative side effects may lead to formulating a greater optimal practice regimen that maximizes benefits and minimizes adverse experiences. The dose-response curves will likely be nonlinear, with ideal parameters varying within and across different populations (Van Dam et al., 2018b).

Engaging with larger and more diverse population studies of mindfulness, methodological issues related to the analysis of gene expression data and social gradients in health will need to be considered (Belsky & Synder-Mackler, 2017). Levine and colleagues (2017) demonstrated preliminary evidence that older adults with lower socioeconomic status (SES) exhibited a pattern of chronic increased inflammatory and
decreased antiviral peripheral-blood gene expression, including NF-κB transcription factor, related to the NLRP3 inflammasome. This chronic pattern, referred to as “conserved transcriptional response to adversity” (CTRA), presumably evolved to help adapt molecular physiology to the types of sporadic and transient threats that generally characterized humans' ancestral environments (Cole, 2014). There is a need to take into consideration this genomic response in vulnerable populations, alongside other proposed measurements of proinflammatory biological embedding such as the NLRP# inflammasome (Belsky & Snyder-Mackler, 2017).

As demonstrated in the study by Daubenmier and colleagues (2016), the mindfulness teacher may impact the participants and outcome. The level of teacher training of clinicians offering mind-body practices is not often addressed in research studies, yet based on the findings of Ruijgrok-Lupton and colleagues (2018), could potentially make a significant difference to outcomes. Mixed methods studies incorporating qualitative research to explore the role of teachers in conveying mindfulness and in the therapeutic relationship with course participants might shed greater insight into the process with clarity of outcomes. Ruijgrok-Lupton and colleagues (2018) measured course attendees’ gains after Mindfulness-Based Stress Reduction (MBSR) instruction of 31 participants were correlated with teacher training and experience. Gains in well-being and reductions in perceived stress were significantly larger for the participant cohort taught by teachers who had completed an additional year of mindfulness-based teacher training and assessment.

Including information on teacher training levels of study clinicians may facilitate more equitable evaluations of mindfulness studies in the future (Ruijgrok-Lupton, Crane &
Dorjee, 2018). The challenge remains that teachers within particular meditation tradition are often authorized to teach individually by their own teacher, and there does not presently exist anything comparable to national or regional licensing or certification. Such future research would be helpful in guiding selection of teachers for any planned study (Davidson & Kaszniak, 2015).

Results from functional genomics such as the studies mentioned in this chapter, demonstrate how avenues of emotional, cognitive, and psychosocial factors can influence health outcomes and well-being, and this knowledge has significantly contributed to a developing a psychophysiological theory of mindfulness. These results have already contributed to a clearer notion of the role of mindfulness practices for stress reduction; however, Muehsam and colleagues (2017) call for researchers to better understand the fundamentals of these therapies and to further explore gene profiling and associated proteomics needed to support this hypothesis. Clear, replicated data are lacking on clinically important questions regarding the interpretation and relevance of results, variability across populations; therefore, controlled longitudinal studies are needed to assess the causality and determine the efficacy and potential therapeutic specificity of mindfulness interventions (Muehsam et al., 2017).

**Implications for NLRP3 Inflammasome and Social Genome Research**

Although huge advances have been made in understanding the role of the NLRP3 inflammasome as an intracellular safeguard to sense and limit altered metabolite production in the early stages of its production, NLRP3 regulation and its relationship to homeostasis in healthy individuals remains to be fully elucidated (Hughes & O’Neill, 2017). In the context of health and inflammatory disease, Coll and colleagues (2016) noted
that the basic mechanism of NLRP3 activation is still incompletely characterized. Accurate modeling of the entire inflammasome complex would facilitate understanding and design of NLRP3 inhibitors, including those mediated by psychological stress (Alcocer-Gómez, & Cordero, 2017; Coll, O’Neill, & Schroder, 2016; Fleshner, Frank, & Maier, 2017).

Furthermore, there is a paucity of data on the function of NLRP3 in homeostasis in healthy animals and humans, as until now, the focus has been the pathological roles of NLRP3 in murine disease which elicits a different pathway than that in the human (Coll, O’Neill, & Schroder, 2016; Hughes & O’Neill, 2017). Inquiry is also called for regarding the mechanisms by which NLRP3 interacts with different organs and organelles of the body such as the gut microbiota and mitochondria as well as physiological states such as obesity. Altered metabolism in activated macrophages and associated metabolic components can drive inflammation or age-associated inflammation where endogenous metabolites and nutrients trigger IL-1β cleavage. All four of these are related to both psychological stress and the NLRP3 inflammasome (Allen, Dinan, Clarke, & Cryan, 2017; Hughes & O’Neill, 2017; Picard, McEwen, Epel, & Sandi, 2018; Place & Kanneganti, 2018). Further unraveling the role of NLRP3 inflammasome activation has the potential to lead to novel therapeutics, including life-style interventions such as mindfulness to limit the broad swath and ever-growing inflammation-related NCD pathologies and facilitate greater well-being (Hughes & O’Neill, 2017).

Specifically, in terms of the present study, there are several variables that may have played a role in the non-statistically significant findings. First, tissue sampling may play a role. There is no biological reason to expect that gene expression dynamics in one cell or tissue such as PBMCs would necessarily parallel those in another cell or tissue (Cole,
Cole (2017) noted that as a general rule, the RNA analyzed in transcriptome profiling studies should come from the specific cells and tissues that mediate the general biological processes under study, essential for mechanistic studies in which the aim is to identify the molecular processes mediating a particular behavioral or physiological process. Instead of looking at PBMCs, perhaps it would have been more instructive to look at subcutaneous adipose tissue for NLRP3 presence. This was elucidated in a systemic review of 19 studies that evaluated the association of NLRP3 with obesity and insulin resistance, focused on NLRP3 expression/polymorphism, analyses indicated that obesity and insulin resistance are associated with increased NLRP3 expression in AT. (Rheinheimer, de Souza, Cardoso, Bauer, & Crispim, 2017).

The remarkable ability of human beings to adaptive and survive is attributable in large part to the capacity of individuals to self-organize into complex social systems. Research in this area of study, such as this stress and the NLRP3 inflammasome study is referred to as human social genomics. This area of research has begun to clarify how extraorganismic social systems reciprocally regulate one’s intraorganismic physiologic function by modulating tissue-specific programs of gene expression (Cole, 2014). Studies of human social genomics are now clarifying which particular types of human genes are subject to social regulation and mapping their social signal transduction pathways. The results of these analyses are shedding new light on the molecular basis for social influences on individual health, leading to a clearer understanding of the genomic basis for human thriving (Cole, 2014). There is a great deal of potential to further explore the mRNA expression of the NLRP3 inflammasome gene to help individuals thrive. As Cole (2014) noted, mapping the evolved regulatory logic of the human genome should have more to tell
us about human well-being and the biology of thriving than it does about disease per se if one seeks to avoid the chronic activation of costly molecular defense programs such as the CTRA and mRNA NLRP3 inflammasome-related inflammation.

**Implications for Nursing Practice and Research**

Healthcare is in transition, from a focus on acute care in which infection and trauma play major roles in reactive care, initiating interventions once an individual is on the verge of or has actually suffered a negative health event, to a new lens addressing preventative healthcare to response to an increase in noncommunicable disease, which can be caused and exacerbated by inappropriate lifestyle choices throughout the healthspan (Debusk & Snapp, 2016; Sagner et al., 2017). Major advances in science, particularly in genomics and lifestyle, accompany this new era of participatory healthcare, where the patient is engaged in one's own well-being (Sagner et al., 2017). Behavioral health specialists including nurses, will increasingly play a valuable role in educating patients on the key lifestyle factors that trigger chronic disease, and assisting patients in making changes to prevent disease and improve health, including the incorporating genomics and stress reduction via interventions such as mindfulness (Debusk & Snapp, 2016).

Genetic and genomic discoveries impact all areas of healthcare practice, from providing insight into new treatments to supporting disease prevention strategies and guiding the development of personalized health interventions (Henderson & Mudd-Martin, 2018). Incorporating genomics, a biobehavioral nursing model integrates both theoretical and empirical knowledge of psycho-behavioral and physiological patterns that contribute to health outcomes (Driscoll, Lyon, & McCain, 2011). Nurses working in multiple settings
may play an integral role in generating and translating this new knowledge into clinically relevant applications (Alexander, 2017).

One note to consider in the sharing of genetic risk information, nurses and other healthcare professionals need to recognize the responsibility that accompanies risk knowledge, and that ultimately this responsibility lies with the patient, not the provider. Falahee and colleagues (2018) suggest that professionals’ evaluation of the utility of predictive genetic testing should be influenced not only by consideration of resource deficits in patient understanding but also mindful of challenging ethical and social issues associated with genetic risk, as well as demonstrating opportunities to facilitate participatory care.

Another essential aspect of participatory care is addressing the role of mindfulness and other integrative medicine in serving vulnerable populations. Mindfulness and other integrative medicine practices have come to be thought of as pricey, nonessential healthcare practices for the overserved. The 2500-year-old historic roots are often overlooked as is the realization that these treatments are impactful, in large part, because of their cultural availability and financial accessibility (Chao & Adler, 2018). It is clear that vulnerable populations are facing life stressors such as poverty, food insecurity, and unstable housing, factors that are well beyond the scope of integrative medicine practices such as mindfulness; however, this emerging field of healthcare can contribute uniquely to reducing the burden of disease for those negatively impacted by health disparities by providing tools to build resilience and health self-efficacy through patient-centered care, and reducing the deleterious effects of chronic stress and trauma (Chao & Adler, 2018). For integrative medicine to achieve its potential, it is essential to address health equity.
explicitly through clinical delivery, educational efforts, and research. Developing group-based treatment of mindfulness to improve access and affordability is critical to expanding the reach and broadening the impact of this discipline. Educating integrative medicine nurses about social determinants of health and structural competency will provide much-needed awareness and skills to address health equity as care is delivered (Chao & Adler, 2018).

In regard to nursing research, from genomics to epigenomics, immunity to immunodeficiency, and apoptosis to cancer, nurse scientists are beginning to explore the biological underpinnings of health and disease across the lifespan (Corwin, 2011). The healthcare industry must undo so much in the way of process and care models, requiring new leadership, thinking, and direction unprecedented in this slow-to-change environment (Reynolds & Jones, 2016). Incorporating genomics has the potential to guide the discipline of nursing research through exploring contributions to individual variability; thereby, advance the understanding of the complex pathophysiology of disease susceptibility and different patient responses to interventions (Alexander, 2017; Lee, Gill, Barr, Yun, & Kim, 2017). From bench to bedside to population health, nursing research is at the forefront of translating basic science into clinical and community applications (Calzone et al., 2013; Henderson & Mudd-Martin, 2018). Improvement initiatives such as incorporating genomics or lifestyle interventions such as mindfulness in healthcare settings may deliver meaningful and necessary changes to patient care and outcomes; however, many improvement initiatives fail to sustain to a point where their full benefits can be realized. This points to the importance of nurse researchers and healthcare practitioners to develop frameworks, models, and tools to support and monitor the sustainability of these vital
integrative practices (Lennox, Maher, & Reed, 2018). Guidelines for this research path have been established in The Genomic Nursing State of the Science Initiative, a broad range of genomic nursing care and research topics mapped to the National Institute of Nursing Research Strategic Plan (2016) of health promotion and disease prevention, along with nursing practice innovation and training (Calzone et al., 2013). Though all nursing care has the potential to be affected by genomics and precision health, by taking an inclusive approach to diversity in this area of research, nurses will be well placed to be leaders in reducing health disparities through research, practice, and education (Taylor, & Mendoza, 2018).

Just as importantly as addressing individuals on the cellular level, proactively including underrepresented minorities in all phases of mindfulness and integrative medicine research is necessary to increase the generalizability of evidenced-based care. Waldron, Hong, and Moskowitz (2018) examined the socio-economic and demographic characteristics of adults enrolled in United States-based randomized controlled trials of mindfulness-based stress reduction and mindfulness-based cognitive therapy, finding a paucity of studies addressing vulnerable populations. Exploring 69 randomized controlled trials, the authors (2018) found only one study specifically aimed to test the mindfulness interventions in racial/ethnic minority or lower socio-economic status populations and none reported their effectiveness in these populations. Respecting the values of diversity, equity, and inclusion, along with developing an inclusive, pluralistic approach to health and well-being can advance understanding of the role that mindfulness and integrative medicine can play in achieving health justice and inclusion for vulnerable populations (Chau & Adler, 2018).
Summary

Chapter Five has addressed the findings and limitations of the study, as well as implications for future research. With new studies, with improved designs including larger sample and effect sizes, higher quality teaching training, measure of more than one inflammasome mRNA, and more accurate measures of the constructs of mindfulness and stress, there may be more significant findings on stress, mindfulness and mRNA-NLRP3 inflammasome genomic expression in this emerging field relevant to nursing science and all aspects of wellbeing.
APPENDIX A

Perceived Stress Scale
Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?

2. In the last month, how often have you felt that you were unable to control the important things in your life?

3. In the last month, how often have you felt nervous and “stressed”?

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

5. In the last month, how often have you felt that things were going your way?

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

7. In the last month, how often have you been able to control irritations in your life?

8. In the last month, how often have you felt that you were on top of things?

9. In the last month, how often have you been angered because of things that were outside of your control?
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
APPENDIX B

Five Factor Mindfulness Questionnaire
Five Factor Mindfulness Questionnaire

Please rate each of the following statements using the scale provided. Write the number in the blank that best describes your own opinion of what is generally true for you.

<table>
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<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td></td>
<td>never or very rarely true</td>
<td>rarely true</td>
<td>sometimes true</td>
<td>often true</td>
<td>very often or always true</td>
</tr>
</tbody>
</table>

_____ 1. When I’m walking, I deliberately notice the sensations of my body moving.
_____ 2. I’m good at finding words to describe my feelings.
_____ 3. I criticize myself for having irrational or inappropriate emotions.
_____ 4. I perceive my feelings and emotions without having to react to them.
_____ 5. When I do things, my mind wanders off and I’m easily distracted.
_____ 6. When I take a shower or bath, I stay alert to the sensations of water on my body.
_____ 7. I can easily put my beliefs, opinions, and expectations into words.
_____ 8. I don’t pay attention to what I’m doing because I’m daydreaming, worrying, or otherwise distracted.
_____ 9. I watch my feelings without getting lost in them.
_____ 10. I tell myself I shouldn’t be feeling the way I’m feeling.
_____ 11. I notice how foods and drinks affect my thoughts, bodily sensations, and emotions.
_____ 12. It’s hard for me to find the words to describe what I’m thinking.
_____ 13. I am easily distracted.
14. I believe some of my thoughts are abnormal or bad and I shouldn’t think that way.

15. I pay attention to sensations, such as the wind in my hair or sun on my face.

16. I have trouble thinking of the right words to express how I feel about things.

17. I make judgments about whether my thoughts are good or bad.

18. I find it difficult to stay focused on what’s happening in the present.

19. When I have distressing thoughts or images, I “step back” and am aware of the thought or image without getting taken over by it.

20. I pay attention to sounds, such as clocks ticking, birds chirping, or cars passing.

21. In difficult situations, I can pause without immediately reacting.

22. When I have a sensation in my body, it’s difficult for me to describe it because I can’t find the right words.

23. It seems I am “running on automatic” without much awareness of what I’m doing.

24. When I have distressing thoughts or images, I feel calm soon after.

25. I tell myself that I shouldn’t be thinking the way I’m thinking.

26. I notice the smells and aromas of things.

27. Even when I’m feeling terribly upset, I can find a way to put it into words.

28. I rush through activities without being really attentive to them.

29. When I have distressing thoughts or images I am able just to notice them without reacting.

30. I think some of my emotions are bad or inappropriate and I shouldn’t feel them.
31. I notice visual elements in art or nature, such as colors, shapes, textures, or patterns of light and shadow.

32. My natural tendency is to put my experiences into words.

33. When I have distressing thoughts or images, I just notice them and let them go.

34. I do jobs or tasks automatically without being aware of what I’m doing.

35. When I have distressing thoughts or images, I judge myself as good or bad, depending what the thought/image is about.

36. I pay attention to how my emotions affect my thoughts and behavior.

37. I can usually describe how I feel at the moment in considerable detail.

38. I find myself doing things without paying attention.

39. I disapprove of myself when I have irrational ideas.
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