Social Stress, Inflammatory Reactivity, and Depressive Symptoms

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy

in the Graduate School of The Ohio State University

By

Annelise A. Madison, M.A.

Graduate Program in Psychology

The Ohio State University

2023

Dissertation Committee:

Janice K. Kiecolt-Glaser, Advisor

Charles F. Emery

Michael Vasey

Abstract

Background: Approximately one-third of depression cases feature clinically elevated inflammation. The Social Signal Transduction Theory of Depression outlines one pathway to inflammation-driven depressive symptoms. It posits that those who report more frequent social stress and who have heightened inflammatory responses to an acute laboratory social stressor will have the greatest depressive symptom increases over time.

Aims: This series of studies features secondary analyses of data from parent trials among samples that were not recruited for clinical depression but rather had a range of depressive symptoms. It first tested the Social Signal Transduction Theory of Depression, and whether this pathway is specific to social stress. It also investigated whether omega-3 supplementation impedes this etiological pathway, especially among those who are socially stressed.

Methods: To test the Social Signal Transduction Theory of Depression, 76 physically healthy adults and 79 breast cancer survivors completed a laboratory social stressor (a marital conflict or the Trier Social Stress Test, respectively), had their blood drawn to assess inflammatory responsivity, and reported their stress exposure at baseline and their depressive symptoms at baseline and follow-up (one month later or four and eight months later, respectively). To test omega-3's effect on inflammatory responses, 138 middle-aged, sedentary adults were randomized to 2.5 g/day of omega-3, 1.25 g/day of omega-3, or placebo for four months. Before and after supplementation, they completed the Trier Social Stress Test and repeatedly had their blood drawn to assess inflammatory responsivity. The final study features secondary analyses from the same randomized, controlled trial to examine whether omega-3 reduced self-reported depressive symptoms among those who reported more social stress.

Results: In the first study, those who reported more frequent social stress, but not other types of stress, and had greater inflammatory responsivity at baseline had heightened depressive

ii

symptoms at follow-up. This effect was specific to social stress. In the second study, omega-3 supplementation promoted a more resilient physiological response to acute social stress, including lower inflammatory responsivity. In the final study, omega-3's antidepressant effect was greatest among overweight and obese individuals who reported frequent social tension, hostility, performance pressure, or lack of social recognition, but not other types of stress. These effects were largely replicated when using plasma levels of omega-3, rather than supplementation group.

Conclusions: Together, these findings provide strong support for the Social Signal Transduction Theory of Depression, showing the unique potency of social stress and inflammatory signaling in depression etiology. Strategies to reduce stress-induced inflammatory responses, such as omega-3 supplementation, may foster stress resilience and prevent depressive symptom worsening, especially among the socially-stressed who may be at risk for inflammation-related depressive symptoms.

Dedication

To my mentor, Dr. Jan Kiecolt-Glaser,

whose innovations laid the groundwork for and inspired my work,

and whose generous mentorship equipped me to forge my own path.

Acknowledgments

First and foremost, I am so grateful to my mentor, Dr. Jan Kiecolt-Glaser. Even with many competing demands, you have always prioritized developing my skills as a scientist, psychologist, and writer, creating new opportunities for me, and promoting my scholarship to others. Your work initially inspired me to pursue a career in psychoneuroimmunology, your mentorship prepared me for it, and your genuine encouragement gave me the confidence to do so. Also, thank you to my co-advisor, Dr. Charles Emery, who has been a continual source of guidance and wisdom. I have learned so much from you in every aspect of my training: coursework, clinical work, and research. Dr. Mike Vasey, thank you for serving on my thesis and dissertation committees. Much of what I learned in your psychopathology class several years ago informed my approach to this integrated dissertation. To all my committee members – your thoughtful feedback certainly enhanced this body of work.

I am thankful for the Department of Psychology and Institute for Behavioral Medicine Research. I have often marveled at the unique combination of rigorous training that I received here. I am also grateful for the funding I have received – both the Dean's Distinguished University Fellowship and the TL1 mentored training grant – which allowed me to develop my program of research. There are more resources and opportunities at The Ohio State University than I could have ever imagined. Many collaborators, including Dr. Baldwin Way, and collaboration opportunities have enriched my time here. I am so happy that I chose to come here for graduate training.

The Stress & Health Lab has been my home at The Ohio State University. I am so thankful to all full- and part-time staff and students who have worked tirelessly on the studies featured in this dissertation. Special thanks to Michael Di Gregorio for your knack in managing multiple studies at once and for your support, care, and encouragement. Thank you also to Dr.

Cathie Atkinson, whose amazing data management skills do not go unnoticed. Also, I very much appreciate Dr. Rebecca Andridge and Juan Peng's statistical expertise; thank you for always providing the most helpful consultation. I am also grateful to the post-doctoral and graduate students who have been in the lab, including Dr. Stephanie Wilson, Dr. Avelina Padin, Dr. Megan Renna, and Dr. Rosie Shrout. You all provided mentorship and wise council during brainstorming sessions.

Lastly, thank you to my family and friends, who have never wavered in your support. Special thanks to my mother and grandmother: I truly could not be where I am today without you both. My friends, you have walked alongside me and cheered me on throughout this journey. I love you all.

Vita

2010……………Norris High School, Firth, NE

2010……………National Merit Scholar

2010……………Better Business Bureau Student of Integrity

2010-2014…….Johnson Leadership Scholarship, Washington and Lee University (W&L)

2014……………Algernon Sydney Sullivan Award for Spiritual Maturity and Service, W&L

2014……………Pinney Prize for Nurturing University Intellectual Life, W&L

2014……………Mapleson Award for Best Overall Record at University, W&L

2014……………B.A. History and Political Science, W&L

2014……………NCAA Division III Academic All-American Women's Track/Cross Country Team

2017-2023……..Dean's Distinguished University Fellowship, The Ohio State University (OSU)

2018……………American Psychosomatic Society Trainee Scholar Award

2018……………Psychoneuroimmunology Research Society Trainee Scholar Award

2018……………Institute for Behavioral Medicine Best Poster, OSU

2019……………Edward F. Hayes Oral Presentation 2nd Place, OSU

2019……………M.A. Psychology, OSU

2019-2020……..Instructor of Record, Introduction to Psychology, OSU

2019-2022……..TL1 Mentored Training Grant, Center for Clinical and Translational Science

2021……………Edward F. Hayes Oral Presentation 1st Place, OSU

2022……………The Matrix Award for Excellence in Clinical Practice, OSU

2022……………Council of Graduate Studies Ray Award for Outstanding University Service

2023-2024……..Clinical Psychology Internship, VA Boston (Behavioral Medicine Track)

Peer-Reviewed Publications

Madison, A.A., Kiecolt-Glaser, J.K., Malarkey, W.B., Belury, M.A. (accepted). Omega-3 fatty acids reduce depressive symptoms only among the socially stressed: A corollary of the Social Signal Transduction Theory of Depression. *Health Psychology.*

Renna, M.E., Madison, A.A., Peng, J., Shrout, M.R., Lustberg, M., Ramaswamy, R.,Wesolowski, R., VanDeusen, J.B., Williams, N.O., Sardesai, S.D., Noonan, A.M., Reinbolt,

R.E., Stover, D.G., Cherian, M., Malarkey, W.B., Andridge, R., Kiecolt-Glaser, J.K. (accepted). Worry and mindfulness differentially impact symptom burden following treatment among breast cancer survivors: Findings from a randomized crossover trial. *Annals of Behavioral Medicine.*

Madison, A.A., Filatov, M., Andridge, R., Haas, G., Povoski, S.P., Agnese, D.M., Lustberg, M., Reinbolt, R.E., Wesolowski, R., Williams, N.O., Malarkey, W.B., Kiecolt-Glaser, J.K. (2023). A troubled heart: Mood disorder history longitudinally predicts faster cardiopulmonary aging among breast cancer survivors. *Plos One,* 18(3), e0283849.

Madison, A.A., Renna, M.E., Andridge, R.A., Peng, J., Shrout, M.R., Sheridan, J., Lustberg, M., Ramaswamy, B., Wesolowski, R., Williams, N.O., Noonan, A.M., Reinbolt, R.E., Stover, D.G., Cherian, M.A., Malarkey, W.B., Kiecolt-Glaser., J.K. (2023). Conflicts hurt: Social stress predicts elevated pain and sadness following mild inflammatory increases. *Pain,* 10-1097.

Shrout, M. R., Black, A. E., Wilson, S. J., Renna, M. E., Madison, A. A., Kiecolt-Glaser, J. K., & Reis, H. T. (2023). How aging couples' emotional and physiological associations change across positive, supportive, and conflictual discussions: Roles of capitalization and responsive behaviors. *Biological Psychology,* 177, 108500.

Shrout, M. R., Renna, M. E., Madison, A. A., Malarkey, W.B., & Kiecolt-Glaser, J.K. (2023). Marital negativity's festering wounds: The emotional, immunological, and relational toll of couples' negative communication patterns. *Psychoneuroendocrinology,* 149, 105989.

Shrout, M.R., Wilson, S.J., Renna, M.E., Madison, A.A., Kiecolt-Glaser, J.K. (2022). "We've got this:" Middle-aged and older couples' satisfying relationships and we-talk promote better physiological, relational, and emotional responses to conflict. *Psychosomatic Medicine,* 85(2), 154-164.

Madison, A.A., Way, B., Ratner, K., Renna, M.E., Andridge, R., Peng, J., Shrout, M.R., Sheridan, J., Lustberg, M., Ramaswamy, B., Wesolowski, R., VanDeusen, J.B., Williams, N.O., Sardesai, S.D., Noonan, A.M., Reinbolt, R.E., Stover, D.G., Cherian, M.A., Malarkey, W.B., Kiecolt-Glaser, J.K. (2022). Typhoid vaccine does not impact feelings of social connection or implicit or explicit social behavior in a randomized, placebo-controlled trial among middle-aged women. *Brain, Behavior, and Immunity,* 107: 124-131.

Renna, M.E., Shrout, M.R., Madison, A.A., Lustberg, M., Povoski, S.P., Agnese, D.M., Reinbolt, R.E., Wesolowski, R., Williams, N.O., Ramaswamy, B., Sardesai, S.D., Noonan, A.M., VanDeusen, J.F., Malarkey, W.B., Kiecolt-Glaser, J.K. (2022). Distress disorder history predicts increased physical symptoms in two samples of breast cancer survivors: Findings across the cancer trajectory. *International Journal of Behavioral Medicine,* 1-10.

Renna, M.E., Shrout, M.R., Madison, A.A., Alfano, C.M., Povoski, S.P., Lipari, A.M., Carson, W.E., Malarkey, W.B., Kiecolt-Glaser, J.K. (2022). Depression and anxiety in colorectal cancer patients: Ties to pain, fatigue, and inflammation. *Psycho-Oncology,* 31:1536–1544.

Madison, A.A., Kiecolt-Glaser, J.K. (2022). Are sick people really more impulsive?: Investigating inflammation-driven impulsivity. *Psychoneuroendocrinology,* 141: 105763.

Renna, M.E., Shrout, M.R., Madison, A.A., Bennett, J.M., Malarkey, W.B., Emery, C.F., Kiecolt-Glaser, J.K. (2022). Distress disorder histories predict heart rate variability trajectories during and after stress. *Psychoneuroendocrinology,* 135: 105575.

Madison, A.A., Andridge, R., Shrout, M.R., Renna, M.E., Bennett, J.M., Jaremka, L.M., Fagundes, C.P, Belury, M.A., Malarkey, W.B., Kiecolt-Glaser, J.K. (2022). Frequent interpersonal stress and inflammatory reactivity predict depressive symptom increases: Two tests of the Social Signal Transduction Theory of Depression. *Psychological Science,* 33:152-164.

Shrout, M. R., Madison, A.A., Renna, M. E., Alfano, C. M., Povoski, S. P., Lipari, A. M., Agnese, D. Carson III, W. E., Malarkey, W. B., Bailey, M. T., & Kiecolt-Glaser, J. K. (2021). The gut connection: Intestinal permeability as a pathway from breast cancer survivors' relationship satisfaction to inflammation across treatment. *Brain, Behavior, and Immunity,* 98: 46-47.

Madison, A.A. (2021). Boosting stress resilience using flexibility as a framework to reduce depression risk. *Brain, Behavior & Immunity – Health,* 18, 100357.

Madison, A.A., Peng, J., Renna, M.E., Shrout, M.R., Alfano, C., Povoski, S., Lipari, A., Agnese, D., Carson, W., Malarkey, W., Kiecolt-Glaser, J.K. (2021). Psychological distress trajectories in Black and White breast cancer survivors: From diagnosis to survivorship. *Psychoneuroendocrinology,* 131, 105288.

Madison, A.A., Belury, M.A., Andridge, R., Renna, M.E., Shrout, M.R., Malarkey, W.B., Lin, J., Epel, E.S., Kiecolt-Glaser, J.K (2021). Omega-3 supplementation and stress reactivity of cellular aging biomarkers: An ancillary substudy of a randomized, controlled trial in midlife adults. *Molecular Psychiatry,* 26: 3034-3042.

Madison, A.A., Way, B.M., Beauchaine, T.P., Kiecolt-Glaser, J.K. (2021). Risk assessment and heuristics: How cognitive shortcuts can fuel the spread of COVID-19. *Brain, Behavior, and Immunity,* 94, 6-7.

Kiecolt-Glaser, J.K., Wilson, S.J., Shrout, M.R., Madison, A.A., Andridge, R., Peng, J., Malarkey, W.B., Bailey, M.L. (2021). The gut reaction to couples' relationship troubles: A route to gut dysbiosis through changes in depressive symptoms. *Psychoneuroendocrinology,* 125, 105132.

Madison, A.A., Kiecolt-Glaser, J.K. (2021). The gut microbiota and nervous system: Age-defined and age-defying. *Seminars in Cell and Developmental Biology,* 116: 98-107.

Madison, A.A, Shrout, M.R., Renna, M.., Kiecolt-Glaser, J.K (2021). Psychological and behavioral predictors of vaccine responsiveness: Considerations for COVID-19. *Perspectives on Psychological Science,* 16(2), 191-203.

Shrout, M. R., Renna, M. E., Madison, A.A., Alfano, C. M., Povoski, S. P., Lipari, A. M., Agnese, D. M., Farrar, W. B., Carson III, W. E., & Kiecolt-Glaser, J. K. (2020). Breast cancer survivors'

satisfying marriages predict better psychological and physical health: A longitudinal comparison of satisfied, dissatisfied, and unmarried women. *Psycho-Oncology*, 30: 699-707.

Renna, M.E., Shrout, M.R., Madison, A.A., Jaremka, L.M., Alfano, C.M., Povoski, S.P., Agnese, D.M., Carson, W.E., Kiecolt-Glaser, J.K. (2021). Fluctuations in depression and anxiety predict dysregulated leptin among obese breast cancer survivors. *Journal of Cancer Survivorship, 15*(6).

Renna, M.E., Peng, J., Shrout, M.R., Madison, A.A., Andridge, R., Alfano, C.M., Povoski, S., Lipari, A., Malarkey, W.B., Kiecolt-Glaser, J.K. (2020). Childhood abuse histories predict steeper inflammatory trajectories across time. *Brain, Behavior, and Immunity,* 91, 541-545.

Renna, M.E., Shrout, M.R., Madison, A.A., Lustberg, M., Povoski, S.P., Agnese, D.M., Reinbolt, R.E., Wesolowski, R., Williams, N.O., Ramaswamy, B., Sadesai, S.D., Noonan, A.M., VanDeusen, J.B., Malarkey, W.B., Kiecolt-Glaser, J.K. (2020). Worry and rumination in breast cancer survivors: perseveration worsens self-rated health. *Journal of Behavioral Medicine,* 44, 253-259.

Renna, M.E., Shrout, M.R., Madison, A.A, Alfano, C.M., Povoski, S.P., Lipari, A.M., Agnese, D.M., Carson, W.E., Kiecolt-Glaser, J.K. (2020). Within-person changes in cancer-related distress predict breast cancer survivors' inflammation across treatment. *Psychoneuroendocrinology,* 121, 104866.

Madison, A.A., Andridge, R., Padin, A., Wilson, S., Bailey, M., Alfano, C., Povoski, S., Lipari, A., Agnese, D., Carson, W., Malarkey, W., Kiecolt-Glaser, J.K. (2020). Endotoxemia coupled with heightened inflammation predicts future depressive symptoms. *Psychoneuroendocrinology,* 122, 104864.

Shrout, M. R., Renna, M. E., Madison, A.A., Jaremka, L. M., Fagundes, C. P., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2020). Cortisol slopes and conflict: a spouse's stress matters. *Psychoneuroendocrinology,* 121, 104839.

Madison, A.A., Vasey, M., Emery, C., Kiecolt-Glaser, J.K. (2020). Social anxiety symptoms, Heart rate variability, and vocal emotion recognition in women: evidence for parasympathetically-mediated positivity bias. *Anxiety, Stress & Coping,* 34:3, 243-257.

Shrout, M. R., Renna, M. E., Madison, A.A., Alfano, C. M., Povoski, S. P., Lipari, A. M., Agnese, D. M., Yee, L. D., Carson III, W. E., & Kiecolt-Glaser, J. K. (2020). Relationship satisfaction predicts lower stress and inflammation in breast cancer survivors: A longitudinal study of within-person and between-person effects. *Psychoneuroendocrinology,* 118, 104708.

Kiecolt-Glaser, J.K., Renna, M.E., Shrout, M.R., Madison, A.A. (2020). Stress reactivity: what pushes us faster, higher, and longer – and why it matters. *Current Directions in Psychological Science.* 29(5), 492-498.

Madison, A.A., Belury, M.A., Andridge, R., Shrout, M.R., Renna, M.E., Malarkey, W.B., Bailey, M.T., Kiecolt-Glaser, J.K. (2020). Afternoon distraction: A high saturated fat meal and

endotoxemia impact post-meal attention in a randomized crossover trial. *American Journal of Clinical Nutrition*, 111(6), 1150-1158.

Madison, A.A., Woody, A., Bailey, B., Lustberg, M., Ramaswamy, B., Wesolowski, R., Williams, N., Reinbolt, R., VanDeusen, J., Sardesai, S, Malarkey, W., Kiecolt-Glaser, J.K. (2020). Cognitive problems of breast cancer survivors on proton pump inhibitors. *Journal of Cancer Survivorship,* 14(2): 226-234.

Madison, A.A., Kiecolt-Glaser, J.K. (2019). Stress and depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Current Opinion in Behavioral Sciences*, 28, 105-110.

Madison, A.A., Andersen, B., Ajam, A. (2019). Response to: "A somatization comorbidity phenotype impacts response to therapy in rheumatoid arthritis: post-hoc results from the certolizumab pegol phase 4 PREDICT trial." *Arthritis Research and Therapy,* 21(1), 65.

Kiecolt-Glaser, J., Wilson, S., Madison, A.A. (2018). Marriage and gut (microbiome) feelings: Tracing novel dyadic pathways to accelerated aging. *Psychosomatic Medicine,* 81(8), 704-710.

Book Chapter

Belury, M.A., Madison, A.A. (in press). Cardiometabolic Diseases. In J. Rippe (Ed.), *Lifestyle Medicine in Mental Health*. CRC.

Fields of Study

Major Field: Psychology

Concentrations: Clinical Psychology

                      Health Psychology

                      Graduate Interdisciplinary Specialization in Biomedical, Clinical, and Translational Science

Table of Contents

List of Tables

List of Supplemental Tables

List of Figures

List of Supplemental Figures

Chapter 1: Introduction

Subthreshold depressive symptoms that, by definition, do not meet Diagnostic and Statistical Manual – V (DSM-V) criteria for a mood disorder, are clinically meaningful and predict poorer outcomes over time (Lewinsohn et al., 2000). For example, adults with subthreshold depression had increased limitations in performing independent activities of daily living three to four years later (Hybels et al., 2009). Indeed, there is evidence of a dose-response relationship between depressive symptoms and health (Cronin-Stubbs et al., 2000; Lyness et al., 2006; Wagner et al., 2000). Moreover, there was a 1.65% work productivity loss for every one-point increase in Patient Health Questionnaire 9-item screen score, such that even relatively minor depressive symptomology was associated with productivity losses (Beck et al., 2011). Also, subthreshold depressive symptoms can be persistent (Meeks et al., 2011) and have the propensity to convert to clinical depression (Cuijpers et al., 2005; Cuijpers & Smit, 2004; Wagner et al., 2000). Once that happens, clinical depression is notoriously difficult to treat (Souery et al., 2006). Therefore, pinpointing factors that worsen and mitigate depressive symptoms is meaningful from the perspectives of public health and of the individual.

This compendium of work examines two such factors that may modulate depressive symptoms: the magnitude of the body's inflammatory response to stress, as well as frequency of social stress. The Social Signal Transduction Theory of Depression posits that those who often experience social tension in their daily lives and show exaggerated inflammatory reactivity to an acute psychosocial stressor in the lab may be the most at risk for depressive symptom increases over time (Slavich & Irwin, 2014). Chapter 2 provides the first empirical test of this theory in two samples. The combination of heightened reactivity to each stressor and frequent stress exposure may prevent recovery, or return to baseline, of key inflammatory markers. Chronic systemic inflammation is a harbinger and concomitant of inflammatory diseases (Liu et

al., 2017), including a subtype of depression that appears to be driven by inflammation (Beijers et al., 2019).

Using the Social Signal Transduction Theory of Depression as a guide, it follows that reducing inflammatory reactivity to social stress could be a clinically meaningful goal. Chapter 3 examines a widely-available, inexpensive, over-the-counter supplement – omega-3 fatty acid – as one strategy to reduce inflammatory reactivity. Lastly, Chapter 4 builds on the prior two chapters to explore whether omega-3's antidepressant effect is most pronounced among those who frequently experience social stress. The remainder of this introduction provides more detail on the theoretical framework and biological underpinnings relevant to the studies featured in the next three chapters. Notably, the studies focus on the continuum of depressive symptoms in non-clinical populations, rather than clinical depression, due to their prevalence, persistence, negative health impact, and tendency to worsen to clinical depression. Thus, this work speaks to symptom alleviation and depression prevention rather than treatment of clinical depression.

Biological Underpinnings: From Psychological Stress to Inflammation

The immune system responds to stressors with a similar, yet muted, inflammatory response as it does to pathogens. In fact, although other laboratory paradigms (e.g., the Stroop Color-Word Interference Test) can induce the physiological stress response, the Trier Social Stress Test (Kirschbaum et al., 1993), featuring a speech and challenging arithmetic task in the presence of straight-faced judges, is widely used due to its nearly universal neuroendocrine and inflammatory stress response generation. The neuroendocrine stress response directly interacts with the immune system. For instance, catecholamines (e.g., adrenaline, noradrenaline) that are released almost immediately upon stress exposure can directly bind to receptors on lymphocytes and increase proinflammatory signaling. The endocrine system eventually

counterbalances this proinflammatory state with the slow and gradual release of cortisol, which exerts an anti-inflammatory effect.

From an evolutionary standpoint, a strong physiological response to psychological stress was highly advantageous, as those who could fight better or flee faster were more likely to survive acute, life-threatening situations. Although there are still some modern-day situations that require swift action (e.g., a car speeding toward an individual in a crosswalk), most stressors are chronic and non-life threatening, such as a nagging boss. In fact, there are many modern stressful situations in which the best response is neither fighting nor fleeing, and yet the strong phylogenetically-preserved "fight or flight" physiological response still occurs. Such a response is not sustainable over an extended timeframe, and the body fatigues. Depleted reserves do not allow for situation-dependent responses; rather, the body may respond in a stereotyped manner to any kind of perceived threat. These inflexible responses do not align with the intensity or type of stressor and ultimately fuel poor recovery and a higher baseline (e.g., of inflammation) (Madison, 2021). In particular, early life stress, such as childhood adversity or trauma can prime immune cells to mount exaggerated responses to psychological stress even into adulthood (Cole, 2019).

A heightened inflammatory response to stress may not be adaptive in the context of modern stressors, which are generally chronic, not life-threatening, and do not usually result in wounding. In their Pathogen Host Theory of Depression, Raison and Miller (2013) highlight the fact that the same genetic alleles that facilitate a robust innate immune response to pathogens are also associated with greater depression risk (Raison & Miller, 2013) This theory helps to explain why these alleles have been preserved in the genome across generations despite their connection to depression (Raison & Miller, 2013). Although these alleles are adaptive in the fight against infectious disease, they may fuel the inflammatory subtype of depression among

3

people living in places where non-life threatening, chronic psychosocial stress, rather than pathogens, is the biggest threat (Raison & Miller, 2013). In some cases, though, a strong inflammatory response to even non-life-threatening social stressors may be adaptive. Although the response does not directly address or "fight" the psychosocial stressor, it can facilitate the same behavioral sequalae that ultimately conserve energy and promote recovery (Kemeny et al., 2004). In addition, the immune system's response to social stressors, particularly those that may result in social exclusion – like negative evaluation or conflict –, prepares the body for a possible attack or wounding that is more likely to happen when separated from the group (Slavich & Irwin, 2014).

Individuals differ in the magnitude and length of their physiological stress response to psychological stress (Marsland et al., 2017; Prather et al., 2009). Although few moderators have been examined more than once, results show that psychosocial (e.g., loneliness, high effort-reward imbalance) and biological (e.g., smoking, poor physical fitness, obesity, sleep deficiency) health risk factors associate with greater stress-induced changes in inflammatory markers (Marsland et al., 2017). Individual-level factors, such as depression, rumination, early life adversity, and high perceived stress, and dyadic-level factors, such as co-rumination and hostility, can heighten and prolong stress responses (Kiecolt-Glaser et al., 2020). Stressor type and duration can also impact the response trajectory, as personally relevant stressors over which the individual has little control or few resources with which to cope, are particularly detrimental (Kiecolt-Glaser et al., 2020).

The Relevance of Social Stress

Psychological stress is a non-specific term that refers to many types of situations with differing severity and duration. Many researchers have asserted the need for more specific terminology related to stress, with some arguing to abandon the term altogether. There is even

a lack of consensus surrounding whether stress is a universally negative experience, as the term "eustress" describes when positive life events trigger the physiological stress response (Szabo, 2020). There are many perspectives on how to classify stress, including: (1) degree of change or upheaval (Holmes & Rahe, 1967); (2) degree of controllability (Seligman, 1972); (3) degree of balance between demands and resources (Lazarus & Folkman, 1984); (4) degree that the stressor matches individual's cognitive vulnerability (e.g., a rejection-sensitive person is not invited to a party) (Clark et al., 2000); and (5) degree of threat to social status (Dickerson et al., 2004). These perspectives create a largely non-overlapping framework for classifying stress. in a recent review, Slavich (2020) proposed that the way that physiological stress response systems respond to a variety of situations may provide clues for a more unified conceptualization of stress. In his Social Safety Theory, he asserted that the physiological stress response is primed to strongly respond to situations that threaten fundamental social bonds because such bonds are critical to reproduction and survival in a world filled with physical and microbial challenges (Slavich, 2020). Although a recent meta-analysis did not find a difference between inflammatory responses generated by social stress versus non-social stress (Marsland et al., 2017), we have found that those who report chronic or repetitive social stress, especially conflict, are more psychologically vulnerable to inflammation (Madison, Andridge, et al., 2021; Madison et al., 2022). In short, each social stressor promotes inflammation, and chronic, underlying social stress predicts lower mood following inflammatory rises.

Biological Underpinnings: From Inflammation to Mood

The pathway from psychosocial stress to inflammation is well-established and not as novel as the subsequent pathway – from inflammation to mood. One-third to one-half of depressed patients have clinically elevated systemic inflammation (e.g., C-reactive protein or CRP > 3 mg/L), suggesting an inflammation-associated subtype of depression (Raison & Miller,

2011; Rethorst et al., 2014). This relationship exists even when the depressive symptoms are not severe enough to meet a diagnostic threshold: a recent meta-regression showed that higher levels of two common inflammatory markers (CRP and interleukin-6 or IL-6) longitudinally predicted depressive symptom increases (Mac Giollabhui et al., 2020). Additionally, even when inflammation is not clinically elevated, certain individuals may be psychologically sensitive to inflammation's mood and behavioral effect, such that low levels of inflammation drive depressive symptoms (Pariante, 2021). Experimental paradigms (e.g., lipopolysaccharide administration in randomized, controlled trials among healthy individuals) and treatment paradigms (e.g., interferon alpha administration for chronic viral infections or cancer) support the causal nature of this relationship, such that even one administration of a potent inflammatory stimulus can provoke negative affect, anhedonia, and social withdrawal, and multiple administrations can onset clinical depression (Capuron et al., 2004; Eisenberger et al., 2009, 2010; Raison et al., 2006).

Mechanistically, heightened inflammation can decrease dopamine synthesis and release, which helps to explain why anhedonia may be a primary symptom of the inflammatory subtype of depression (Felger, 2016; Haroon et al., 2018). Essentially, inflammation can dysregulate reward and motivation circuitry (Felger, 2016). It can also reduce serotonin synthesis, as it metabolizes tryptophan, the serotonin-precursor, into kynurenine, which can ultimately lead to the production of neurotoxic compounds and increased glutamate (Dantzer et al., 2011). Heightened levels of glutamate can alter neurocircuits involved in depression, including the basal ganglia to prefrontal circuit responsible for reward responses and motivation (Felger, 2018). Moreover, inflammation increases oxidative stress, and reactive oxygen species inhibit the synthesis of monoamines (serotonin, norepinephrine, dopamine) (Felger, 2018). Peripheral inflammation can also impact the brain and behavior via signaling from afferent vagal

nerve endings (Pavlov & Tracey, 2012). After an onslaught of inflammation, the resulting

behavioral phenotype depends upon many factors including: timing of the inflammatory spike,

other neurocircuitry (e.g., amgydala function and level of trait anxiety), as well as environmental

context (Madison & Kiecolt-Glaser, 2022).

Inflammation in the central nervous system can directly interact with the brain and

therefore may seem to be more relevant to mood and behavior. Central inflammation, measured

in cerebral spinal fluid (CSF) collected in a lumbar puncture, is much more costly and difficult to

collect than peripheral inflammation, measured from serum or plasma collected via a simple

blood draw. A recent study suggests that lumbar punctures may not be necessary: Among

depressed patients, peripheral CRP was strongly related to CSF CRP (r=0.855), which itself

tracked with CSF cytokine receptors (Felger et al., 2018). Additionally, peripheral IL-6 and

another proinflammatory cytokine, tumor necrosis factor-alpha (TNF-α), also correlated with

CSF CRP, although not as strongly (r=0.442, r=0.360, respectively) (Felger et al., 2018).

Peripheral CRP was also associated with a cluster of CSF inflammatory markers that

themselves predicted depression severity, especially symptoms related to reward and

motivation (Felger et al., 2018). These findings suggest that peripheral inflammatory markers,

which are cheaper and non-invasive, provide a window into the brain's inflammatory state.

Thus, peripheral inflammation is the focus of the three studies included in this document.

Detailed Outline and Justification of the Current Work

The Social Signal Transduction Theory of Depression

As noted above, Slavich's Social Signal Transduction Theory of Depression explains

how repetitive social stress can promote depressive symptoms (Slavich & Irwin, 2014).

Specifically, this theory suggests that heightened inflammatory reactivity to social stress is

problematic and depressogenic in the context of frequent interpersonal conflict and tension

(Slavich & Irwin, 2014). Chronic and repeated conflict or other social stressors may prevent full recovery and drive up systemic inflammation (Madison, 2021). There has not been a satisfactory test of this theory, as the only test to date used salivary cytokine reactivity to social stress (Slavich et al., 2020), which may reflect oral hygiene and pathology more so than systemic inflammation (Korte & Kinney, 2016). Chapter 2 features a test of this theory via secondary analyses of two different samples, each with a distinct laboratory psychosocial stressor – the Trier Social Stress Test and a marital conflict. These studies assessed depressive symptoms repeatedly across time and use serum cytokines to index inflammatory reactivity. Ultimately, both samples provided evidence to support this theory, and these results highlight frequent social stress – rather than non-social stress – as paramount in depression symptom worsening. This article appeared in *Psychological Science* (Madison et al., 2021)*.*

Reducing the Inflammatory Response to Psychosocial Stress To Prevent Depression

The idea that inflammation can provoke low mood is appealing due to the relatively straightforward treatment and prevention options. For example, three infusions of the proinflammatory cytokine antagonist infliximab reduced depressive symptoms among those who were resistant to other antidepressant treatments and had clinically-elevated levels of inflammation prior to treatment initiation (Raison et al., 2013). However, each infliximab infusion costs nearly $1,000 and takes about two hours in the clinic to administer (Bonafede et al., 2012). Therefore, even among depressed patients who are known to have clinically-elevated levels of inflammation, proinflammatory cytokine antagonist treatment is currently too costly and burdensome to become a first-line treatment. In short, for most patients with the inflammatory subtype of depression, proinflammatory cytokine antagonists are not an option. Instead, such patients fail multiple traditional antidepressant treatments and assume the label of "treatment-resistant."

Identifying anti-inflammatory agents that are safe, easy-to-use, and available over-the-counter is one depression prevention strategy. Omega-3 supplementation reduces basal inflammation, can be administered at home, and is safe and well-tolerated at even relatively high doses (Kiecolt-Glaser et al., 2012). In our omega-3 randomized, placebo-controlled clinical trial, four months of supplementation with 2.5 g/d of omega-3 resulted in a 12% decrease in IL-6, compared to a 36% increase in the placebo group, and 2.3% decrease in TNF-α, compared to a 12% increase in the placebo group. There were no group differences in participant reports of nonserious adverse events, and participants returned less than 4% of pills, suggesting high adherence.

Based on the results featured in Chapter 2, reducing inflammatory responsivity to social stress may be particularly relevant to depression risk. In the same omega-3 randomized, controlled trial discussed above, participants completed the Trier Social Stress Test at baseline and then again after four months of supplementation or placebo. At these visits, reactivity of biomarkers relevant to aging were measured, including salivary cortisol, telomerase from peripheral blood lymphocytes, and serum anti-inflammatory and pro-inflammatory cytokines. In secondary analyses, I found that 2.5 g/d of omega-3 reduced cortisol by 19% and IL-6 by 33% throughout the stressor, compared to the placebo, and it prevented the 26% post-stress decline in interleukin-10 (IL-10; an anti-inflammatory cytokine) that was observed in the placebo group. Chapter 3 features the resulting paper, which was published in *Molecular Psychiatry* (Madison et al., 2021).

Omega-3 Supplementation, Social Stress, and Depression

If omega-3 supplementation can reduce inflammatory reactivity to an acute psychosocial stressor, then given the above literature review, it might follow that it can also reduce depressive symptoms. Multiple meta-analyses have found that omega-3 supplementation reduces depressive symptoms (Liao et al., 2019; Mocking et al., 2016). In these randomized, controlled trials, omega-3 supplementation typically exerts a medium effect, compared to placebo, with certain groups of people (i.e., antidepressant users) finding more benefit (Liao et al., 2019; Mocking et al., 2016). However, several individual randomized, controlled trials, including two from our lab (Kiecolt-Glaser, Belury, Andridge, Malarkey, & Glaser, 2011; Kiecolt-Glaser et al., 2012), have failed to find an anti-depressant effect, suggesting that there may be key moderators. As the Social Signal Transduction Theory of Depression (and Chapter 2's results) suggest, frequency of social stress may modulate omega-3 supplementation's effect on depressive symptoms. That is, those who experience more frequent social stress may have the greatest depressive symptom reduction when they supplement with omega-3, given that it reduces acute inflammatory reactivity, to which they are more psychologically susceptible (Madison, Andridge, et al., 2021; Madison et al., 2022). Chapter 4 features secondary analyses of our randomized, controlled trial to examine whether omega-3's antidepressant effect depends on frequency of social stress exposure. In these analyses, I found that omega-3 had an anti-depressant effect only among those who reported more frequent hostility, social tension, performance pressure, and lack of social recognition. However, this effect was not evident among those with frequent work-related stress. Importantly, these findings were largely corroborated when substituting plasma omega-3 levels for supplementation group. Thus, Chapter 4's results provide additional support for the Social Signal Transduction Theory of Depression.

Chapter 1 References

Beck, A., Crain, A. L., Solberg, L. I., Unützer, J., Glasgow, R. E., Maciosek, M. V., & Whitebird, R. (2011). Severity of depression and magnitude of productivity loss. *The Annals of Family Medicine*, *9*(4), 305–311.

Beijers, L., Wardenaar, K. J., van Loo, H. M., & Schoevers, R. A. (2019). Data-driven biological subtypes of depression: Systematic review of biological approaches to depression subtyping. *Molecular Psychiatry*, *24*(6), 888–900.

Bonafede, M. M., Gandra, S. R., Watson, C., Princic, N., & Fox, K. M. (2012). Cost per treated patient for etanercept, adalimumab, and infliximab across adult indications: A claims analysis. *Advances in Therapy*, *29*(3), 234–248.

Capuron, L., Ravaud, A., Miller, A. H., & Dantzer, R. (2004). Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. *Brain, Behavior, and Immunity*, *18*(3), 205–213.

Clark, D. A., Beck, A. T., Alford, B. A., Bieling, P. J., & Segal, Z. V. (1999). *Scientific foundations of cognitive theory and therapy of depression.* Wiley.

Cole, S. W. (2019). The conserved transcriptional response to adversity. *Current Opinion in Behavioral Sciences*, *28*, 31–37.

Cronin-Stubbs, D., De Leon, C. F. M., Beckett, L. A., Field, T. S., Glynn, R. J., & Evans, D. A. (2000). Six-year effect of depressive symptoms on the course of physical disability in community-living older adults. *Archives of Internal Medicine*, *160*(20), 3074–3080.

Cuijpers, P., & Smit, F. (2004). Subthreshold depression as a risk indicator for major depressive disorder: A systematic review of prospective studies. *Acta Psychiatrica Scandinavica*, *109*(5), 325–331.

Cuijpers, P., Smit, F., & Willemse, G. (2005). Predicting the onset of major depression in

    subjects with subthreshold depression in primary care: A prospective study. *Acta*

    Psychiatrica Scandinavica, *111*(2), 133–138.

Dantzer, R., O'Connor, J. C., Lawson, M. A., & Kelley, K. W. (2011). Inflammation-associated

    depression: From serotonin to kynurenine. *Psychoneuroendocrinology*, *36*(3), Article 3.

Dickerson, S. S., Gruenewald, T. L., & Kemeny, M. E. (2004). When the social self is

    threatened: Shame, physiology, and health. *Journal of Personality*, *72*(6), 1191–1216.

Eisenberger, N. I., Berkman, E. T., Inagaki, T. K., Rameson, L. T., Mashal, N. M., & Irwin, M. R.

    (2010). Inflammation-induced anhedonia: Endotoxin reduces ventral striatum responses

    to reward. *Biological Psychiatry*, *68*(8), 748–754.

Eisenberger, N. I., Inagaki, T. K., Rameson, L. T., Mashal, N. M., & Irwin, M. R. (2009). An fMRI

    study of cytokine-induced depressed mood and social pain: The role of sex differences.

    Neuroimage, *47*(3), 881–890.

Felger, J. C. (2016). The role of dopamine in inflammation-associated depression: Mechanisms

    and therapeutic implications. *Inflammation-Associated Depression: Evidence,*

    Mechanisms and Implications, 199–219.

Felger, J. C. (2018). Imaging the role of inflammation in mood and anxiety-related disorders.

    Current Neuropharmacology, *16*(5), 533–558.

Felger, J. C., Haroon, E., Patel, T. A., Goldsmith, D. R., Wommack, E. C., Woolwine, B. J., Le,

    N.-A., Feinberg, R., Tansey, M. G., & Miller, A. H. (2018). What does plasma CRP tell us

    about peripheral and central inflammation in depression? *Molecular Psychiatry*, 1–11.

Haroon, E., Chen, X., Li, Z., Patel, T., Woolwine, B. J., Hu, X. P., Felger, J. C., & Miller, A. H.

    (2018). Increased inflammation and brain glutamate define a subtype of depression with

decreased regional homogeneity, impaired network integrity, and anhedonia. *Translational Psychiatry*, *8*(1), Article 1.

Holmes, T. H., & Rahe, R. H. (1967). The social readjustment rating scale. *Journal of Psychosomatic Research*.

Hybels, C. F., Pieper, C. F., & Blazer, D. G. (2009). The complex relationship between depressive symptoms and functional limitations in community-dwelling older adults: The impact of subthreshold depression. *Psychological Medicine*, *39*(10), 1677–1688.

Kemeny, M. E., Gruenewald, T. L., & Dickerson, S. S. (2004). Shame as the emotional response to threat to the social self: Implications for behavior, physiology, and health. *Psychological Inquiry*, *15*(2), 153–160.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., & Glaser, R. (2011). Omega-3 supplementation lowers inflammation and anxiety in medical students: A randomized controlled trial. *Brain, Behavior, and Immunity*, *25*(8), Article 8.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., Hwang, B. S., & Glaser, R. (2012). Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: A randomized controlled trial. *Brain, Behavior, and Immunity*, *26*(6), 988–995.

Kiecolt-Glaser, J. K., Renna, M. E., Shrout, M. R., & Madison, A. A. (2020). Stress reactivity: What pushes us higher, faster, and longer—And why it matters. *Current Directions in Psychological Science*, *29*(5), 492–498.

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81.

Korte, D. L., & Kinney, J. (2016). Personalized medicine: An update of salivary biomarkers for periodontal diseases. *Periodontology 2000*, *70*(1), 26–37.

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer publishing

    company.

Lewinsohn, P. M., Solomon, A., Seeley, J. R., & Zeiss, A. (2000). Clinical implications of

    "subthreshold" depressive symptoms. *Journal of Abnormal Psychology*, *109*(2), 345.

Liao, Y., Xie, B., Zhang, H., He, Q., Guo, L., Subramaniapillai, M., Fan, B., Lu, C., & McIntyer,

    R. (2019). Efficacy of omega-3 PUFAs in depression: A meta-analysis. *Translational*

    Psychiatry, *9*(1), 1–9.

Liu, C. H., Abrams, N. D., Carrick, D. M., Chander, P., Dwyer, J., Hamlet, M. R., Macchiarini, F.,

    PrabhuDas, M., Shen, G. L., & Tandon, P. (2017). Biomarkers of chronic inflammation in

    disease development and prevention: Challenges and opportunities. *Nature*

    immunology, 18(11), 1175-1180.

Lyness, J. M., Heo, M., Datto, C. J., Ten Have, T. R., Katz, I. R., Drayer, R., Reynolds III, C. F.,

    Alexopoulos, G. S., & Bruce, M. L. (2006). Outcomes of minor and subsyndromal

    depression among elderly patients in primary care settings. *Annals of Internal Medicine*,

    144(7), 496–504.

Mac Giollabhui, N., Ng, T. H., Ellman, L. M., & Alloy, L. B. (2020). The longitudinal associations

    of inflammatory biomarkers and depression revisited: Systematic review, meta-analysis,

    and meta-regression. *Molecular Psychiatry*, 1–13.

Madison, A. A. (2021). Boosting stress resilience using flexibility as a framework to reduce

    depression risk. *Brain, Behavior, & Immunity-Health*, 100357.

Madison, A. A., Andridge, R., Shrout, M. R., Renna, M. E., Bennett, J. M., Jaremka, L. M.,

    Fagundes, C. P., Belury, M. A., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2021). Frequent

    interpersonal stress and inflammatory reactivity predict depressive symptom increases:

Two tests of the social signal transduction theory of depression. *Psychological Science*, *33*(1), 152–164.

Madison, A. A., Belury, M. A., Andridge, R., Renna, M. E., Shrout, M. R., Malarkey, W. B., Lin, J., Epel, E. S., & Kiecolt-Glaser, J. K. (2021). Omega-3 supplementation and stress reactivity of cellular aging biomarkers: An ancillary substudy of a randomized, controlled trial in midlife adults. *Molecular Psychiatry*, 1–9.

Madison, A. A., & Kiecolt-Glaser, J. (2022). Are sick people really more impulsive?: Investigating the inflammation-present focus link. *Psychoneuroendocrinology*, 105763.

Madison, A. A., Renna, M., Andridge, R., Peng, J., Shrout, M. R., Sheridan, J., Lustberg, M., Ramaswamy, B., Wesolowski, R., & Williams, N. O. (2022). Conflicts hurt: Social stress predicts elevated pain and sadness after mild inflammatory increases. *Pain*, 10–1097.

Marsland, A. L., Walsh, C., Lockwood, K., & John-Henderson, N. A. (2017). The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *64*, 208–219.

Meeks, T. W., Vahia, I. V., Lavretsky, H., Kulkarni, G., & Jeste, D. V. (2011). A tune in "a minor" can "b major": A review of epidemiology, illness course, and public health implications of subthreshold depression in older adults. *Journal of Affective Disorders*, *129*(1), Article 1.

Mocking, R., Harmsen, I., Assies, J., Koeter, M., Ruhé, H., & Schene, A. (2016). Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. *Translational Psychiatry*, *6*(3), e756–e756.

Pariante, C. M. (2021). Increased Inflammation in Depression: A Little in All, or a Lot in a Few? *American Journal of Psychiatry*, *178*(12), 1077–1079.

Pavlov, V. A., & Tracey, K. J. (2012). The vagus nerve and the inflammatory reflex—Linking immunity and metabolism. *Nature Reviews Endocrinology*, *8*(12), 743–754.

Prather, A. A., Carroll, J. E., Fury, J. M., McDade, K. K., Ross, D., & Marsland, A. L. (2009).

Gender differences in stimulated cytokine production following acute psychological

stress. *Brain, Behavior, and Immunity*, *23*(5), 622–628.

Raison, C. L., Capuron, L., & Miller, A. H. (2006). Cytokines sing the blues: Inflammation and

the pathogenesis of depression. *Trends in Immunology*, *27*(1), Article 1.

Raison, C. L., & Miller, A. (2013). The evolutionary significance of depression in Pathogen Host

Defense (PATHOS-D). *Molecular Psychiatry*, *18*(1), Article 1.

Raison, C. L., & Miller, A. H. (2011). Is depression an inflammatory disorder? *Current Psychiatry*

Reports, *13*(6), 467–475.

Raison, C. L., Rutherford, R. E., Woolwine, B. J., Shuo, C., Schettler, P., Drake, D. F., Haroon,

E., & Miller, A. H. (2013). A randomized controlled trial of the tumor necrosis factor

antagonist infliximab for treatment-resistant depression: The role of baseline

inflammatory biomarkers. *JAMA Psychiatry*, *70*(1), Article 1.

Rethorst, C. D., Bernstein, I., & Trivedi, M. H. (2014). Inflammation, obesity and metabolic

syndrome in depression: Analysis of the 2009–2010 National Health and Nutrition

Survey (NHANES). *The Journal of Clinical Psychiatry*, *75*(12), e1428.

Seligman, M. E. (1972). Learned helplessness. *Annual Review of Medicine*, *23*(1), 407–412.

Slavich, G. M. (2020). Social safety theory: A biologically based evolutionary perspective on life

stress, health, and behavior. *Annual Review of Clinical Psychology*, *16*.

Slavich, G. M., Giletta, M., Helms, S. W., Hastings, P. D., Rudolph, K. D., Nock, M. K., &

Prinstein, M. J. (2020). Interpersonal life stress, inflammation, and depression in

adolescence: Testing Social Signal Transduction Theory of Depression. *Depression and*

Anxiety, *37*(2), 179–193.

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive

  disorder: A social signal transduction theory of depression. *Psychological Bulletin*,

  140(3), Article 3.

Souery, D., Papakostas, G. I., & Trivedi, M. H. (2006). Treatment-resistant depression. *Journal*

  of Clinical Psychiatry, *67*, 16.

Szabo, S. (2020). The Origins & Evolution of Stress Research: From Distress to Eustress. *The*

  FASEB Journal, *34*(S1), 1–1.

Wagner, H. R., Burns, B., Broadhead, W., Yarnall, K., Sigmon, A., & Gaynes, B. (2000). Minor

  depression in family practice: Functional morbidity, co-morbidity, service utilization and

  outcomes. *Psychological Medicine*, *30*(6), 1377–1390.

Chapter 2: Initial Empirical Test of the Social Signal Transduction Theory

(Published in *Psychological Science*)

Frequent Interpersonal Stress and Inflammatory Reactivity

Predict Depressive Symptom Increases:

Two Tests of the Social Signal Transduction Theory of Depression

Annelise A. Madison, M.A.[1,2], Rebecca Andridge, Ph.D. [1,3], M. Rosie Shrout, Ph.D. [1], Megan

E. Renna, Ph.D. [1], Jeanette M. Bennett, Ph.D.[4], Lisa M. Jaremka, Ph.D. [5], Christopher P.

Fagundes, Ph.D. [6], Martha A. Belury, Ph.D. [1,7], William B. Malarkey, M.D. [1,8], Janice K.

Kiecolt-Glaser, Ph.D.[1,9]

(1) Institute for Behavioral Medicine Research, The Ohio State University College of Medicine,

(2) Department of Psychology, The Ohio State University, (3) College of Public Health, The

Ohio State University, (4) Department of Psychological Science, University of North Carolina –

Charlotte, (5) Department of Psychology and Brain Sciences, University of Delaware, (6)

Department of Psychology, Rice University, (7) Department of Human Sciences, College of

Education and Human Ecology, The Ohio State University (8) Department of Internal Medicine,

The Ohio State University College of Medicine (9) Department of Psychiatry and Behavioral

Health, The Ohio State University College of Medicine

Corresponding author: Janice Kiecolt-Glaser, Institute for Behavioral Medicine Research, The
Ohio State University College of Medicine, 460 Medical Center Drive, Columbus, OH 43210,
614-293-3499 (telephone), 614-366-2097 (fax), Janice.Kiecolt-Glaser@osumc.edu.
Short Title: Interpersonal Stress, Inflammatory Reactivity, and Depression

Abstract

The Social Signal Transduction Theory of Depression asserts that those who experience ongoing interpersonal stressors and mount a greater inflammatory response to social stress are at higher risk for depression. The current study tests this theory among two adult samples. In Study 1, physically healthy adults (N=76) who reported more frequent interpersonal tension had heightened depressive symptoms at Visit 2, but only if they had greater inflammatory reactivity to a marital conflict at Visit 1. Similarly, in Study 2, depressive symptoms increased among lonelier and less socially supported breast cancer survivors (N=79). This effect was most pronounced among those with higher inflammatory reactivity to a social-evaluative stressor at Visit 1. In both studies, non-interpersonal stress did not interact with inflammatory reactivity to predict later depressive symptoms.

Statement of Relevance

Psychological stress triggers depression in some people but not others. Characteristics of the stressor and of the individual can help to determine depression onset. For instance, depression is more likely if the stressor is social in nature, long-lasting, or frequent. The Social Signal Transduction Theory of Depression suggests that those who have more frequent social stress and who have higher levels of inflammation in response to this stress are more likely to develop depression over time. The current study tests this theory in two distinct samples – physically healthy adults and breast cancer survivors – and findings support the theory. In line with prior research, our results suggest that social stress is more relevant than non-social stress to depression. These findings point to social stress and inflammation as prime targets for depression treatment and prevention.

Keywords: Inflammation, Depression, Social, Interpersonal, Stress

Introduction

Although inflammation is an adaptive response to injury meant to facilitate healing, chronic inflammation can erode mental and physical health. A significant subset of depressed individuals has chronic inflammation (Kiecolt-Glaser, Derry, et al., 2015), and depressed individuals have greater inflammatory reactivity to laboratory social stressors than their non-depressed peers (Fagundes et al., 2013; G. E. Miller et al., 2005; Pace et al., 2006). The Social Signal Transduction Theory of Depression posits that elevated inflammatory reactivity is not only a correlate, but also a risk factor for depression – especially in the context of chronic or repetitive interpersonal stress (Slavich & Irwin, 2014).

Interpersonal stress, including objective threats like social isolation or perceived threats like loneliness, robustly predicts depression (Slavich, 2020). For instance, individuals who experienced chronic interpersonal stress or a recent major interpersonal stressful life event had an enhanced risk of depression onset, compared to their less-stressed peers (Vrshek-Schallhorn et al., 2015). In addition, among those who were already experiencing high depressive symptoms, interpersonal stressors preceded a symptom spike, which did not occur following non-interpersonal stressors (Gunthert et al., 2007).

Inflammation may mechanistically link interpersonal stress and depression. Inflammatory cytokines are the primary transducers of social signals, as they can mediate context-appropriate physiological, cognitive, and behavioral shifts (Slavich, 2020). For example, healthy young women who reported higher levels of interpersonal stress had greater expression of pro- and anti-inflammatory signaling molecules six months later (Miller, Rohleder, & Cole, 2009). Acute social stress also provokes a strong but transient inflammatory spike (Marsland et al., 2017). An influx of inflammation can increase threat-related neural sensitivity to negative social interactions and boost reward-related neural sensitivity to positive social interactions, leading to

withdraw from distant or negative relationships and affiliation with close, supportive others –

behaviors characteristic of sick people (Eisenberger et al., 2017). This marked change in social

goals is an appropriate and adaptive response, conserving energy and facilitating recovery.

Even so, the Social Signal Transduction Theory of Depression suggests that heightened

inflammatory reactivity to social stress is problematic and depressogenic in the context of

frequent interpersonal conflict and tension (Slavich & Irwin, 2014). Chronic and repeated conflict

can prevent a return to the initial homeostatic set point and drive up systemic inflammation,

ultimately motivating more sustained disengagement, like what is observed in depression.

The cross-sectional relationship between depression and heightened inflammatory

reactivity has been replicated multiple times (Fagundes et al., 2013; G. E. Miller et al., 2005;

Pace et al., 2006), but more longitudinal data are needed to determine whether greater

inflammatory reactivity precedes and increases risk for depression. One study found that those

with greater inflammatory reactivity to a laboratory social stressor had elevated depressive

symptoms one year later (Aschbacher et al., 2012). According to the Social Signal Transduction

Theory of Depression, those with heightened inflammatory reactivity to social stress may be

especially at risk for depression when they have chronic or repetitive interpersonal stress

(Slavich & Irwin, 2014).

The Current Study

The current study tests The Social Signal Transduction Theory of Depression using

inflammatory reactivity to a laboratory social stressor, self-reported interpersonal stress, and

repeated measures of depressive symptoms in two different adult samples – physically healthy

married couples (Study 1) and breast cancer survivors (Study 2). In both studies, participants

completed a laboratory social stressor at the baseline visit and reported their depressive

symptoms. At follow-up visits, participants reported their depressive symptoms and stress levels

Study 1 assessed the frequency of social stressors, whereas Study 2 assessed perceived social support and loneliness. We hypothesized that those with greater chronic interpersonal stress (as reported at the follow-up visits) *and* heightened inflammatory reactivity to the laboratory social stressor at baseline would have elevated depressive symptoms at follow-up. We expected that this relationship would be unique to interpersonal stress and would not exist for non-interpersonal stress. The Ohio State University Institutional Review Board approved both studies, and all participants provided written informed consent.

Study 1: Method

Participants

For a parent study on inflammatory and metabolic responses to high-fat meals (Kiecolt-Glaser, Jaremka, et al., 2015), we used print- and web-based announcements to recruit 43 physically healthy couples, ages 24-61, who had been married for at least three years. The exclusionary criteria related to chronic health conditions and medications are detailed elsewhere (Kiecolt-Glaser, Jaremka, et al., 2015).

Procedure

Participants completed two full-day study visits spaced 30.07 (*SD*=29.57) days apart at The Ohio State University Clinical Research Center. At both admissions, couples arrived at 7:30 AM after fasting for 12 hours, a catheter was inserted into each person's arm, and a baseline blood draw was taken. Participants completed a brief relaxation period and then ate a standardized high-fat meal made with either saturated fat or oleic sunflower oil for the parent study's aim. Blood was also drawn once after the meal but this measure was not included in our models due to our desire to have only one pre-stress, true baseline value.

Couples then engaged in a 20-minute problem-solving discussion. Prior to the discussion, experimenters conducted a brief interview to identify mutually contentious topics

22

(e.g., finances, sex, in-laws). Blood samples were collected approximately 90 and 300 minutes

post-conflict. At both visits, participants reported their depressive symptoms approximately three

hours before the problem discussion, and at Visit 2, they also reported their interpersonal and

overall stress levels. Trunk fat was measured dual X-ray absorptiometry (DXA) at Visit 1.

Self-Report Measures

The 20-item Center for Epidemiological Studies Depression Scale (CES-D) indexed the

frequency of depressive symptoms over the past week (Radloff, 1977) (Visit 1: α =0.86, Visit 2:

α =0.88). The revised Test of Negative Social Exchange (TENSE) scale assessed frequency of

interpersonal conflict with important others over the past month (Ruehlman & Karoly, 1991)

(Visit 2: α=.97). The subscales include anger, insensitivity, and interference. Participants

responded on a 10-point Likert scale ranging from "not at all" to "frequently." The 30-item short-

form Trier Inventory of Chronic Stress (TICS-S) (Schulz & Schlotz, 2002), assessed frequency

of work-related and interpersonal chronic stressors over the past three months, using a five-

point Likert scale ranging from "never" to "very often" (Visit 2: α =.92). The social stress

subscales indexed interpersonal stress and the work-stress subscales measured non-

interpersonal stress. Participants also completed the short-form Perceived Stress Scale (PSS-4)

(Cohen, 1988) as a general measure of perceived stress that does not specifically address

interpersonal stress (Visit 2: α= .78).

Inflammatory Markers

Serum IL-6 and TNF-α were assayed as previously described (Kiecolt-Glaser et al.,

2015). Each subject's samples were assayed for all cytokine markers in one run, thus using the

same controls for all time points. The sensitivity for these serum cytokines was 0.3 pg/mL For

IL-6 and TNF-α, the intra-assay coefficients of variation were 3.42% and 2.59%, respectively,

and the inter-assay coefficients of variation were 8.43% and 8.14%, respectively.

Analytic Strategy

Eight participants were missing at least one inflammatory marker measurement, which precluded the calculation of their inflammatory reactivity; thus, they were excluded from analyses. Additionally, two individuals did not report their depressive symptoms at Visit 2, and therefore were also excluded. Excluded individuals were younger (*M*=33.20, *SD*=5.57) than those included in the models (*M*=38.88, *SD*=8.26, *t(*14.8)=2.84, *p*=.01) but did not differ from individuals included in the models on any other variable of interest at Visit 1 (*ps*>.48).

We first modeled the trajectories of inflammatory markers surrounding the marital discussion using linear mixed-effects models with (categorical) time as the sole predictor. These models had an unstructured subject-level residual covariance matrix to account for within-subject measurement correlations and a random couple-level intercept to account for clustering within spousal pairs. IL-6 and TNF-α values were log-transformed to better approximate normality of residuals. We then modeled the trajectory of depressive symptoms over time with a similar model, substituting visit (Visit 1, Visit 2) as the predictor.

Next, we created an inflammatory reactivity variable by (1) calculating the IL-6 and TNF-α slopes from baseline to 90 minutes post-discussion and from baseline to 300 minutes post-discussion, and (2) averaging the two slopes for each inflammatory marker. We then z-scored the individual inflammatory marker slope variables and averaged the two z-scores for a composite measure of inflammatory reactivity. We also created a baseline inflammatory burden covariate to account for regression to the mean; to do so, pre-stress values of log-transformed IL-6 and TNF-α were z-scored and averaged.

For the primary analyses, we used linear mixed-effects models with Visit 2 depressive symptoms as the outcome, the main effects of Visit 2 chronic interpersonal stress and Visit 1 inflammatory reactivity, as well as their interaction term. Visit 1 depressive symptoms was

24

included as a covariate so that these models capture the change in depression from Visit 1 to Visit 2. Separate models were constructed for various measures of interpersonal stress (TICS and TENSE subscales). To test the specificity of interpersonal stress, these models were re-run with measures of non-interpersonal stress (TICS work-related subscales, PSS). The primary and alternative models controlled for age and trunk fat, which are both linked to depression (Kessler et al., 1992; Speed et al., 2019), as well as meal type, baseline inflammatory burden, and sex. Our previous analyses (Kiecolt-Glaser, Habash, et al., 2015; Kiecolt-Glaser, Jaremka, et al., 2015) showed a post-meal increase in IL-6, but not TNF-α, but no differences between meal types. The random effect for each couple captured the within-couple correlation. When there was a significant interaction, we probed it at the 25th and 75th percentiles of inflammatory reactivity, and the resulting simple slope tests are depicted in Figure 2.2. In follow-up analyses, we tested these interactions with individual inflammatory slopes (e.g., TNF-α from baseline to 90-minutes post stress) rather than the inflammatory reactivity composite score (Table S2.1). Thus, in total, we ran 13 primary models and 52 follow-up models. Due to the exploratory nature of this theory's first empirical test, we report p-values that are not multiple-test corrected. However, we also note in the results and tables which results remained significant following False Discovery Rate (FDR) correction using the Benjamini-Hochberg procedure (adjusting as a group all p-values reported in each table). In all models, the Kenward-Roger degrees of freedom adjustment was used. All analyses were done with SAS 9.4 (Cary, NC). Two-tailed tests were conducted, and the alpha level was set at .05.

Study 1: Results

Preliminary Analyses

Participants were young (*M*=38.88 years old, SD=8.26) and mostly white (82%). Couples had been married for an average of 12.17 years (SD=6.66) (Table 2.1). On average, TNF-α did

not change after the stressor (F(2,74)=1.89, p=.16) but IL-6 increased following the stressor (F(74)=69.04, p<.0001). Specifically, IL-6 increased from pre-stress to 90 minutes post-stress (B=0.62, SE=0.08, t(75)=8.03, ps<.0001) and continued to increase from 90 minutes to 300 minutes post-stress (B=0.40, SE=0.07, t(75)=5.84, p<.0001) (Figure 2.1). On average, depressive symptoms did not change from Visit 1 to Visit 2 (p=.33).

Interpersonal Stress, Inflammatory Reactivity, and Depression

Those with higher levels of social tension on the TICS had greater depressive symptoms at Visit 2, but this relationship was only true for those with high inflammatory reactivity to the social stressor at Visit 1 (B=0.93, SE=0.36, t(66)=2.57, p=.01). Similarly, those who reported more angry, insensitive, or interfering interactions on the TENSE had more depressive symptoms at Visit 2, but only if they had high levels of inflammatory reactivity at Visit 1 (angry: B=0.20, SE=0.09, t(66)=2.29, p=0.03; insensitive: B=0.08, SE=0.03, t(66)=2.5, p=0.01; interfering: B=0.36, SE=0.08, t(66)=4.49, p<.001) (Figure 2.2). Upon multiple-test correction, the main effects of the TENSE subscales and the interaction of TENSE interference and inflammatory reactivity remained significant. Inflammatory reactivity to social stress at Visit 1 marginally interacted with lack of social recognition to predict later depressive symptoms (B=0.70, SE=0.37, t(66)=1.89, p=.06), but did not interact with any other TICS social stress subscale (ps>.63) (Table 2.2). Follow-up analyses revealed that IL-6 reactivity from baseline to 90 minutes post-stress primarily drove significant results (Table S2.1).

Non-Interpersonal Stress, Inflammatory Reactivity, and Depression

Although those with higher PSS scores had higher depressive symptoms (B=0.96, SE=0.22, t(64.8)=4.31, p<.0001), this effect did not depend on inflammatory reactivity to social stress (interaction p=.34). The main effect of PSS on depression remained significant even after multiple-test correction. Work-related stress subscales on the TICS did not predict depressive

symptoms (*p*s>.16), nor did they interact with inflammatory reactivity to predict depressive symptoms (interaction *p*s>.36) (Table 2.2).

Study 2: Method

Participants

Participants (*N*=100) were female stage 0 to IIIa breast cancer survivors from the waitlist control condition of a parent randomized controlled trial of hatha yoga (Clinical trials identifier: NCT00486525). They had completed cancer treatment (except for hormonal therapy) between two months and three years prior to the study. They were recruited through oncologists' referrals, community print and web-based announcements, and breast cancer groups and events. The exclusionary criteria and randomization procedure are described elsewhere (Kiecolt-Glaser et al., 2014).

Procedure

The waitlist control group was told to continue their normal activities and refrain from starting a yoga practice. They completed three visits. Visits 2 and 3 were 4.47 (*SD*=1.08) and 7.68 (*SD*=1.11) months after Visit 1. After their final assessment, they were offered yoga classes. At Visit 1, women had a fasting baseline blood draw between 7:00 and 9:00 AM to control for diurnal variability. After the blood draw, they ate a standardized breakfast.

Around 9:00 AM at Visit 1 only, they underwent the Trier Social Stress Test, a well-validated psychosocial stressor consisting of a speech and a mental arithmetic task (Kirschbaum et al., 1993). They had their blood drawn 45 and 120 minutes post-stress to assess their inflammatory reactivity. At all visits, women reported their depressive symptoms, loneliness, social support, and perceived stress levels. They reported their depressive symptoms shortly before the stressor and their loneliness, social support, and perceived stress levels one hour after the stressor. We gathered cancer-related information (time since

27

treatment, cancer stage at diagnosis, treatment type) from participants' medical charts and assessed sagittal abdominal diameter, a measure of belly fat.

Self-Report Measures

The CES-D, as described in Study 1, indexed depressive symptoms at each visit (.85 < αs < .91 at all visits). The UCLA Loneliness Scale assessed perceptions of social isolation (Russell, 1996) (.92 < αs < .94 at all visits). Social support was measured with the 40-item Interpersonal Support Evaluation List (ISEL) (Cohen & Hoberman, 1983) (αs=.94 at all visits). To measure stress that was not specifically interpersonal, participants completed the full-length PSS (Cohen et al., 1994) (.88 < αs < .91 at all visits).

Inflammatory Markers

Serum cytokine levels, assessed undiluted in duplicate, were determined using Meso Scale Discover Proinflammatory II Ultra Sensitive 4-plex per kit instructions. The lower limits of detection for IL-6 and TNF-α were 0.26 pg/mL and 0.37 pg/mL, respectively. The intra-assay coefficients of variation for IL-6 and TNF-α were 1.43% and 4.32%, respectively, with inter-assay coefficients of variation of 4.42% and 5.30%, respectively.

Analytic Strategy

Upon randomization, 100 breast cancer survivors were allocated to the waitlist control group. Overall, 90 women returned for Visit 1 and 87 for Visit 2, and 11 of these women did not have complete inflammatory reactivity data at the baseline visit, largely due to blood draw issues. Therefore, 79 were included in the primary models, as the mixed models described below include all participants with at least one observation of the outcome variable. Those excluded from the models did not differ on key variables of interest at baseline (ps>.11).

We used the same analytic strategy as in Study 1. However, to model depressive symptoms at the two follow-up visits, linear mixed-effects models with an unstructured subject-

level covariance matrix were used for the moderation models to account for the high correlation

between each participant's repeated measurements. These models adjusted for age, sagittal

abdominal diameter, inflammatory burden, baseline CES-D scores, time since treatment, cancer

stage (0-I, I-II, IIIA), chemotherapy treatment (yes or no), and radiation treatment (yes or no).

Unlike Study 1, there was no clustering of subjects in spousal pairs, thus no models contained a

random effect for couple. Significant interactions were probed at the 25th and 75th percentiles of

inflammatory reactivity, and the resulting simple slope tests are depicted in Figure 2.4. In follow-

up analyses, we tested these interactions with individual inflammatory slopes rather than the

inflammatory reactivity composite score (Table S2.2). As an alternative model, to test the time

bound of the theory, we re-ran the primary Study 2 models with Visit 1 values of chronic stress.

The Study 1 chronic stress measures were not administered at Visit 1, so we were unable to run

these alternative models using that sample. Thus, in total, we ran 3 primary models, 3

alternative models, and 12 follow-up models. As in Study 1, we noted which results remained

significant upon FDR correction. In all models, the Kenward-Roger degrees of freedom

adjustment was used. All analyses were done with SAS 9.4 (Cary, NC). Two-tailed tests were

conducted, and the alpha level was set at .05.

<div align="center">**Study 2: Results**</div>

Preliminary Analyses

Women were middle-aged (*M*=51.11 years old, *SD*=8.90) and 12.04 months (*SD*=8.25)

post-treatment. Overall, 85% were white, 51% had been diagnosed with early stage (0-I) breast

cancer, and a majority had received chemotherapy (63%) or radiation (61%) (Table 2.3). Across

participants, TNF-α declined from pre-stress to 45 minutes post-stress (B=-0.089, SE=0.03,

t(78)=-2.78, *p*=.007) but not from 45 to 120 minutes post-stress (B=0.017, SE=0.03, *t*(78)=-0.69,

p=.49). IL-6 marginally increased from pre-stress to 45 minutes post-stress (B=0.077, SE=0.04,

t(78)=1.83, *p*=0.07) and continued to increase from 45 to 120 minutes post-stress (B=0.24, SE=0.05, *t*(78)=4.37, *p*s<.0001) (Figure 2.3). Depressive symptoms did not change throughout the study (*p*=.16).

Interpersonal Stress, Inflammatory Reactivity, and Depression

Women who had lower social support had heightened depressive symptoms at the follow-up visits, and this effect was intensified if they had greater inflammatory reactivity to the laboratory social stressor at the baseline visit (B=-0.11, SE=0.05, *t*(86.2) = -2.2, *p*=.03). Similarly, those who were lonelier had greater depressive symptoms at the follow-up visits, and this effect was marginally amplified among those with greater inflammatory reactivity to the social stressor at the baseline visit (B=0.13, SE=0.07, *t*(98.8) = 1.9, *p*=.06) (Figure 2.4). Only the main effects of loneliness and social support remained significant upon multiple-test correction. Follow-up analyses revealed that TNF-α stress reactivity primarily drove these results (Table S2.2).

Non-Interpersonal Stress, Inflammatory Reactivity, and Depression

Although those who reported more stress on the PSS had higher depressive symptoms (B=0.41, SE=0.07, *t*(133)=5.58, *p*<.0001), the effect of inflammatory reactivity on later depressive symptoms did not depend on PSS scores (B=-0.08, SE=0.09, t(103)=-0.82, *p*=.41) (Table 2.4). The main effect of PSS remained significant after multiple-test correction.

Alternative Longitudinal Model

When using chronic stress measures at baseline rather than the follow-up visits, those who were lonelier at baseline had depressive symptom increases at follow-up, and this effect was stronger among those with high inflammatory reactivity (B=0.22, SE=0.09, t(67.2)=2.35, *p*=.02). Similarly, those who had less social support at baseline had elevated depressive symptoms at follow-up, and it marginally interacted with inflammatory reactivity to predict later

depressive symptoms (B=-0.10, SE=0.05, $t(67.1)$=-1.93, p=.06). Non-interpersonal stress, as measured by the PSS, did not predict later depressive symptoms (p=.39), nor did it interact with inflammatory reactivity to predict depressive symptoms (p=.17).

Discussion

Across two samples, we found evidence supporting the Social Signal Transduction Theory of Depression (Slavich & Irwin, 2014). Those who reported more chronic interpersonal stress – both objective (Study 1) and perceived (Study 2) – had elevated depressive symptoms, but this effect depended on the level of inflammatory reactivity to a laboratory social stressor. Among Study 1's physically healthy couples, frequent interpersonal stress, particularly conflict-related social stress, predicted increased depressive symptoms only among those with exaggerated inflammatory reactivity to the social stressor; there was no association between interpersonal stress and later depressive symptoms among those with lower inflammatory reactivity. Among Study 2's breast cancer survivors, lonelier and less socially supported women had greater depressive symptoms no matter their inflammatory reactivity, but those with high inflammatory reactivity combined with elevated interpersonal stress experienced even greater increases in depressive symptoms over time. Importantly, these findings were specific to interpersonal stress, pointing to the unique role of social relationships in depression etiology. These results generalized across physically healthy individuals and cancer survivors, two laboratory social stressors, multiple measures of chronic interpersonal stress administered at baseline and follow-up, different follow-up periods, and varying timeframes of post-stress inflammation, demonstrating the theory's robustness.

These results show that inter-individual variability in stress exposure and inflammatory reactivity to social stress may play a role in depression risk. In turn, depression and related cognitive biases (e.g., catastrophizing) may augment stress exposure and inflammatory

reactivity, propagating a vicious cycle. According to the stress generation hypothesis, depressed individuals may unintentionally generate additional stressors due to the nature of depressive symptoms (Hammen, 2006). For instance, someone who is depressed and does not have the motivation to do household chores may have more conflict with their spouse. Notably, our sample differences, such as sex, physical health status, and marriage status can influence stress exposure and perception. Other factors associated with depression, such as lower subjective social status (Derry et al., 2013), and heightened emotional reactivity (Carroll et al., 2011) are risk factors for elevated inflammatory reactivity to social stress. Given the role that stress exposure and exaggerated inflammatory reactivity may play in depression etiology, further investigation into factors that modulate exposure and reactivity is warranted.

These results extend the developing inflammation-depression narrative. Current evidence suggests that inflammation and depression fuel one another – a vicious cycle (Kiecolt-Glaser, et al., 2015; Mac Giollabhui et al., 2020). Along with higher basal inflammation, depressed individuals have greater inflammatory reactivity to laboratory stressors (Fagundes et al., 2013; Miller et al., 2005; Pace et al., 2006). Our findings suggest that exaggerated inflammatory reactivity is not only a correlate of, but also a risk factor for heightened depressive symptoms, especially in the context of chronic or frequent interpersonal stress.

Our preliminary analyses showed that unlike TNF-α, IL-6 consistently increased following laboratory social stressors, in line with meta-analytic evidence that found greater elevations in IL-6 following acute stressors (Marsland et al., 2017). Even so, in Study 2, TNF-α reactivity primarily drove the results; although it did not increase on average across the sample, there was significant variability in TNF-α responses such that some individuals had a steep rise following the stressor. Results from Study 1 also indicate that IL-6 may continue to increase even five hours post-stress. Few studies have measured inflammatory markers for longer than

two hours after a laboratory stressor (Marsland et al., 2017); thus, these data add to the literature by showing sustained post-stress IL-6 reactivity. Notably, IL-6 has a diurnal rhythm and typically rises in the afternoon, but the elevations we observed five-hours post-stressor were greater than what would be expected at that time of day (Nilsonne et al., 2016), pointing to the stressor's sustained effect. Follow-up analyses in the supplementary material showed that inflammatory reactivity one to two hours post-stress may be especially important.

One other notable difference between studies is that IL-6 reactivity primarily drove the effects among physically healthy men and women in Study 1, whereas TNF-α reactivity did so among female cancer patients in Study 2. Sample differences – including sex and cancer status – may help to explain this difference. For instance, although a comprehensive meta-analysis did not find evidence that sex moderates cytokine reactivity to stress (Marsland et al., 2017), there are prior reports of stronger IL-6 reactivity among women and stronger TNF-α reactivity among men (e.g., Steptoe, Owen, Kunz-Ebrecht, & Mohamed-Ali, 2002). Also, TNF-α not only provokes inflammation but also mediates cell death and is particularly relevant to cancer, which may help to explain why it was more central to Study 2's sample.

Findings from our alternative model in Study 2 push the time bounds of the Social Signal Transduction Theory of Depression, suggesting that chronic interpersonal stress – even from several months prior – can provoke depression. While our primary models tested the effect of interpersonal stress reported at the follow-up visit(s), the alternative model showed that interpersonal stress that occurred before the baseline visit also predicted depressive symptom increases at follow-up. These alternative models utilized data only from our breast cancer sample, a demographic that may disproportionately benefit from social support and suffer the consequences of social stress. These results warrant further investigation of interpersonal stress timing and its relationship with later depression among other samples.

33

We found that social stress, rather than general stress, predicted depressive symptom increases over time. Consistent with these findings, a plethora of prior evidence indicates that humans are highly motivated to form and maintain social bonds, and therefore, threats to social safety most profoundly impact physical and mental health (Slavich, 2020). Social threat is more closely tied to inflammation than are other stressors, perhaps because the body is readying itself for possible wounding and infection, which is more likely when separated from the group (Slavich & Irwin, 2014). Our results extend this prior work by demonstrating the aptness of the Social Signal Transduction Theory of Depression's focus on social stress rather than all life stress.

Our longitudinal findings have prevention and treatment implications. They suggest a role for anti-inflammatory treatments, at least in certain cases of depression (e.g., Kappelmann et al., 2018). Moreover, they corroborate longstanding central tenets of the interpersonal theory of depression, namely that interpersonal stress drives depression, and that resolving interpersonal stress may help to quell depression. This idea led to the development of Interpersonal Therapy for Depression (IPT), which has strong empirical support (Cuijpers et al., 2011). Our results suggest that inflammation and interpersonal stress may be vital targets not only for depression treatment – but also for prevention.

The strengths of these studies include repeated measurement of inflammatory markers before and after well-controlled laboratory psychosocial stressors, as well as longitudinal depressive symptoms measurement. Additionally, results generalized across multiple methodological variations, revealing the theory's robustness. Although results were similar among physically healthy couples and female breast cancer survivors, both samples were mostly White and living in the Midwestern United States, so it is unclear whether these results generalize to other populations. Moreover, the inflammatory composite scores were

conceptualized a priori to capture both initial and sustained stress reactivity as a unitary predictor variable, but it is a novel way of conceptualizing reactivity, and it deserves further exploration and replication. Also, the combination of inflammatory reactivity and social stress may not be unique risk factors for depression; in fact, they may predispose to other forms of psychopathology – an area for further research. Lastly, due to the exploratory nature of our hypotheses as an initial test of the Social Signal Transduction Theory, we ran many statistical tests, and most of our significant findings did not survive correction for multiple tests. Even so, these findings form a foundation for future work, and need to be replicated in more diverse samples and with other social stress paradigms.

Conclusion

In two different samples, we found support for the Social Signal Transduction Theory of Depression. Breast cancer survivors and physically healthy married couples who had greater interpersonal stress had increased depressive symptoms over time, and this relationship was especially pronounced among those with high inflammatory reactivity to the laboratory social stressor at baseline. These results demonstrate the clinical significance and predictive validity of both chronic interpersonal stress and inflammatory reactivity to laboratory social stressors in depression. Accordingly, this research lends support for inflammation and social stress as prime targets for depression prevention and treatment.

Chapter 2 References

Aschbacher, K., Epel, E., Wolkowitz, O., Prather, A., Puterman, E., & Dhabhar, F. (2012).

    Maintenance of a positive outlook during acute stress protects against pro-inflammatory

    reactivity and future depressive symptoms. *Brain, Behavior, and Immunity*, *26*(2), 346–

    352.

Carroll, J. E., Low, C. A., Prather, A. A., Cohen, S., Fury, J. M., Ross, D. C., & Marsland, A. L.

    (2011). Negative affective responses to a speech task predict changes in interleukin (IL)-

    6. *Brain, Behavior, and Immunity*, *25*(2), 232–238.

Cohen, S. (1988). *Perceived stress in a probability sample of the United States.* In S. Spacapan

    & S. Oskamp (Eds.), The social psychology of health (pp. 31–67). Sage Publications, Inc.

Cohen, S., & Hoberman, H. M. (1983). Positive events and social supports as buffers of life

    change stress 1. *Journal of Applied Social Psychology*, *13*(2), 99–125.

Cohen, S., Kamarck, T., & Mermelstein, R. (1994). Perceived stress scale. *Measuring Stress: A*

    Guide for Health and Social Scientists, *10*, 1–2.

Cuijpers, P., Geraedts, A. S., van Oppen, P., Andersson, G., Markowitz, J. C., & van Straten, A.

    (2011). Interpersonal psychotherapy for depression: A meta-analysis. *American Journal*

    of Psychiatry, *168*(6), 581–592.

Derry, H. M., Fagundes, C. P., Andridge, R., Glaser, R., Malarkey, W. B., & Kiecolt-Glaser, J. K.

    (2013). Lower subjective social status exaggerates interleukin-6 responses to a

    laboratory stressor. *Psychoneuroendocrinology*, *38*(11), 2676–2685.

Eisenberger, N. I., Moieni, M., Inagaki, T. K., Muscatell, K. A., & Irwin, M. R. (2017). In sickness

    and in health: The co-regulation of inflammation and social behavior.

    Neuropsychopharmacology, *42*(1), 242–253.

Fagundes, C. P., Glaser, R., Hwang, B. S., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2013).

Depressive symptoms enhance stress-induced inflammatory responses. *Brain,

Behavior, and Immunity*, *31*, 172–176.

Gunthert, K. C., Cohen, L. H., Butler, A. C., & Beck, J. S. (2007). Depression and next-day

spillover of negative mood and depressive cognitions following interpersonal stress.

Cognitive Therapy and Research, *31*(4), 521–532.

Hammen, C. (2006). Stress generation in depression: Reflections on origins, research, and

future directions. *Journal of Clinical Psychology*, *62*(9), 1065–1082.

Kappelmann, N., Lewis, G., Dantzer, R., Jones, P., & Khandaker, G. (2018). Antidepressant

activity of anti-cytokine treatment: A systematic review and meta-analysis of clinical trials

of chronic inflammatory conditions. *Molecular Psychiatry*, *23*(2), 335–343.

Kessler, R. C., Foster, C., Webster, P. S., & House, J. S. (1992). The relationship between age

and depressive symptoms in two national surveys. *Psychology and Aging*, *7*(1), 119.

Kiecolt-Glaser, J. K., Bennett, J. M., Andridge, R., Peng, J., Shapiro, C. L., Malarkey, W. B.,

Emery, C. F., Layman, R., Mrozek, E. E., & Glaser, R. (2014). Yoga's impact on

inflammation, mood, and fatigue in breast cancer survivors: A randomized controlled

trial. *Journal of Clinical Oncology*, *32*(10), 1040.

Kiecolt-Glaser, J. K., Derry, H. M., & Fagundes, C. P. (2015). Inflammation: Depression fans the

flames and feasts on the heat. *American Journal of Psychiatry*, *172*(11), 1075–1091.

Kiecolt-Glaser, J. K., Habash, D. L., Fagundes, C. P., Andridge, R., Peng, J., Malarkey, W. B., &

Belury, M. A. (2015). Daily stressors, past depression, and metabolic responses to high-

fat meals: A novel path to obesity. *Biological Psychiatry*, *77*(7), 653–660.

Kiecolt-Glaser, J. K., Jaremka, L., Andridge, R., Peng, J., Habash, D., Fagundes, C. P., Glaser,

R., Malarkey, W. B., & Belury, M. A. (2015). Marital discord, past depression, and

metabolic responses to high-fat meals: Interpersonal pathways to obesity. *Psychoneuroendocrinology*, *52*, 239–250.

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81.

Mac Giollabhui, N., Ng, T. H., Ellman, L. M., & Alloy, L. B. (2020). The longitudinal associations of inflammatory biomarkers and depression revisited: Systematic review, meta-analysis, and meta-regression. *Molecular Psychiatry*, 1–13.

Marsland, A. L., Walsh, C., Lockwood, K., & John-Henderson, N. A. (2017). The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *64*, 208–219.

Miller, G. E., Rohleder, N., Stetler, C., & Kirschbaum, C. (2005). Clinical depression and regulation of the inflammatory response during acute stress. *Psychosomatic Medicine*, *67*(5), 679–687.

Miller, G., Rohleder, N., & Cole, S. W. (2009). Chronic interpersonal stress predicts activation of pro-and anti-inflammatory signaling pathways six months later. *Psychosomatic Medicine*, *71*(1), 57.

Nilsonne, G., Lekander, M., Åkerstedt, T., Axelsson, J., & Ingre, M. (2016). Diurnal variation of circulating interleukin-6 in humans: A meta-analysis. *PLoS One*, *11*(11), e0165799.

Pace, T. W., Mletzko, T. C., Alagbe, O., Musselman, D. L., Nemeroff, C. B., Miller, A. H., & Heim, C. M. (2006). Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *American Journal of Psychiatry*, *163*(9), 1630–1633.

Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the

    general population. *Applied Psychological Measurement*, *1*(3), 385–401.

Ruehlman, L. S., & Karoly, P. (1991). With a little flak from my friends: Development and

    preliminary validation of the Test of Negative Social Exchange (TENSE). *Psychological*

    Assessment: A Journal of Consulting and Clinical Psychology, *3*(1), 97.

Russell, D. W. (1996). UCLA Loneliness Scale (Version 3): Reliability, validity, and factor

    structure. *Journal of Personality Assessment*, *66*(1), 20–40.

Schulz, P., & Schlotz, W. (2002). Das Trierer Inventar zur Erfassung von chronischem Stress—

    Version 2 (TICS 2). *Trierer Psychologische Berichte*, *29*.

Slavich, G. M. (2020). Social safety theory: A biologically based evolutionary perspective on life

    stress, health, and behavior. *Annual Review of Clinical Psychology*, *16*.

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive

    disorder: A social signal transduction theory of depression. *Psychological Bulletin*,

    140(3), 774.

Speed, M. S., Jefsen, O. H., Børglum, A. D., Speed, D., & Østergaard, S. D. (2019).

    Investigating the association between body fat and depression via Mendelian

    randomization. *Translational Psychiatry*, *9*(1), 1–9.

Steptoe, A., Owen, N., Kunz-Ebrecht, S., & Mohamed-Ali, V. (2002). Inflammatory cytokines,

    socioeconomic status, and acute stress responsivity. *Brain, Behavior, and Immunity*,

    16(6), 774–784.

Vrshek-Schallhorn, S., Stroud, C. B., Mineka, S., Hammen, C., Zinbarg, R. E., Wolitzky-Taylor,

    K., & Craske, M. G. (2015). Chronic and episodic interpersonal stress as statistically

    unique predictors of depression in two samples of emerging adults. *Journal of Abnormal*

    Psychology, *124*(4), 918.

Table 2.1. Visit 1 characteristics of physically healthy individuals included in analyses (*N*=76)

	Mean (SD)	Number (%)
Age	38.88(8.26)	
Trunk Fat, g	19326.76(7300.78)	
Race		
White		62 (82%)
Black		14 (18%)
Years Married	12.17(6.66)	
Sex (% Female)		38 (50%)
Serum IL-6, pre-stress (pg/mL)	2.12(5.68)	
Serum TNF-alpha, pre-stress (pg/mL)	4.74(1.18)	
Inflammatory reactivity	0.01(0.72)	
CES-D	6.86(6.33)	
TENSE, revised*		
Anger	8.45(7.64)	
Insensitivity	19.29(20.43)	
Interference	9.74(10.69)	
TICS-S*		
Social overload	4.09(2.63)	
Social performance pressure	5.00(2.38)	
Social isolation	3.47(2.34)	
Social tension	3.29(2.01)	
Lack of social recognition	4.13(2.17)	
Work overload	6.00(2.57)	
Work performance pressure	6.08(2.70)	
Work discontent	2.84(1.88)	
Overextended at work	1.92(1.66)	
PSS-4*	4.43(2.89)	

Note: SD = standard deviation; CES-D = Center for Epidemiological Studies Depression Scale; TICS-S =Trier Inventory of Chronic Stressors, short form; TENSE=Test of Negative Social Exchange; PSS-4=Perceived Stress Scale-4 item; *measured at Visit 2

Table 2.2. Study 1 regression coefficients from mixed-effects models

Effect	Estimate	(SE)	Test Statistic	P-value
Inflammatory reactivity	-0.74	(1.01)	t(66) = -0.73	0.47
TENSE Anger	0.31	(0.07)	t(66) = 4.56	<.0001*
Inflammatory reactivity x TENSE Anger	0.20	(0.09)	t(66) = 2.29	0.03
Inflammatory reactivity	-0.47	(0.87)	t(66) = -0.55	0.59
TENSE Insensitivity	0.11	(0.03)	t(66) = 4.11	0.0001*
Inflammatory reactivity x TENSE Insensitivity	0.08	(0.03)	t(66) = 2.5	0.01
Inflammatory reactivity	-2.37	(1.07)	t(66) = -2.21	0.03
TENSE Interference	0.14	(0.05)	t(66) = 2.91	0.005*
Inflammatory reactivity x TENSE Interference	0.36	(0.08)	t(66) = 4.49	<.0001*
Inflammatory reactivity	1.50	(1.54)	t(66) = 0.97	0.33
TICS Social overload	0.23	(0.24)	t(66) = 0.97	0.33
Inflammatory reactivity x TICS Social overload	0.03	(0.30)	t(66) = 0.09	0.93
Inflammatory reactivity	0.61	(2.03)	t(66) = 0.3	0.76
TICS Social performance pressure	0.10	(0.25)	t(66) = 0.4	0.69
Inflammatory reactivity x TICS Social performance pressure	0.17	(0.35)	t(66) = 0.48	0.63
Inflammatory reactivity	1.44	(1.49)	t(66) = 0.97	0.34
TICS Social isolation	0.12	(0.28)	t(66) = 0.42	0.67
Inflammatory reactivity x TICS Social isolation	0.03	(0.46)	t(66) = 0.07	0.95
Inflammatory reactivity	-1.34	(1.29)	t(66) = -1.04	0.30
TICS Social tension	0.44	(0.32)	t(66) = 1.38	0.17
Inflammatory reactivity x TICS Social tension	0.93	(0.36)	t(66) = 2.57	0.01
Inflammatory reactivity	-1.88	(1.86)	t(66) = -1.01	0.32
TICS Lack of social recognition	0.45	(0.26)	t(66) = 1.73	0.09
Inflammatory reactivity x TICS Lack of social recognition	0.70	(0.37)	t(66) = 1.89	0.06
Inflammatory reactivity	0.34	(2.28)	t(66) = 0.15	0.88
TICS Work overload	0.10	(0.25)	t(66) = 0.42	0.68
Inflammatory reactivity x TICS Work overload	0.22	(0.37)	t(66) = 0.6	0.55
Inflammatory reactivity	0.80	(2.22)	t(66) = 0.36	0.72

TICS Work performance pressure	0.16	(0.21)	t(66) = 0.77	0.45
Inflammatory reactivity x TICS Work performance pressure	0.10	(0.30)	t(66) = 0.33	0.74
Inflammatory reactivity	0.30	(1.54)	t(66) = 0.19	0.85
TICS Work discontent	0.45	(0.31)	t(66) = 1.43	0.16
Inflammatory reactivity x TICS Work discontent	0.40	(0.43)	t(66) = 0.92	0.36
Inflammatory reactivity	1.98	(1.29)	t(66) = 1.54	0.13
TICS Overextended at work	0.46	(0.39)	t(66) = 1.18	0.24
Inflammatory reactivity x TICS Overextended at work	-0.18	(0.50)	t(66) = -0.36	0.72
Inflammatory reactivity	0.57	(1.27)	t(65) = 0.45	0.65
PSS-4	0.96	(0.22)	t(64.8) = 4.31	<.0001*
Inflammatory reactivity x PSS-4	0.23	(0.24)	t(65) = 0.96	0.34

Note: SE = standard error; TICS-S =Trier Inventory of Chronic Stressors, short form; TENSE = Test of Negative Social Exchange; PSS-4 = Perceived Stress Scale-4 item; models adjusted for age, trunk fat, meal type, baseline inflammatory burden, and sex; *remained significant with False Discovery Rate correction

Table 2.3. Baseline characteristics of breast cancer survivors included in analyses (*N*=79)

	Mean (SD)	Number (%)
Age	51.11 (8.90)	
SAD	20.88 (3.68)	
Months since treatment	12.04 (8.25)	
Race		
White		67 (85%)
Black		10 (13%)
Asian American		2 (3%)
Cancer stage		
0-I		40 (51%)
I-II		31 (39%)
III+		8 (10%)
Chemotherapy treatment		50 (63%)
Radiation treatment		48 (61%)
No longer menstruating		64 (81%)
Serum IL-6, pre-stress (pg/mL)	2.45(2.50)	
Serum TNF-alpha, pre-stress (pg/mL)	7.36(3.46)	
Inflammatory reactivity	-.06(.81)	
CES-D	10.43(7.55)	
UCLA loneliness*	37.35(9.23)	
ISEL*	94.61(15.36)	
PSS*	21.76(8.58)	

Note: SD = standard deviation; SAD = Sagittal Abdominal Diameter; CES-D = Center for Epidemiological Studies Depression Scale; UCLA = University of California-Los Angeles; ISEL = Interpersonal Support Evaluation List; PSS = Perceived Stress Scale; *measured at first follow-up visit

Table 2.4. Study 2 regression coefficients from mixed-effects models

Effect	Estimate	(SE)	Test Statistic	P-value
Inflammatory reactivity	-5.53	(2.78)	t(96.2) = -1.99	0.049
UCLA loneliness	0.33	(0.06)	t(90.5) = 5.9	<.0001*
Inflammatory reactivity x UCLA loneliness	0.13	(0.07)	t(98.8) = 1.9	0.06
Inflammatory reactivity	9.99	(4.76)	t(85.8) = 2.1	0.04
ISEL	-0.22	(0.03)	t(87) = -6.38	<.0001*
Inflammatory reactivity x ISEL	-0.11	(0.05)	t(86.2) = -2.2	0.03
Inflammatory reactivity	1.98	(2.13)	t(97.5) = 0.93	0.36
PSS	0.41	(0.07)	t(133) = 5.58	<.0001*
Inflammatory reactivity x PSS	-0.08	(0.09)	t(103) = -0.82	0.41

Note: SE = standard error; UCLA = University of California-Los Angeles; ISEL = Interpersonal Support Evaluation List; PSS = Perceived Stress Scale; models adjusted for age, sagittal abdominal diameter, baseline inflammatory burden, baseline CES-D scores, time since treatment, cancer stage, and cancer treatment type; *remained significant with False Discovery Rate correction

Table S2.1. Individual Inflammatory Slopes Interact with Interpersonal Stress to Predict
Depressive Symptoms in Study 1

Predictor	P-values for Interactions with Slopes:			
	IL-6 Initial	IL-6 Sustained	TNF Initial	TNF Sustained
TENSE anger	<.0001*	0.02	0.41	0.15
TENSE insensitivity	0.0004*	0.01	0.44	0.02
TENSE interference	0.002*	0.053	0.33	0.09
TICS lack of social recognition	0.21	0.24	0.12	0.71
TICS overextended at work	0.97	0.39	0.61	0.39
TICS social performance pressure	0.95	0.40	0.42	0.73
TICS work performance pressure	0.57	0.25	0.27	0.96
TICS social overload	0.28	0.62	0.26	0.82
TICS social isolation	0.03	0.50	0.24	0.04
TICS social tension	0.002*	0.11	0.24	0.56
TICS work discontent	0.04	0.06	0.53	0.82
TICS work overload	0.54	0.95	0.85	0.81
PSS-4	0.36	0.52	0.80	0.98

Initial = baseline to 90 minutes; Sustained = baseline to 300 minutes; TICS =Trier Inventory of
Chronic Stressors; TENSE = Test of Negative Social Exchange; PSS-4 = Perceived Stress
Scale-4 item; models adjusted for age, trunk fat, meal type, baseline inflammatory burden, and
sex; *remained significant with False Discovery Rate correction

Table S2.2. Individual Inflammatory Slopes Interact with Interpersonal Stress to Predict Depressive Symptoms in Study 2

Predictor	P-values for Interactions with Slopes:			
	IL-6 Initial	IL-6 Sustained	TNF Initial	TNF Sustained
ISEL	0.83	0.46	**0.03**	**0.0004***
PSS	0.35	0.39	0.80	0.55
UCLA loneliness	0.91	0.78	**0.03**	**0.0004***

Initial = baseline to 45 minutes; Sustained = baseline to 120 minutes; UCLA = University of California-Los Angeles; ISEL = Interpersonal Support Evaluation List; PSS = Perceived Stress Scale; models adjusted for age, sagittal abdominal diameter, baseline inflammatory burden, baseline CES-D scores, time since treatment, cancer stage, and cancer treatment type; remained significant with False Discovery Rate correction
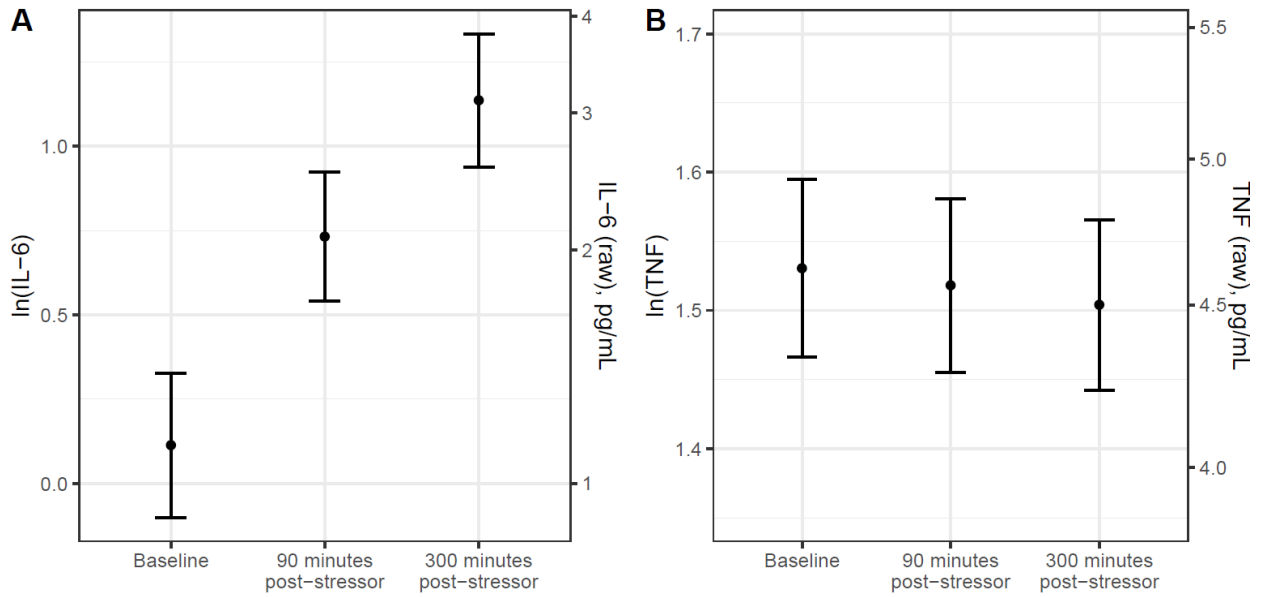
Figure 2.1. Estimated Marginal Mean Trajectories of Inflammatory Markers surrounding Study 1's Marital Conflict. IL-6 rose after the conflict ($p<.0001$), but TNF-α did not ($p=.16$). IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha. Bars represent standard error.

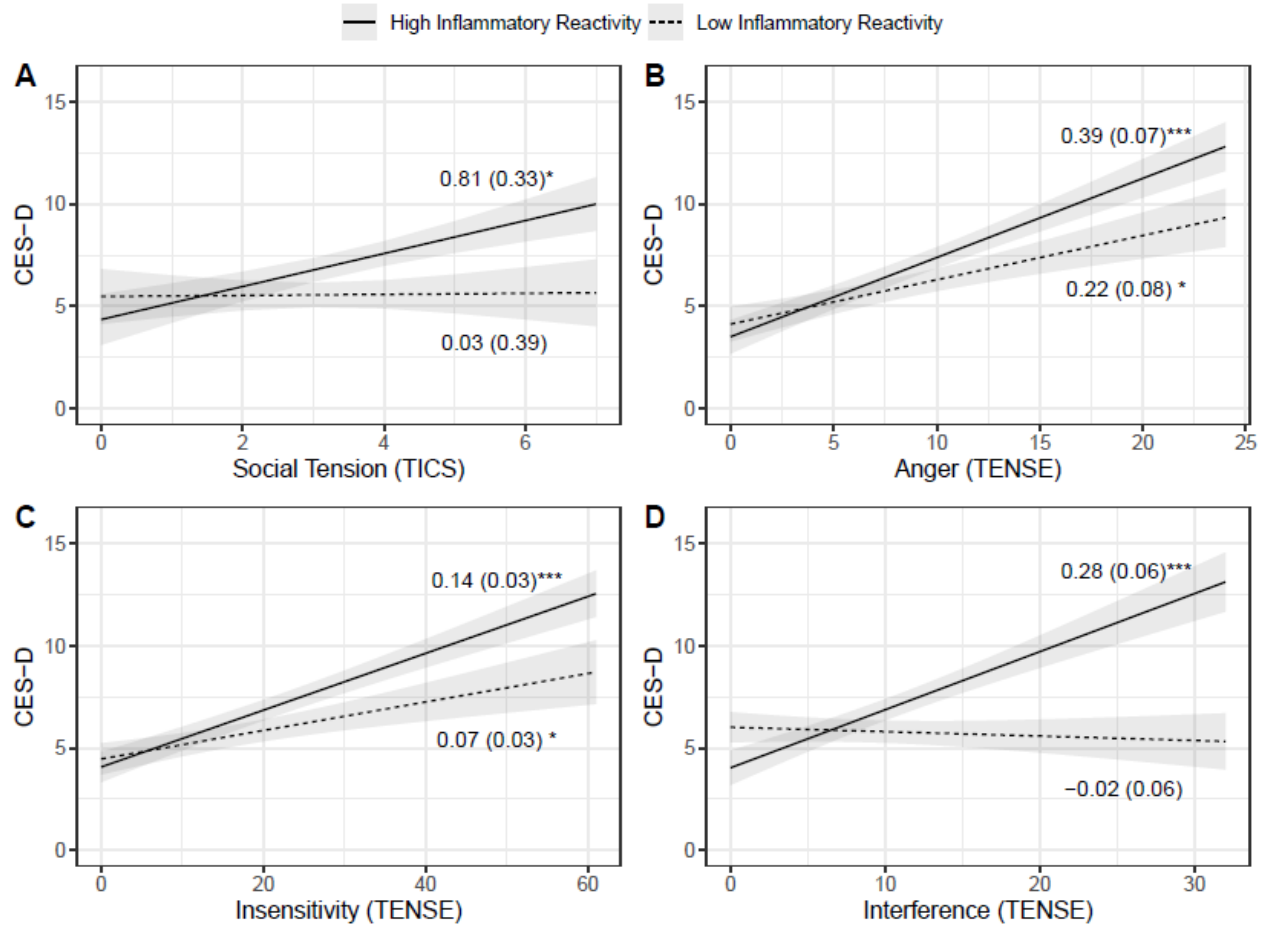Figure 2. Interpersonal Stress Interacts with Inflammatory Reactivity to Predict Depressive Symptoms in Study 1. CES-D = Center for Epidemiological Studies Depression Scale; TICS =Trier Inventory of Chronic Stressors; TENSE = Test of Negative Social Exchange. ***p<.001, **p<.01, *p<.05; Shaded area represents standard error. Models adjusted for age, trunk fat, meal type, baseline inflammatory burden, and sex.
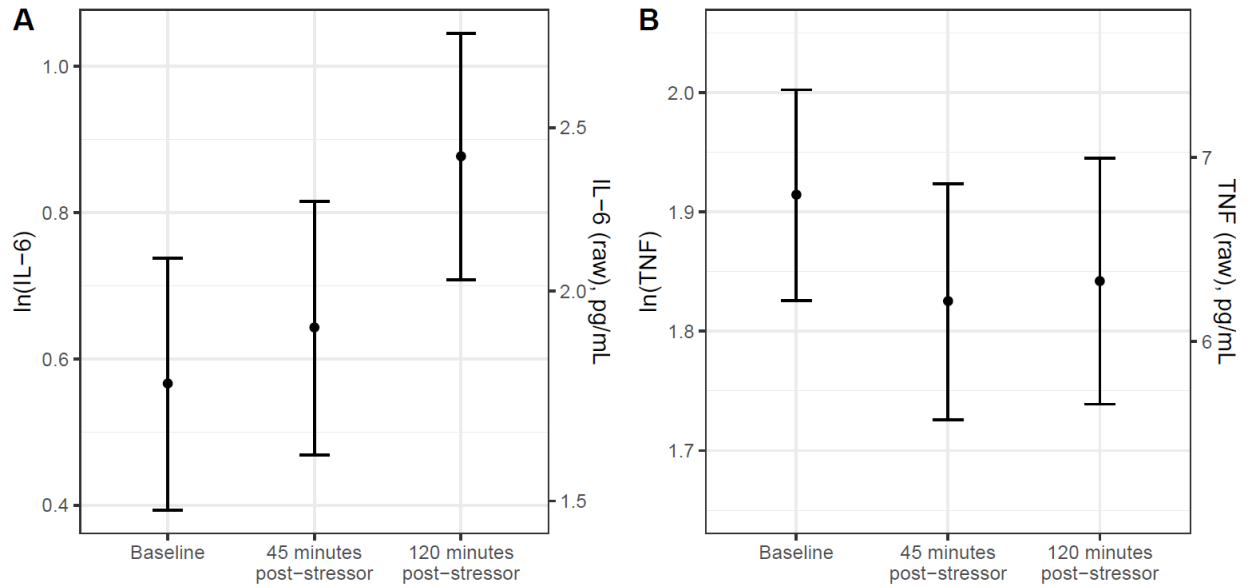
Figure 2.3. Estimated Marginal Mean Trajectories of Inflammatory Markers surrounding Study 2's Social-Evaluative Speech Stressor. IL-6 rose after the stressor ($p<.0001$), while TNF-α fell ($p=.03$). IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha. Bars represent standard error.
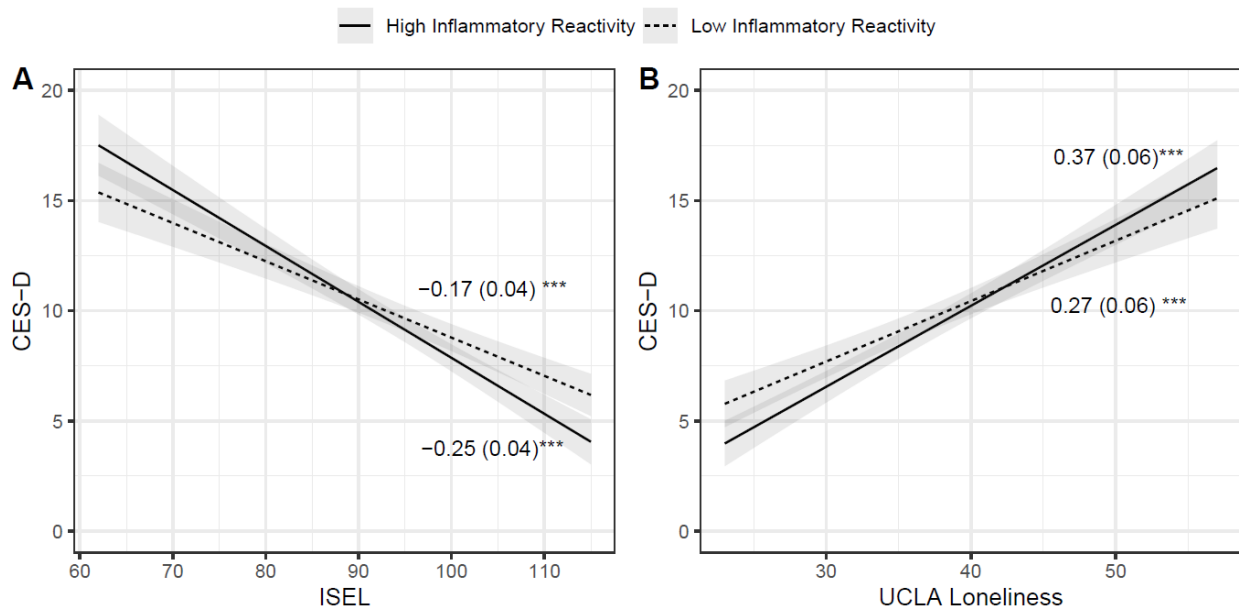
Figure 2.4. Social Support and Loneliness Interact with Inflammatory Reactivity to Predict Depressive Symptoms in Study 2. CES-D = Center for Epidemiological Studies Depression Scale; UCLA = University of California-Los Angeles; ISEL = Interpersonal Support Evaluation List. ***p<.001; Shaded area represents standard error. Models adjusted for age, sagittal abdominal diameter, baseline inflammatory burden, baseline CES-D scores, time since treatment, cancer stage, and cancer treatment type.

Chapter 3: Omega-3 Supplementation to Blunt Physiological Reactivity to Stress

(Published in *Molecular Psychiatry*)

Omega-3 Supplementation and Stress Reactivity of Cellular Aging Biomarkers:
An Ancillary Substudy of a Randomized, Controlled Trial in Midlife Adults

Annelise A. Madison, M.A. [1,2], Martha A. Belury, Ph.D. [1,3], Rebecca Andridge, Ph.D. [1,4],

Megan E. Renna, Ph.D. [1], M. Rosie Shrout, Ph.D. [1], William B. Malarkey, M.D. [1,5], Jue Lin,

Ph.D.[6], Elissa S. Epel [7], Janice K. Kiecolt-Glaser, Ph.D.[1,8]

(1) Institute for Behavioral Medicine Research, The Ohio State University College of Medicine,
(2) Department of Psychology, The Ohio State University, (3) Department of Human Sciences,
College of Education and Human Ecology, The Ohio State University, (4) College of Public
Health, The Ohio State University, (5) Department of Internal Medicine, The Ohio State
University College of Medicine, (6) Department of Biochemistry and Biophysics, University of
California, San Francisco, (7) Department of Psychiatry, University of California, San Francisco,
(8) Department of Psychiatry and Behavioral Health, The Ohio State University College of
Medicine

Corresponding author: Janice Kiecolt-Glaser, Institute for Behavioral Medicine Research, The

Ohio State University College of Medicine, 460 Medical Center Drive, Columbus, OH 43210,

614-293-3499 (telephone), 614-366-2097 (fax), Janice.Kiecolt-Glaser@osumc.edu.

Short Title: Omega-3 and Stress Reactivity

Abstract

Higher levels of omega-3 track with longer telomeres, lower inflammation, and blunted sympathetic and cardiovascular stress reactivity. Whether omega-3 supplementation alters the stress responsivity of telomerase, cortisol, and inflammation is unknown. This randomized, controlled trial examined the impact of omega-3 supplementation on cellular aging-related biomarkers following a laboratory speech stressor. In total, 138 sedentary, overweight, middle-aged participants (n=93 women, n=45 men) received either 2.5 g/d of omega-3, 1.25 g/d of omega-3, or a placebo for 4 months. Before and after the trial, participants underwent the Trier Social Stress Test. Saliva and blood samples were collected once before and repeatedly after the stressor to measure salivary cortisol, telomerase in peripheral blood lymphocytes, and serum anti-inflammatory (interleukin-10; IL-10) and proinflammatory (interleukin-6; IL-6, interleukin-12, tumor necrosis factor-alpha) cytokines. Adjusting for pre-supplementation reactivity, age, sagittal abdominal diameter, and sex, omega-3 supplementation altered telomerase (p=0.05) and IL-10 (p=0.05) stress reactivity; both supplementation groups were protected from the placebo group's 24% and 26% post-stress declines in the geometric means of telomerase and IL-10, respectively. Omega-3 also reduced overall cortisol (p=0.03) and IL-6 (p=0.03) throughout the stressor; the 2.5 g/d group had 19% and 33% lower overall cortisol levels and IL-6 geometric mean levels, respectively, compared to the placebo group. By lowering overall inflammation and cortisol levels during stress and boosting repair mechanisms during recovery, omega-3 may slow accelerated aging and reduce depression risk. ClinicalTrials.gov identifier: NCT00385723

Introduction

Omega-3 fatty acid consumption may lessen accelerated aging and early mortality. Men and women in the top quintile of omega-3 fatty acid intake had 15% and 18% lower cardiovascular disease mortality 16 years later, respectively, compared to those in the lowest quintile (Zhang et al., 2018). At a cellular level, higher blood levels of omega-3 track with longer telomeres (Farzaneh-Far et al., 2010; Kiecolt-Glaser et al., 2013), which are DNA repeats at the end of chromosomes that help to maintain genomic integrity during cell division (Blackburn et al., 2015), thereby promoting healthy cellular aging. Results from genome-wide association studies suggest a causal role of short telomeres in age-related disease, especially cardiovascular disease (Codd et al., 2013; Haycock et al., 2017). Thus, there is evidence that omega-3 promotes longevity at both the epidemiological and cellular level.

A dysregulated physiological stress response is a risk factor for many physical and mental diseases, including depression (Kiecolt-Glaser et al., 2020; Turner et al., 2020), and omega-3 may reduce morbidity by regulating stress-responsive systems. University students with higher serum omega-3 levels were buffered from an uptick in stimulated proinflammatory cytokine release during a high-stress exam period (Maes et al., 2000). Heightened inflammatory reactivity to acute stress may increase risk for depression (Aschbacher et al., 2012), and therefore, omega-3's anti-inflammatory properties may help to break the link between stress exposure and depression. Intriguingly, omega-3 supplementation also reduces sympathetic and cardiovascular reactivity to an acute stressor (Ginty & Conklin, 2012; Monahan et al., 2004; Rousseau et al., 1998).

Prior publications from this randomized, placebo-controlled trial (RCT) showed that four months of omega-3 supplementation lowered basal inflammation and oxidative stress (Kiecolt-Glaser et al., 2012, 2013), but this pre-planned ancillary substudy investigated whether it altered

the stress responsivity of biomarkers relevant to telomere length and cellular aging (i.e., cortisol, inflammatory cytokines, and telomerase). Cortisol and proinflammatory cytokines naturally rise after acute stress, but exaggerated cortisol responses are associated with shorter telomeres both cross-sectionally and longitudinally (Steptoe et al., 2017; Tomiyama et al., 2012), and proinflammatory cytokines fuel oxidative stress (De Biase et al., 2003; Gidron et al., 2006), which shortens telomeres (Kurz et al., 2004). We hypothesized that omega-3 supplementation would reduce cortisol and inflammatory stress reactivity. Telomerase is an enzyme that maintains and restores telomeres, and Epel and colleagues found differences in response to an acute laboratory stressor – the Trier Social Stress Test [TSST; 20]. We predicted that telomerase levels would not change following an acute stressor among those taking omega-3. In accord with the post-stress trajectory observed in Epel et al.'s low-stress, non-caregiving cohort (Epel et al., 2010), we expected that the placebo group's telomerase would first rise within 45 minutes, and then fall at 120 minutes post-stress. Importantly, we investigated these questions in a sedentary, overweight sample of middle-aged adults – a high-risk group for accelerated aging (Horvath et al., 2014).

Subjects and Methods

Participants

Overall, 138 individuals (93 women, 45 men), ages 40–85, participated in this RCT. Campus and community print and web-based announcements were used for recruitment. The Ohio State University biomedical institutional review board approved this study. Each participant provided written informed consent. These ancillary hypotheses were pre-specified and are distinct from the primary results (Kiecolt-Glaser et al., 2012, 2013).

Due to our desire to study sedentary, overweight individuals, only those who engaged in less than 2 hours of vigorous physical activity per week and had a body mass index (BMI) between 22.5 and 50 were included. The parent study's exclusionary criteria, described

elsewhere, yielded a sample that was free of metabolic, autoimmune, and inflammatory diseases and did not take medications that alter mood, cardiovascular, or immune function (Kiecolt-Glaser et al., 2012, 2013).

Across groups, 63% of participants were women, 79% were white, and 16% were black. Participants' ages ranged from 40 to 85 with a mean of 51 years. Using the BMI cut point of 25 kg/m^2, 125 (91%) were overweight (Table 3.1).

Randomization and Blinding

At the baseline visit, participants were randomly assigned to a group using a permuted block randomization sequence and given their first month's supply. At every subsequent visit, participants returned unused supplements and received the next month's supply. Adherence was high and did not differ between groups, with 3.3%, 2.0%, and 2.6% percent of unused supplements returned in the placebo, low, and high dose groups, respectively (p = 0.31). As previously described, participants and experimenters were adequately blinded (Kiecolt-Glaser et al., 2012).

Procedure

At the baseline and post-intervention visits, participants arrived at The Ohio State University's Clinical Research Center, a hospital research floor, at 07:45 and completed mood questionnaires, ate a standardized breakfast, had a baseline blood draw around 08:50 to assess pre-stressor telomerase and cytokine levels, and provided saliva for a baseline cortisol measure. Around 10:10, participants completed a 20-minute stressor, detailed below. Participants had their blood drawn to measure telomerase and cytokine levels 0.75 and 2 hours post-stress. Participants also provided saliva to measure cortisol immediately post-stressor, as well as 0.75, 1.25, 1.75, and 2 hours post-stress. They also reported their state anxiety levels before and after the stressor.

Supplement and Placebo

In this 3-arm parallel group RCT, participants received either (A) 2.496 g/d omega-3 (*n*=46), (B) 1.25 g/d omega-3, and placebo (*n*=46), or (C) placebo (*n*=46). All participants took 6 pills (3 g oil) daily. For the two supplement groups, each 500 mg gel capsule contained 347.5 mg eicosapentaenoic acid (EPA) and 58 mg docosahexaenoic acid (DHA). Thus, the high dose group took 2085 g/d of EPA and 348 g/d of DHA and the low dose group took 1042.5 g/d or EPA and 174 g/d of DHA. The placebo was a mixture of palm, olive, soy, canola, and coco butter oils that approximated the saturated:monounsaturated:polyunsaturated (SMP) ratio consumed by US adults, 37:42: 21 (USDA Continuing Survey of Food Intake by Individuals, 1994–1996). OmegaBrite (Waltham, MA) supplied both the omega-3 and the matching placebo. See Table S3.1 for fatty acid analysis of supplement.

Psychosocial Stressor

The Trier Social Stress Test (TSST) is a well-validated, widely-used psychosocial laboratory acute stress paradigm (Kirschbaum et al., 1993). After participants were given instructions, they had 10 minutes to prepare a speech about why they were the best job candidate. Without using notes or aides, participants then delivered a 5-minute speech in front of a video camera and a panel of two judges wearing white lab coats who were told to maintain a neutral facial expression. If participants' speeches ended early, they were told to continue until the full five minutes had passed. After the speech, participants completed a five-minute serial-subtraction task out loud in front of the same panel. When participants made mistakes, they were told to restart from the beginning. The TSST reliably provokes strong neuroendocrine and inflammatory responses (Allen et al., 2014).

State Anxiety Index

Participants reported their state anxiety levels before and after the TSST on the widely-used Speilberger 20-item State Anxiety Index (Spielberger et al., 1983). The measure asks

participants to rate on a four-point scale how strongly they are experiencing each feeling (e.g., calmness, jitteriness) "right now, in this moment," ranging from "not at all" to "very much."

Cellular Aging Biomarkers

Salivary cortisol was assayed using the Cortisol Coat-A-Count Radioimmunoassay (Diagnostic Products Corporation). This plasma kit was modified to measure free cortisol in saliva per the manufacturers' directions. The assay was counted and calculated on the Packard Cobra II Gamma Counter (Packard Instrument Company). The sensitivity was .025 ul/dl and the inter-assay coefficient variation was 5.2%. This assay method for telomerase and these cytokines are described elsewhere (Kiecolt-Glaser et al., 2012, 2013). The inter-assay coefficients of variation were 6.8% for telomerase, 12.5% for IL-6, 12.1% for TNF-α, 6.4% for IL-10, and 10.5% for IL-12.

Power Analysis

There were no prior data on omega-3 supplementation's effect on cortisol and telomerase stress reactivity, so we based our a priori power analysis on past reports of its association with inflammatory reactivity. In groups similar to our placebo group, stress boosted TNF-α by 0.6 standard deviations (effect size=0.6) (Altemus et al., 2001; Maes et al., 2000). However, among those with higher serum omega-3 levels, TNF-α increased only 0.1 standard deviations after stress, six times lower than those who have low serum levels of omega-3 (Maes et al., 2000). Using these results and an estimated standard deviation of 0.79 pg/mL based on pilot data from our laboratory results in an estimated reduction in stress-related increase of 0.46 pg/mL in the lower dose group versus placebo. Thus, a sample size of 46 per group would allow us to detect this reduction (effect size 0.6) with 80% power.

Analytical Plan

Zero-order correlations between variables of interest at the baseline visit were performed. All physiological outcomes were natural-log transformed due to their positive skew to

ensure homoscedastic residuals. A time variable was created to index telomerase and cytokine reactivity time points (80 minutes before the stressor, 45 minutes after stress onset, and 120 minutes after stress onset). Because cortisol was assessed at six timepoints throughout the stressor, we calculated area under the curve with respect to ground (AUCg) as an index of total cortisol release as well as area under the curve with respect to increase (AUCi) as an index of cortisol reactivity to the stressor.

To test group differences in cortisol at the post-intervention visit, we used linear regression models with group predicting post-intervention cortisol reactivity (AUGi) and total cortisol (AUGg), adjusting for baseline values. To assess whether there were group differences in telomerase and cytokine stress reactivity and overall levels at the post-intervention visit, we used hierarchical linear models with group, time, and a group by time interaction variable to predict telomerase, pro-inflammatory cytokine (i.e., IL-6, TNF-α, IL-12), and anti-inflammatory cytokine (i.e., IL-10) trajectories, adjusting for baseline values (at each reactivity time point). To account for sex-based differences in stress reactivity (Bekhbat & Neigh, 2018), we also tested sex as a moderator in all primary models. However, there were no sex-based differences in the effect of omega-3 supplementation on overall levels (ps>.22) or reactivity (ps>.11) of the outcomes of interest, so sex was not included as a moderator in final models. These hierarchical linear models used a subject-specific random intercept to account for the within-subject correlation of the repeated measurements within the visit, and the Kenward-Roger adjustment to the degrees of freedom. As in our study on basal telomere length and oxidative stress (Kiecolt-Glaser et al., 2013), all models were adjusted for age, sagittal abdominal diameter, and sex.

Our analytic strategy required the inclusion of participants' post-intervention outcome measurements in the models. We added telomerase and cortisol assessments after the trial began, so only 103 and 118 had baseline telomerase and cortisol data, respectively. Two

58

participants were lost to follow-up (*n*=1 in placebo, *n*=1 in 1.25 g/d) and three (*n*=1 in placebo, *n*=2 in 2.5 g/d) discontinued the intervention. In addition, one participant did not have a sagittal abdominal diameter measurement and therefore was excluded from all analyses. Data from 97 participants were included in the telomerase model, 110 in the cortisol models, and 120-131 in the cytokine analyses (IL-6 n=131, TNF n=131, IL-10 n=127, IL-12 n=120). Figure S3.1 shows the participant flow through the trial.

Since the resulting analyses did not use all randomized subjects, we compared baseline outcome levels across the groups to ensure there were no differences. The same modeling strategy was used for these analyses as for the primary analyses (linear regression for cortisol AUCg and AUCi; hierarchical linear models for telomerase and cytokines; adjustment for age, sagittal abdominal diameter, and sex).

When a significant group by time interaction occurred, the following pre-planned contrasts were performed to probe the interaction: 1. Between-group mean differences at each time point (pre-stress, 45 minutes post-stress, 120 minutes post-stress) and 2. Within-group changes between each time point. When a significant group main effect occurred (with non-significant interaction), we contrasted overall group means (pooled across time points). Two-tailed tests of significance were conducted and all alpha levels were set at $\alpha=0.05$. Data were analyzed with SAS version 9.4 (SAS Institute, Cary, NC). Relevant data and statistical code will be made available upon written request.

Results

Manipulation Check

At both visits, participants reported higher state anxiety levels immediately after the TSST than they did before the stressor (Baseline visit: paired $t(133)=8.7$, $p<.0001$; Post-intervention visit: paired $t(122)=6.8$, $p<.0001$), indicating that the manipulation was successful.

Omega-3 Supplementation and Telomerase Reactivity

At the baseline visit, there were no group differences in overall levels (ps>0.17) or reactivity (ps>0.08) of the outcomes of interest. Estimated marginal means are shown in Tables S3.2 and S3.3. However, omega-3 supplementation impacted post-intervention telomerase reactivity to the laboratory stressor (p=.05) (Figure 3.1) but not overall telomerase levels (p=.98). Among those in the two supplement groups, telomerase did not change throughout the measurement period (ps>.07). In contrast, among those in the placebo group, telomerase did not change from pre-stress to 45 minutes post-stress (p=.90), but sharply declined from 45 minutes to 120 minutes post-stress (p=.001). Pre-planned contrasts showed that there were no between-group differences in telomerase before the stressor (ps>.58), 45 minutes (ps>.26), or 120 minutes (ps>.13) post-stress onset.

Omega-3 Supplementation and Cortisol Reactivity

Omega-3 supplementation did not impact cortisol reactivity to the laboratory stressor, as measured by the area under the curve increase (p=.44). However, omega-3 supplementation lowered total cortisol levels throughout the stressor in a dose-response manner, as indexed by AUCg (p=.04); those receiving the high dose had the lowest overall cortisol levels, and those in the placebo group had the highest (Figure 3.2). Those taking the high dosage of omega-3 had significantly lower cortisol compared to the placebo group (p=.01); those taking the low dose did not differ from the placebo group (p=.29). Across all participants, the TSST caused a significant rise in cortisol at both visits (Baseline visit: paired t(119)=5.6, p<.0001; Post-intervention visit: paired t(112)=4.1, p<.0001).

Omega-3 Supplementation and Inflammatory Reactivity

Omega-3 supplementation influenced IL-10 stress reactivity (interaction effect F(4, 206=2.44, p=0.05) but not overall levels (p=0.30). Although there were no group differences before (ps>0.71) or 45 minutes after the stressor (ps>.14), those in the omega-3 groups had

higher levels of IL-10 120 minutes after the stressor (ps<.05) (Figure 3.3). In the placebo group, IL-10 declined from pre-stress to 120 minutes post-stress (change=-0.199, SE=0.067, t(206)=-2.95, p=0.004), but the omega-3 supplementation groups did not have this decline (ps>0.34).

Omega-3 supplementation did not affect IL-6 stress reactivity (interaction effect p=0.11), but it did lower overall IL-6 levels (main effect F(2, 226)=3.73, p=0.03). The high dosage group had lower IL-6 levels than the placebo group (B=0.288, SE=0.106, t(226)=2.71, p=0.007) but there were no other group differences (ps>0.10) (Figure 3.4). Omega-3 supplementation did not impact TNF-α or IL-12 reactivity to the stressor (interaction effect ps>0.34) or overall levels (main effect ps>0.59). The TSST triggered an increase in IL-6 at both visits across all participants (Baseline visit: paired t(129)=10.0, p<.0001; Post-intervention visit: paired t(123)=8.5, p<.0001).

Discussion

In this RCT, omega-3 supplementation blocked stress-related decreases in telomerase and anti-inflammatory cellular signaling, while reducing overall cortisol and IL-6 levels among sedentary, overweight middle-aged adults. Specifically, the high dose (2.5 g/d) lowered overall cortisol and IL-6, while the 1.25 g/d dose was sufficient to ward off a post-stress drop in telomerase and IL-10 levels. These findings complement and extend our prior work, which showed that omega-3 supplementation reduced basal inflammation and oxidative stress (Kiecolt-Glaser et al., 2012, 2013). Our current findings were dose-dependent, such that those receiving the high dose had the greatest differences compared to the placebo group – suggesting a causal relationship. Taken together, these results provide initial evidence that omega-3 may have a unique stress-buffering effect on biomarkers relevant to cellular aging and mental health among a sedentary, overweight middle-aged sample.

Omega-3 Supplementation and Telomerase Reactivity

Among those in the supplement groups, telomerase levels did not change in response to an acute stressor. In contrast, the placebo group's geometric mean of telomerase dropped 24% between 45 and 120 minutes after the stressor. To our knowledge, this is the longest post-stress follow-up measurement of telomerase reported in the literature. Epel previously showed a slight numerical telomerase decline among non-caregiver control group between 50 and 90 minutes post-stress (Epel et al., 2010). Data from our longer follow-up period suggest that without the buffering effects of omega-3, telomerase may ultimately drop below pre-stress levels two hours post-stressor. However, this finding is preliminary due to a paucity of research in this domain and replication is needed.

If telomerase does decline following stress, it could be intensified with repeated or chronic stressors. Indeed, chronically-stressed caregivers had lower overall telomerase throughout an acute stressor, compared to non-caregivers (Epel et al., 2010). Several samples experiencing adversity have shown a pattern of short telomeres with high telomerase activity (Chen et al., 2014; Zalli et al., 2014) – a compensatory profile of short but stable telomeres. However, some individuals under chronic or repeated stress may have the detrimental combination of short telomeres and low telomerase activity, leading to accelerated telomere shortening.

Intriguingly, in the control group, we did not replicate Epel's finding of increased telomerase within one hour after the stressor (Epel et al., 2010). Unlike Epel's sample, ours was overweight and rarely engaged in vigorous physical activity, two risk factors for accelerated aging (Du et al., 2012; Horvath et al., 2014); these factors may blunt the post-stress telomerase rise – a question for future exploration.

Omega-3 Supplementation and Cortisol

Omega-3 supplementation reduced total cortisol levels throughout the stressor. The high dose of omega-3 (2.5 g/d), but not the low dose (1.25 g/d), produced a significant (19%) reduction in total cortisol release compared to the placebo group. Importantly, across all participants, cortisol levels rose in response to the stressor, and therefore it is significant that those who received the omega-3 supplementation maintained lower cortisol levels throughout the stressor. These findings are especially notable in light of the growing literature implicating exaggerated cortisol stress reactivity in many clinically important outcomes, including hypertension (Hamer & Steptoe, 2012), coronary artery calcification (Hamer et al., 2012), and depression (Burke et al., 2005). In fact, heightened cortisol reactivity uniquely predicted coronary artery calcification, whereas sympathetic nervous system reactivity did not (Hamer et al., 2012). This literature suggests that our finding is clinically meaningful; through lower stress-induced cortisol release, omega-3 supplementation may help to prevent common chronic diseases and depression.

In addition to these disease outcomes, a greater cortisol response to acute stress dovetails with shorter telomeres (Steptoe et al., 2017; Tomiyama et al., 2012; Turner et al., 2020). Therefore, telomerase may rise in tandem with cortisol to protect telomeres. Indeed, Epel et al. (Epel et al., 2010) reported that those with greater cortisol reactivity had higher total telomerase levels throughout the stressor. In this sample, the placebo group had higher cortisol levels compared to the omega-3 groups throughout the stressor, but we observed a telomerase drop 120 minutes post-stress. This finding aligns with the observation that cortisol suppresses lymphocyte telomerase activity in vitro (Choi et al., 2008). Without a compensatory telomerase rise, telomeres may ultimately shorten at a faster rate overtime. In contrast, those in the omega-3 supplementation groups had lower total cortisol release and maintained stable levels of

telomerase throughout the stressor, a combination that could help to maintain telomere length across time.

Omega-3 Supplementation and Inflammation

The high 2.5 g/d dose of omega-3 lowered the overall pro-inflammatory IL-6 geometric mean throughout the stressor by 33%. The stressor triggered an increase in IL-6 across all participants, but even so, omega-3 supplementation decreased overall IL-6 levels during this stressful period. Although the 1.25 g/d dose did not lower overall IL-6 levels compared to the placebo, it was sufficient to prevent a 18% post-stress drop in the anti-inflammatory cytokine IL-10 geometric mean. In contrast, neither omega-3 dosage impacted TNF-α or IL-12 reactivity or overall levels. These RCT findings extend evidence from an observational study in which those with higher serum omega-3 levels had lower proinflammatory responses during high stress periods (Maes et al., 2000); our results suggest that omega-3 may directly modulate the inflammatory stress response.

Meta-analytic evidence suggests that IL-6 robustly increases following acute stress with a moderate to large effect size, while TNF-α shows a smaller increase (Marsland et al., 2017), which may explain our pattern of results. There were too few studies to assess IL-12 reactivity in the meta-analysis (Marsland et al., 2017). However, contrary to our placebo group's trajectory, IL-10 usually increases following acute stress, but publication bias is a concern (Marsland et al., 2017). Our sample characteristics (i.e., overweight, sedentary) may be responsible for the placebo group's post-stress decline in IL-10. If this is the case, omega-3 supplementation appears to reverse this effect, which could help to weaken the link between obesity and accelerated aging.

According to the concept of inflammaging, people with heightened inflammatory reactivity and an imbalance of pro- and anti-inflammatory cellular signaling may age too quickly on a cellular level (Franceschi et al., 2000). Proinflammatory cytokines provoke the release of

reactive oxygen species (De Biase et al., 2003), contributing to oxidative stress, inefficient cellular functioning, and more inflammation. However, the anti-inflammatory cytokine IL-10 has anti-oxidant properties (Haddad & Fahlman, 2002). By balancing pro- and anti-inflammatory cytokine release throughout an acute stressor, omega-3 promotes healthy cellular aging.

Omega-3 may reduce the risk of developing depression via lowered stress-induced inflammation. Meta analyses indicate that omega-3 supplementation can lower depressive symptoms (Liao et al., 2019; Mocking et al., 2016). Several studies suggest that depressed individuals have a heightened inflammatory stress response (Fagundes et al., 2013; G. E. Miller et al., 2005; Pace et al., 2006), and, even more intriguingly, one study found that such elevated reactivity predicted increased depressive symptoms one year later (Aschbacher et al., 2012). Therefore, our finding that omega-3 supplementation blunted the inflammatory stress response may help to explain omega-3 supplementation's antidepressant effect.

Clinical Implications

Most U.S. adults' dietary intake of omega-3 is well below recommended values. The Academy of Nutrition and Dietetics recommends that the general population consume 500 mg/day of EPA and DHA (Vannice & Rasmussen, 2014), but data from a nationally-representative sample revealed that the median intake from food and dietary supplements was 18 and 15 mg/day, respectively (Papanikolaou et al., 2014). For those with pre-existing conditions (e.g., mood disorders, cardiovascular disease), the recommendations are even higher (Freeman et al., 2006; Kris-Etherton et al., 2002). Although it can be difficult for those at high risk for heart disease to implement and sustain dietary changes (Dansinger et al., 2005), our high adherence rate indicates that daily omega-3 supplementation is feasible. Moreover, these findings suggest that apart from other dietary changes, daily omega-3 supplementation alone may help protect cells from the toll of acute stressors, thereby facilitating a healthy biological aging process.

Strengths and Limitations

This RCT's multiple strengths include the 4-month supplementation period, the 3-arm design with a placebo control, the baseline and post-intervention administration of a well-validated laboratory speech stressor, and the repeated biological measurements for 3.5 hours throughout the stressor. Additionally, telomerase measurement extended beyond the previously reported 90-minutes post-stress, which facilitated the discovery of a post-stress telomerase dip in the placebo group that was prevented by omega-3 supplementation. The 3-arm design supports causal claims; the biological outcomes were dose-dependent, in that the high dose and placebo groups had the greatest observed differences. Lastly, because each participant completed the acute laboratory stressor before and after the intervention, we could adjust for baseline reactivity to account for potential inter-individual variability.

One limitation is that our sample was predominately white, female, and highly educated. Even so, one noteworthy feature of our middle-aged sample is that they were overweight and not physically active, and therefore at increased risk for accelerated aging (Du et al., 2012; Horvath et al., 2014); these sample characteristics allowed us to examine whether omega-3 supplementation could lessen the divergence between chronological and biological aging. Another limitation is that the analyses did not use all randomized subjects due to the decision to assess telomerase and cortisol reactivity after the trial had already begun. However, we ensured that those included in these models did not differ in their baseline reactivity. Lastly, we did not connect stress-related telomerase fluctuations to telomere length, which is the biomarker associated with disease risk.

Conclusion

Four months of omega-3 supplementation led to a profile of stress resilience – lower overall levels of cortisol and inflammation during stress, and higher levels of telomerase and anti-inflammatory activity during recovery. This has direct relevance to aging biology and

66

psychiatry. These findings are preliminary, but if replicated, they suggest that omega-3

supplementation may limit the impact of repeated stress on cellular aging and depression risk.

Chapter 3 References

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and

    psychological markers of stress in humans: Focus on the Trier Social Stress Test.

    Neuroscience & Biobehavioral Reviews, *38*, 94–124.

Altemus, M., Rao, B., Dhabhar, F. S., Ding, W., & Granstein, R. D. (2001). Stress-induced

    changes in skin barrier function in healthy women. *Journal of Investigative Dermatology*,

    117(2), 309–317.

Aschbacher, K., Epel, E., Wolkowitz, O., Prather, A., Puterman, E., & Dhabhar, F. (2012).

    Maintenance of a positive outlook during acute stress protects against pro-inflammatory

    reactivity and future depressive symptoms. *Brain, Behavior, and Immunity*, *26*(2), 346–

    352.

Bekhbat, M., & Neigh, G. N. (2018). Sex differences in the neuro-immune consequences of

    stress: Focus on depression and anxiety. *Brain, Behavior, and Immunity*, *67*, 1–12.

Blackburn, E. H., Epel, E. S., & Lin, J. (2015). Human telomere biology: A contributory and

    interactive factor in aging, disease risks, and protection. *Science*, *350*(6265), 1193–

    1198.

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to

    psychological stress: A meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846–856.

Chen, S. H., Epel, E. S., Mellon, S. H., Lin, J., Reus, V. I., Rosser, R., Kupferman, E., Burke, H.,

    Mahan, L., & Blackburn, E. H. (2014). Adverse childhood experiences and leukocyte

    telomere maintenance in depressed and healthy adults. *Journal of Affective Disorders*,

    169, 86–90.

Choi, J., Fauce, S. R., & Effros, R. B. (2008). Reduced telomerase activity in human T

    lymphocytes exposed to cortisol. *Brain, Behavior, and Immunity*, *22*(4), 600–605.

Codd, V., Nelson, C. P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J. L., Hottenga, J. J.,

   Fischer, K., Esko, T., & Surakka, I. (2013). Identification of seven loci affecting mean

   telomere length and their association with disease. *Nature Genetics*, *45*(4), 422–427.

Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., & Schaefer, E. J. (2005).

   Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and

   heart disease risk reduction: A randomized trial. *Jama*, *293*(1), 43–53.

De Biase, L., Pignatelli, P., Lenti, L., Tocci, G., Piccioni, F., Riondino, S., Pulcinelli, F. M.,

   Rubattu, S., Volpe, M., & Violi, F. (2003). Enhanced TNFα and oxidative stress in

   patients with heart failure: Effect of TNFα on platelet O2-production. *Thrombosis and

   Haemostasis*, *90*(08), 317–325.

Du, M., Prescott, J., Kraft, P., Han, J., Giovannucci, E., Hankinson, S. E., & De Vivo, I. (2012).

   Physical activity, sedentary behavior, and leukocyte telomere length in women.

   American Journal of Epidemiology, *175*(5), 414–422.

Epel, E. S., Lin, J., Dhabhar, F. S., Wolkowitz, O. M., Puterman, E., Karan, L., & Blackburn, E.

   H. (2010). Dynamics of telomerase activity in response to acute psychological stress.

   Brain, Behavior, and Immunity, *24*(4), 531–539.

Fagundes, C. P., Glaser, R., Hwang, B. S., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2013).

   Depressive symptoms enhance stress-induced inflammatory responses. *Brain,

   Behavior, and Immunity*, *31*, 172–176.

Farzaneh-Far, R., Lin, J., Epel, E. S., Harris, W. S., Blackburn, E. H., & Whooley, M. A. (2010).

   Association of marine omega-3 fatty acid levels with telomeric aging in patients with

   coronary heart disease. *Jama*, *303*(3), 250–257.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De

   Benedictis, G. (2000). Inflamm-aging: An evolutionary perspective on

   immunosenescence. *Annals of the New York Academy of Sciences*, *908*(1), 244–254.

Freeman, M. P., Hibbeln, J. R., Wisner, K. L., Davis, J. M., Mischoulon, D., Peet, M., Keck Jr, P. E., Marangell, L. B., Richardson, A. J., & Lake, J. (2006). Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. *Journal of Clinical Psychiatry*, *67*(12), 1954.

Gidron, Y., Russ, K., Tissarchondou, H., & Warner, J. (2006). The relation between psychological factors and DNA-damage: A critical review. *Biological Psychology*, *72*(3), 291–304.

Ginty, A. T., & Conklin, S. M. (2012). Preliminary evidence that acute long-chain omega-3 supplementation reduces cardiovascular reactivity to mental stress: A randomized and placebo controlled trial. *Biological Psychology*, *89*(1), 269–272.

Haddad, J. J., & Fahlman, C. S. (2002). Redox-and oxidant-mediated regulation of interleukin-10: An anti-inflammatory, antioxidant cytokine? *Biochemical and Biophysical Research Communications*, *297*(2), 163–176.

Hamer, M., Endrighi, R., Venuraju, S. M., Lahiri, A., & Steptoe, A. (2012). Cortisol responses to mental stress and the progression of coronary artery calcification in healthy men and women. *PloS One*, *7*(2), e31356.

Hamer, M., & Steptoe, A. (2012). Cortisol responses to mental stress and incident hypertension in healthy men and women. *The Journal of Clinical Endocrinology & Metabolism*, *97*(1), E29–E34.

Haycock, P. C., Burgess, S., Nounu, A., Zheng, J., Okoli, G. N., Bowden, J., Wade, K. H., Timpson, N. J., Evans, D. M., & Willeit, P. (2017). Association between telomere length and risk of cancer and non-neoplastic diseases: A Mendelian randomization study. *JAMA Oncology*, *3*(5), 636–651.

Horvath, S., Erhart, W., Brosch, M., Ammerpohl, O., von Schönfels, W., Ahrens, M., Heits, N., Bell, J. T., Tsai, P.-C., & Spector, T. D. (2014). Obesity accelerates epigenetic aging of human liver. *Proceedings of the National Academy of Sciences*, *111*(43), 15538–15543.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., Hwang, B. S., & Glaser, R. (2012). Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: A randomized controlled trial. *Brain, Behavior, and Immunity*, *26*(6), 988–995.

Kiecolt-Glaser, J. K., Epel, E. S., Belury, M. A., Andridge, R., Lin, J., Glaser, R., Malarkey, W. B., Hwang, B. S., & Blackburn, E. (2013). Omega-3 fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial. *Brain, Behavior, and Immunity*, *28*, 16–24.

Kiecolt-Glaser, J. K., Renna, M. E., Shrout, M. R., & Madison, A. A. (2020). Stress Reactivity: What Pushes Us Higher, Faster, and Longer—And Why It Matters. *Current Directions in Psychological Science*, *29*(5), 492–498.

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*, *106*(21), 2747–2757.

Kurz, D. J., Decary, S., Hong, Y., Trivier, E., Akhmedov, A., & Erusalimsky, J. D. (2004). Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *Journal of Cell Science*, *117*(11), 2417–2426.

Liao, Y., Xie, B., Zhang, H., He, Q., Guo, L., Subramaniapillai, M., Fan, B., Lu, C., & Mclntyer, R. (2019). Efficacy of omega-3 PUFAs in depression: A meta-analysis. *Translational Psychiatry*, *9*(1), 1–9.

Maes, M., Christophe, A., Bosmans, E., Lin, A., & Neels, H. (2000). In humans, serum

polyunsaturated fatty acid levels predict the response of proinflammatory cytokines to

psychologic stress. *Biological Psychiatry*, *47*(10), 910–920.

Marsland, A. L., Walsh, C., Lockwood, K., & John-Henderson, N. A. (2017). The effects of acute

psychological stress on circulating and stimulated inflammatory markers: A systematic

review and meta-analysis. *Brain, Behavior, and Immunity*, *64*, 208–219.

Miller, G. E., Rohleder, N., Stetler, C., & Kirschbaum, C. (2005). Clinical depression and

regulation of the inflammatory response during acute stress. *Psychosomatic Medicine*,

67(5), 679–687.

Mocking, R., Harmsen, I., Assies, J., Koeter, M., Ruhé, H., & Schene, A. (2016). Meta-analysis

and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major

depressive disorder. *Translational Psychiatry*, *6*(3), e756–e756.

Monahan, K. D., Wilson, T. E., & Ray, C. A. (2004). Omega-3 fatty acid supplementation

augments sympathetic nerve activity responses to physiological stressors in humans.

Hypertension, *44*(5), 732–738.

Pace, T. W., Mletzko, T. C., Alagbe, O., Musselman, D. L., Nemeroff, C. B., Miller, A. H., &

Heim, C. M. (2006). Increased stress-induced inflammatory responses in male patients

with major depression and increased early life stress. *American Journal of Psychiatry*,

163(9), 1630–1633.

Papanikolaou, Y., Brooks, J., Reider, C., & Fulgoni, V. L. (2014). US adults are not meeting

recommended levels for fish and omega-3 fatty acid intake: Results of an analysis using

observational data from NHANES 2003–2008. *Nutrition Journal*, *13*(1), 31.

Rousseau, D., Moreau, D., Raederstorff, D., Sergiel, J. P., Rupp, H., Müggli, R., & Grynberg, A.

(1998). Is a dietary n-3 fatty acid supplement able to influence the cardiac effect of the

psychological stress? *Molecular and Cellular Biochemistry*, *178*(1–2), 353–366.
72

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P., & Jacobs, G. (1983). *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press.

Steptoe, A., Hamer, M., Lin, J., Blackburn, E. H., & Erusalimsky, J. D. (2017). The longitudinal relationship between cortisol responses to mental stress and leukocyte telomere attrition. *The Journal of Clinical Endocrinology & Metabolism*, *102*(3), 962–969.

Tomiyama, A. J., O'Donovan, A., Lin, J., Puterman, E., Lazaro, A., Chan, J., Dhabhar, F. S., Wolkowitz, O., Kirschbaum, C., & Blackburn, E. (2012). Does cellular aging relate to patterns of allostasis?: An examination of basal and stress reactive HPA axis activity and telomere length. *Physiology & Behavior*, *106*(1), 40–45.

Turner, A. I., Smyth, N., Hall, S. J., Torres, S. J., Hussein, M., Jayasinghe, S. U., Ball, K., & Clow, A. J. (2020). Psychological stress reactivity and future health and disease outcomes: A systematic review of prospective evidence. *Psychoneuroendocrinology*, 104599.

Vannice, G., & Rasmussen, H. (2014). Position of the academy of nutrition and dietetics: Dietary fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics*, *114*(1), 136–153.

Zalli, A., Carvalho, L. A., Lin, J., Hamer, M., Erusalimsky, J. D., Blackburn, E. H., & Steptoe, A. (2014). Shorter telomeres with high telomerase activity are associated with raised allostatic load and impoverished psychosocial resources. *Proceedings of the National Academy of Sciences*, *111*(12), 4519–4524.

Zhang, Y., Zhuang, P., He, W., Chen, J., Wang, W., Freedman, N., Abnet, C., Wang, J., & Jiao, J. (2018). Association of fish and long-chain omega-3 fatty acids intakes with total and cause-specific mortality: Prospective analysis of 421 309 individuals. *Journal of Internal Medicine*, *284*(4), 399–417.

Table 3.1. Baseline characteristics.

		Placebo (n = 46)	1.25 g/d (n = 46)	2.5 g/d (n = 46)
Age (years)*		51.1 (8.6)	51.1 (8.0)	51.0 (6.7)
Female		36 (78%)	28 (61%)	29 (63%)
Race				
	White	33 (72%)	39 (85%)	37 (80%)
	Black	9 (20%)	5 (11%)	8 (17%)
	Asian	2 (4%)	1 (2%)	1 (2%)
	Other	2 (4%)	1 (2%)	0 (0%)
Sagittal abdominal diameter (cm)*		22.8 (3.2)	23.9 (3.4)	22.9 (2.9)

*Data are mean (SD)

Figures
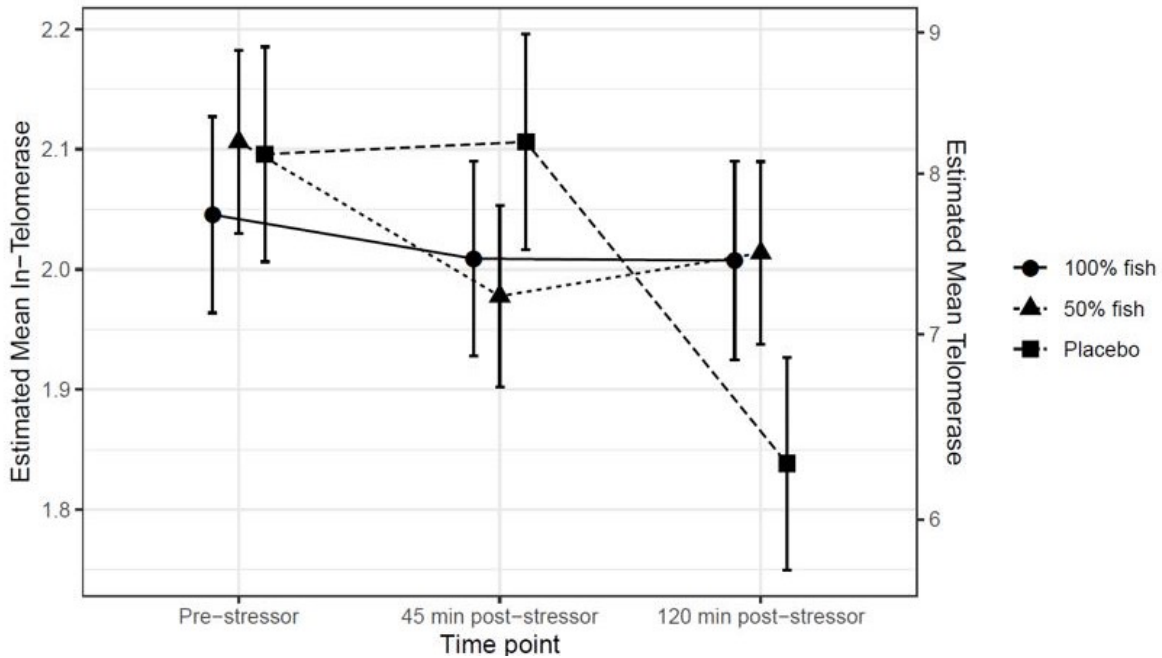
Figure 3.1. Omega-3 supplementation impacted telomerase reactivity to an acute stressor (p=0.05). Supplementation with either 2.5 g/d and 1.25 g/d of omega-3 prevented changes in telomerase following an acute stressor (ps>0.07). In contrast, those in the placebo group had a 24% decline in the geometric mean of telomerase from 45 minutes to 120 minutes after the stressor (p=0.001). Error bars are ± 1 standard error.

Figure 3.2. Omega-3 supplementation lowered total salivary cortisol output throughout an acute stressor (p=0.04). Specifically, supplementation with 2.5 g/d of omega-3 resulted in 19% lower total salivary cortisol throughout the stressor compared to the placebo group (p=0.01), but supplementation with 1.25 g/d of omega-3 did not affect cortisol levels compared to placebo group (p=0.29). Error bars are 95% confidence interval.
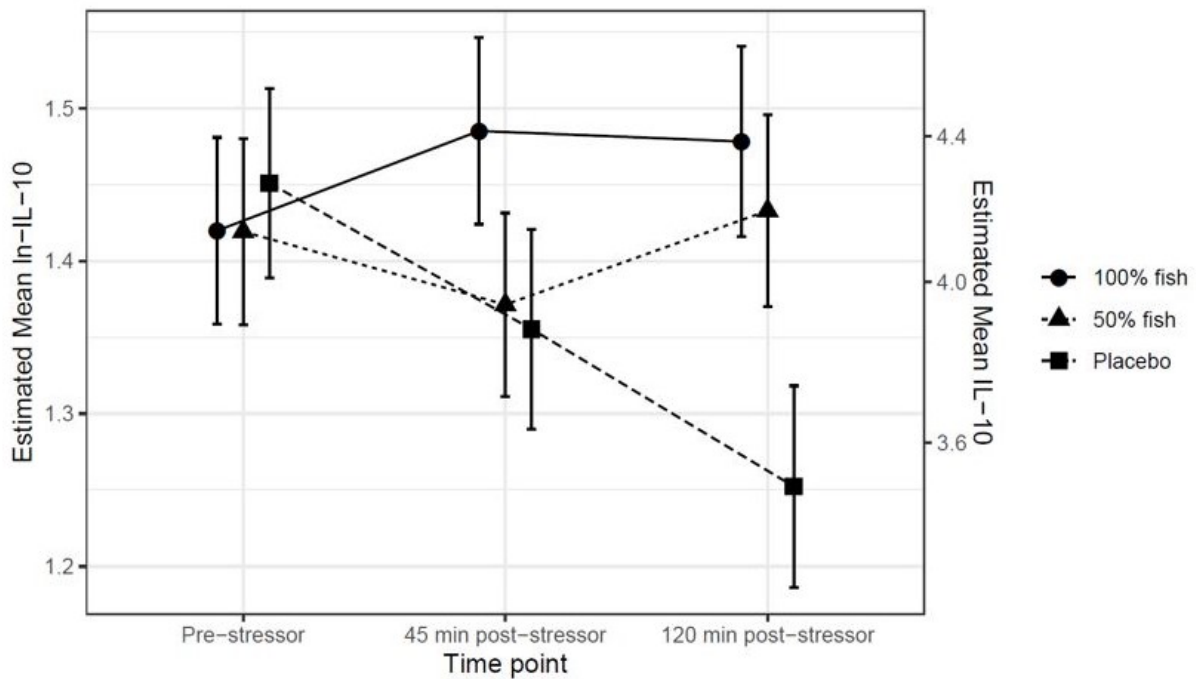
Figure 3.3. Omega-3 supplementation influenced IL-10 stress reactivity (p=0.047). Supplementation with either 2.5 g/d and 1.25 g/d of omega-3 prevented changes in IL-10 following an acute stressor (ps>0.31). In contrast, those in the placebo group had an 18% decline in the geometric mean of IL-10 from pre-stress to 120 minutes after the stressor (p=0.004), such that their IL-10 geometric mean was 26% lower than the high dose group (p=0.012) and 20% lower than the low dose group (p=0.047) 120 minutes after the stressor. Error bars are ± 1 standard error.
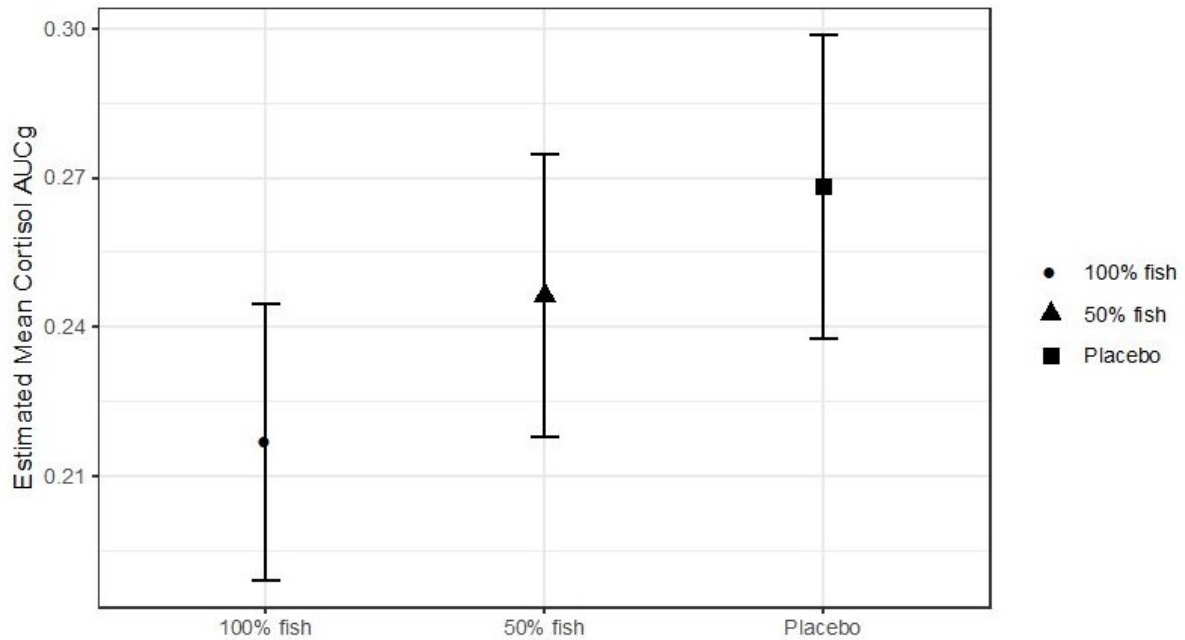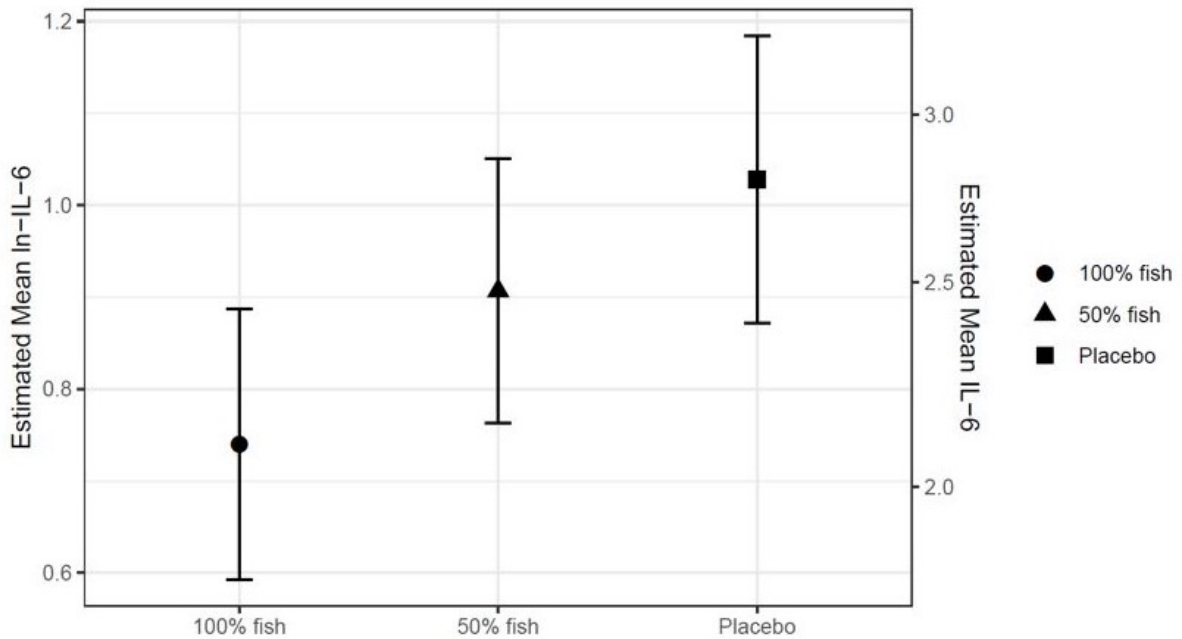
Figure 3.4. Omega-3 supplementation lowered overall IL-6 release throughout an acute stressor (p=0.03). Specifically, supplementation with 2.5 g/d of omega-3 resulted in a 33% lower geometric mean of IL-6, compared to the placebo group (p=0.007), but supplementation with 1.25 g/d of omega-3 did not affect IL-6 levels, compared to placebo (p=0.26). Error bars are 95% confidence interval.

# Supplemental Tables and Figures

Figure S3.1. Participant Flow by Group.
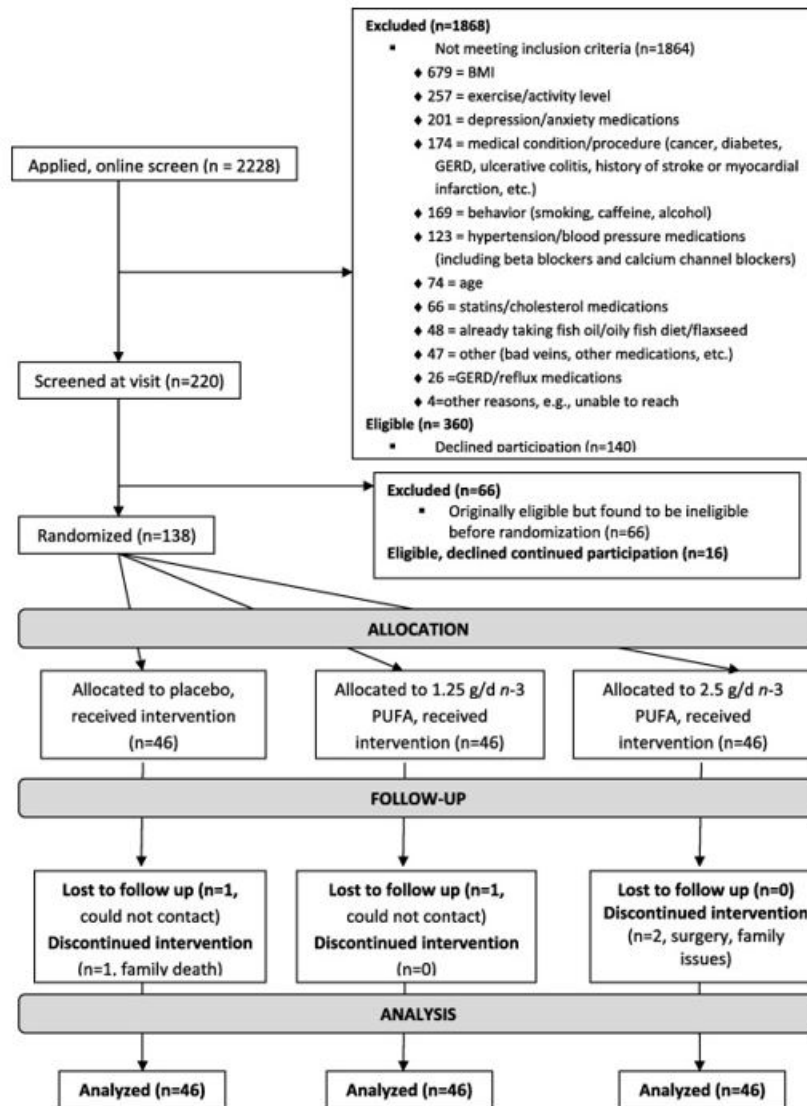
Table S3.1. Fatty Acid Composition of Dietary Oil Supplements (% Fatty Acids).

		Placebo	Supplement
		% Fatty acid	% Fatty acid
C14:0	Myristic acid	3.1	0.0
C16:0	Palmitic acid	16.4	0.1
C18:0	Stearic acid	3.2	0.5
C18:1n9	Oleic acid	48.7	0.7
C18:1n7	Vaccenic acid	1.6	0.3
C18:2n6	Linoleic acid	21.5	0.2
C18:3n3	Alpha linolenic acid	3.3	0.2
C18:4n3	Stearidonic acid	0.1	6.4
C20:4n6	Arachidonic acid	0.1	3.2
C20:4n3	Eicosatetraenoic acid	0.0	1.0
C20:5n3	Eicosapentaenoic acid	1.0	76.8
C22:6n3	Docosahexaenoic acid	0.1	8.5
Total Reported		100.0	100.0
Saturated Fatty Acids[1]		29.1	0.7
Monounsaturated Fatty Acids[2]		45.3	1.2
Omega 3 Fatty Acids[3]		4.8	93.3
Omega 6 Fatty Acids[4]		20.5	4.6

[1]C14:0, C16:0, C18:0

[2]C16:1n7, C18:1n7, C18:1n9

[3]Sum of (C18:3n3+C18:4n3+C20:4n3+C20:5n3+C22:5n3+C22:6n3)

[4]Sum of (C18:2n6+C18:3n6+C20:2n6+C20:3n6+C20:4n6+C22:4n6)

Table S3.2. Estimated Marginal Means for Telomerase and IL-10 Over Time.

| | | | | | P-values for Within-Group Changes | | |
| | | | | | Pre to 45 min | 45 min to 120 min | Pre to 120 min |
Outcome	Group	Pre-Stress	45 min Post	120 min Post			
ln(Telomerase)	100% fish	2.05 (0.082)	2.01 (0.081)	2.01 (0.083)	0.64	0.98	0.63
	50% fish	2.11 (0.076)	1.98 (0.075)	2.01 (0.076)	0.07	0.62	0.20
	placebo	2.10 (0.089)	2.11 (0.090)	1.84 (0.089)	0.90	**0.001**	**0.002**
ln(IL-10)	100% fish	1.42 (0.061)	1.49 (0.061)	1.48 (0.062)	0.31	0.92	0.38
	50% fish	1.42 (0.061)	1.37 (0.060)	1.43 (0.063)	0.46	0.35	0.84
	placebo	1.45 (0.062)	1.36 (0.065)	1.25 (0.066)	0.15	0.14	**0.004**

Data shown as Mean (SE). P-values are from contrasts in the hierarchical linear models.

Table S3.3. Estimated Marginal Means for Cortisol and IL-6

Outcome	Group	Mean (SE)		P-value vs. Placebo
Cortisol AUCg	100% fish	0.217	(0.014)	0.01
	50% fish	0.246	(0.014)	0.29
	placebo	0.268	(0.015)	--
ln(IL-6)	100% fish	0.740	(0.075)	**0.007**
	50% fish	0.907	(0.073)	0.26
	placebo	1.028	(0.079)	--

Data shown as Mean (SE). P-values are from linear regression model (cortisol) and contrasts in the hierarchical linear model (IL-6).

Chapter 4: Omega-3 Reduces Depressive Symptoms among the Socially-Stressed

(Accepted at *Health Psychology)*

Omega-3 Fatty Acids Reduce Depressive Symptoms Only Among the Socially Stressed:
A Corollary of the Social Signal Transduction Theory of Depression

Annelise A. Madison, M.A. [1,2], Janice K. Kiecolt-Glaser, Ph.D.[1,3], William B. Malarkey, M.D.[1,4], Martha A. Belury, Ph.D.[1,5]

(1) Institute for Behavioral Medicine Research, The Ohio State University College of Medicine, (2) Department of Psychology, The Ohio State University, (3) Department of Psychiatry and Behavioral Health, The Ohio State University College of Medicine, (4) Department of Internal Medicine, The Ohio State University College of Medicine, (5) Department of Human Sciences, College of Education and Human Ecology, The Ohio State University

ORCID IDs:
Annelise A. Madison: 0000 – 0001 – 6091 – 6844
Janice K. Kiecolt-Glaser: 0000 – 0003 – 4900 – 9578
William B. Malarkey: 0000 – 0002 – 1021 – 995X
Martha A. Belury: 0000 – 0002 – 6580 - 4281

Corresponding author: Annelise A. Madison, Institute for Behavioral Medicine Research, The Ohio State University College of Medicine, 460 Medical Center Drive, Columbus, OH 43210, 614-293-5184, annelise.madison@osumc.edu

Abstract

Objective: There is mixed evidence about whether omega-3 fatty acids reduce depressive symptoms. We previously reported that four months of omega-3 supplementation reduced inflammatory responsivity to a lab-based social stressor. In another study, we showed that those with exaggerated inflammatory responsivity to a social stressor had the greatest depressive symptom increases over time, especially if they experienced frequent social stress. Here we tested whether omega-3 supplementation reduced subthreshold depressive symptoms among those who experienced frequent social stress.

Methods: Healthy, sedentary, generally overweight middle-aged and older adults (*N*=138) were randomly assigned to four-months of pill placebo (*n*=46), 1.25 grams per day (g/d) omega-3 (*n*=46), or 2.5 g/d omega-3 (*n*=46). At a baseline visit and monthly follow-up visits, they reported depressive symptoms and had their blood drawn to assess plasma levels of omega-3 fatty acids. Participants completed the Trier Inventory of Chronic Stress at Visit 2 and the Test of Negative Social Exchange at Visit 3.

Results: Among those who were overweight or obese, both doses of omega-3 reduced depressive symptoms only in the context of frequent hostile interactions and social tension, and 2.5 g/d of omega-3 lowered depressive symptoms among those with less social recognition or more performance pressure (*p*s<.05). Findings were largely corroborated with plasma omega-3 fatty acids. No other social stress or work stress measure moderated omega-3 fatty acids' relationship with depressive symptoms (*p*s>.05).

Conclusions: Omega-3 fatty acids' antidepressant effect may be most evident among those who experience frequent social stress, perhaps because omega-3 fatty acids reduce inflammatory reactivity to social stressors.

Introduction

Chronic inflammation is common in the United States. More than 30% of U.S. adults have chronically elevated inflammation (C-reactive protein > 3 mg/L), which may be present even in the absence of disease (Ong et al., 2013). This inflammation is relevant not only to physical health outcomes, but also to depressive symptoms. Inflammation can play a role in sub- or supra-threshold depressive symptoms, and even slightly elevated inflammation can be problematic (Pariante, 2021).

Subthreshold depression is common and clinically important (Cuijpers et al., 2007). Effective treatment of subthreshold depression may prevent the onset of major depressive disorder. Although proinflammatory cytokine antagonists can effectively treat intractable cases of major depressive disorder when elevated inflammation is present (Raison et al., 2013), such biologic anti-inflammatory treatments are expensive and carry risks (Bonafede et al., 2012); therefore, they are unwarranted for subthreshold depressive symptoms. Inexpensive, over-the-counter agents and dietary approaches that can reduce inflammation may be effective depression-prevention strategies.

Omega-3 fatty acids may be good candidates, as they are available in diet and supplemental forms. Although several common foods are rich in omega-3 fatty acids (e.g., salmon, mackerel, chia and flax seeds, walnuts), most Americans do not have sufficient intake from foods alone. Median intake in one nationally-representative sample was 33 mg/day (Papanikolaou et al., 2014), which is well below the Academy of Nutrition and Dietetics recommendation of 500 mg/day (Vannice & Rasmussen, 2014). Among people who eat diets that are richer in omega-3 fatty acids, such as the Mediterranean diet, there is some evidence of lower depression burden (Sánchez-Villegas et al., 2013; Shafiei et al., 2019). Omega-3 fatty acid supplementation is a viable option for those who have insufficient dietary intake, as it is

safe and well-tolerated. It can reduce basal levels of inflammation (Kiecolt-Glaser et al., 2012) while also dampening inflammatory responsivity to acute social stress (Madison et al., 2021a).

In line with its anti-inflammatory effect, multiple meta-analyses of randomized controlled trials indicate that omega-3 fatty acid supplementation reduces depressive symptoms (Liao et al., 2019; Mocking et al., 2016). In these randomized controlled trials, omega-3 fatty acid supplementation has a medium effect size on depressive symptoms, compared to placebo, with certain subgroups finding more benefit (i.e., antidepressant users) (Liao et al., 2019; Mocking et al., 2016). However, several individual randomized controlled trials, including two from our lab (Kiecolt-Glaser, Belury, Andridge, Malarkey, & Glaser, 2011; Kiecolt-Glaser et al., 2012), have failed to find an anti-depressant effect. One explanation for these null findings is that participants' depressive symptoms were quite low upon entry into the trials (Kiecolt-Glaser, Belury, Andridge, Malarkey, & Glaser, 2011; Kiecolt-Glaser et al., 2012). The most up-to-date Cochrane review indicates that omega-3 fatty acid supplementation may only reduce depressive symptoms among those with more severe depression (Appleton et al., 2021). This observation suggests that omega-3 fatty acid supplementation may be a useful depression treatment strategy. However, it does not preclude the possibility that omega-3 supplementation may reduce subthreshold depressive symptoms among a subgroup, such as those with more frequent stress exposure.

In light of the Social Signal Transduction Theory of Depression, omega-3 fatty acids' antidepressant effect may be most evident among those who experience frequent social stress (Slavich & Irwin, 2014). This theory posits that those who have elevated inflammatory reactivity to social stress, as well as frequent exposure to social stress, will have the greatest depressive symptom increases across time (Slavich & Irwin, 2014). Using data from two distinct samples, each with a unique laboratory social stressor – one involving conflict with a marital partner and the other involving negative evaluation by a panel of unfamiliar judges (i.e., The Trier Social

86

Stress Test; TSST) – our lab provided evidence in support of this theory (Madison et al., 2021b). Notably, only among those who experienced frequent social stress, especially conflict or exclusion-related stress, did a heightened inflammatory response to acute stress predict depression symptom increases in the following months. This relationship did not hold for those who experienced frequent non-social stress. Similarly, after administering a typhoid vaccine or saline placebo to breast cancer survivors, we found that those who reported more frequent angry, insensitive, or interfering interactions in their daily lives reported more sadness and pain following inflammatory increases (Madison et al., 2023). In contrast, non-social stress did not sensitize these women to inflammation's effect on mood and pain. Thus, across samples, laboratory paradigms, timeframes, and inflammatory stimuli, we have found that chronic or repetitive conflict and exclusion-related stress increases psychological vulnerability to inflammation.

Social stressors can also trigger an inflammatory response, but this response is not unique to social stress. Indeed, meta-analytic evidence suggests that there is not a significant difference between inflammatory response magnitude for social threats versus other types of stressors (Marsland et al., 2017). Our lab showed that omega-3 fatty acid supplementation can reduce proinflammatory cytokine reactivity surrounding the TSST speech stressor (Madison et al., 2021a), yet even though our paradigm did not include a non-social stressor, omega-3 may also reduce inflammatory responses to non-social stressors. Nonetheless, because omega-3 reduces inflammatory responses to acute stress, we suspect that it should have a more potent effect on mood among those who are more psychologically vulnerable to the effects of inflammation (i.e, the socially-stressed).

The Current Study

This study features secondary, exploratory analyses of a parent randomized, placebo-controlled trial of omega-3 fatty acid supplementation among sedentary, overweight adults,

which found that 2.5 g/d and 1.25 g/d omega-3 supplementation reduced oxidative stress and basal inflammation but not depressive symptoms, compared to placebo (Kiecolt-Glaser et al., 2012, 2013). The parent study recruited overweight but otherwise healthy adults because they are at an increased risk for heightened inflammation and depression (Milaneschi et al., 2019; Pereira-Miranda et al., 2017). The current study follows up on the parent trial's null results for depressive symptoms by exploring individual difference factors that could moderate omega-3's effect. The first aim was to investigate social stress as a moderator of the relationship between omega-3 fatty acid supplementation and depressive symptoms. Specifically, we hypothesized that four months of omega-3 fatty acid supplementation would reduce depressive symptoms among those who experienced a higher frequency of social stress – in a dose-response manner. Also, we predicted that observed effects would be specific to social stress, compared to other types of stress (i.e., work-related stress), and that they would be stronger for conflict-related social stress than for other types of social stress (i.e., social overload). As an additional test, we substituted plasma levels of omega-3 fatty acids for supplementation group, and our hypotheses paralleled those above.

Research Design and Methods

Power Analysis

The most recent Cochrane meta-analysis found that omega-3 fatty acid supplementation has a small-to-modest benefit for depressive symptomology, compared to placebo (standardized mean difference= -0.30) (Appleton et al., 2021). We previously reported that social stress – especially social stress related to conflict and tension – was moderately related to depressive symptoms ($.35 < r s < .61$) (Madison et al., 2021). A sensitivity analysis with G*Power 3.1.9.4 revealed that the smallest effect size that we could detect is $f^2 = .11$ given the established sample size of 138 participants, assuming an alpha level of 0.05 with seven predictors (main effects, interaction effect, and covariates listed below). This small-to-medium effect size is on

par with the main effects above; however, it is important to note that the effect size of the interaction is not contingent on the main effects, and no prior work has tested the interactions of interest.

Participants and Research Protocol

Details about the participants, supplements, and procedure are found in the parent publication (Kiecolt-Glaser et al., 2012). In short, at a screening visit, trained experimenters administered the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders – IV (SCID-IV) to assess current and lifetime mood disorder history. The SCID-IV was incorporated after the first several screening visits, so SCID-IV data was not obtained for 15 participants. They were also screened for the following health conditions and medication usage, which were grounds for exclusion: psychoactive drugs, lipid-altering drugs, cardiovascular medications, steroids, prostaglandin inhibitors, heparin, warfarin, regular use of non-steroidal anti-inflammatory drugs other than a daily aspirin, substance abuse (including smoking), pregnancy, diabetes, current omega-3 fatty acid supplementation, digestive disorders, convulsive disorders, autoimmune and/or inflammatory diseases. Individuals were also excluded if they typically engaged in two or more hours of vigorous physical activity per week or had a body mass index (BMI) below 22.5 or over 40. The Ohio State University Institutional Review Board approved the study, and all participants provided written consent.

The 138 healthy, sedentary, generally overweight and obese middle-aged and older adults who were deemed eligible were randomly assigned to the placebo group (n=46), the 1.25 grams per day (g/d) omega-3 supplementation group (n=46), and the 2.5 g/d omega-3 supplementation group (n=46). Participants completed questionnaires and had their blood drawn to assess omega-3 fatty acid levels at the baseline visit as well as at one follow-up visit for each month of the four-month trial, for a total of five visits. Also at the baseline visit, participants' sagittal abdominal diameter was measured to provide data on abdominal fat. Prior

publications from this randomized, controlled trial have shown that four months of omega-3 fatty acid supplementation: (1) lowered basal IL-6 and TNF-α levels (Kiecolt-Glaser et al., 2012); (2) drove down oxidative stress (Kiecolt-Glaser et al., 2013); (3) reduced responsivity of key cellular aging biomarkers, including IL-6, during and after a laboratory speech stressor (Madison et al., 2021a); and (4) did not lower depressive symptoms, on average, across the entire sample (Kiecolt-Glaser et al., 2012).

Self-Report Measures

At each visit, the 20-item Center for Epidemiological Studies Depression Scale (CES-D) measured the frequency of depressive symptoms over the past week (Radloff, 1977) ($0.84 < \alpha$s < 0.91 at each visit). A cut score of 16 was used to index clinically significant depressive symptoms (Weissman et al., 1977). At Visit 2, the 57-item Trier Inventory of Chronic Stress (TICS-S) (Schulz & Schlotz, 2002), assessed frequency of work-related and interpersonal chronic stressors over the past three months, using a five-point Likert scale ranging from "never" (0) to "very often" (4) ($\alpha=0.96$). Five social stress subscales (social overload, lack of social recognition, social tension, performance pressure, and social isolation) indexed interpersonal stress, and three work stress subscales (work overload, work dissatisfaction, and overextended at work) measured non-interpersonal stress. Subscales ranged in length from four to nine items, which were summed for the total subscale score. Frequency of interpersonal conflict with important others over the past month was assessed with the revised 18-item Test of Negative Social Exchange (TENSE) scale at visit 3 (Ruehlman & Karoly, 1991) ($\alpha=0.93$). The subscales included hostility, ridicule, insensitivity, and interference, and ranged in length from three to six items. On a Likert scale, participants reported how often various tense interpersonal interactions occurred in the past month, spanning from 'not at all' (0) to 'about every day' (4). Items were averaged, such that the score for each subscale ranged from 0 to 4.

Omega-3 Fatty Acid Levels in Plasma

Chloroform: methanol (2:1, v/v) with 0.2 vol. 0.88% KCl was used to extract lipids from plasma (Bligh & Dyer, 1959). To prepare fatty acid methyl esters of the fractions, they were incubated with tetramethylguanidine at 100 °C (Shantha et al., 1993) and analyzed by gas chromatography (Shimadzu, Columbia, MD) using a 30-m Omegawax 320 (Supelco-Sigma) capillary column. The helium flow rate was 30 ml/min and oven temperature was held at 175 °C for 4 min and increased to 220 °C at a rate of 3°C/min as previously described (Belury & Kempa-Steczko, 1997). Retention times were compared to authentic standards for fatty acid methyl esters (Supelco-Sigma, St. Louis, MO and Mireya, Inc., Pleasant Gap, PA). For this study, the variable of interest was the sum of omega-3 fatty acids (Alpha linolenic Acid, Stearidonic Acid, Eicosatetraenoic Acid, Eicosapentaenoic Acid, Docosapentaenoic Acid, Docosahexaenoic Acid) as a percentage of plasma fatty acids.

Analytic Strategy

We first performed zero-order correlations between variables of interest at the baseline visit. We also conducted analysis of variance and chi-square to see whether supplementation groups differed on variables of interest. If a chi-square test was significant, we then calculated cell percentages and adjusted residuals (z-scores). If an analysis of variance test was significant, least significant difference post-hoc comparisons were performed. The TENSE variables were not normally distributed, so the Kruskal-Wallis one-way analysis of variance tested for group differences.

For the primary hypothesis, we used generalized estimating equation (GEE) models with robust standard error estimates (Ballinger, 2004; Zeger & Liang, 1986) to test whether the three-way interaction of supplementation group by stress by visit predicted depressive symptoms. GEE models were appropriate for these repeated measures analyses because they modeled participants' average responses, thereby providing efficient estimates of how much the average

response changed for every one-unit increase in a predictor variable (Ballinger, 2004; Zeger & Liang, 1986). Importantly, measures of social stress and non-social stress were run in separate models to determine whether any observed effects were unique to social stress. When the three-way interaction was non-significant, it was removed from models to test the two-way interaction of supplementation group by social stress, controlling for visit, to examine whether omega-3 fatty acids' antidepressant effect depended on social stress regardless of supplementation duration. In a second round of analyses, we used the same modelling strategy, but this time substituting total plasma omega-3 fatty acid levels for supplementation group. Blood was collected at each visit, so we used this time-varying variable in the interaction with stress and did not include visit in the interaction (but statistically adjusted for it) because we did not expect the effect of time-varying plasma omega-3 on depressive symptoms, conditioned on stress levels, to vary by visit. The TICS was assessed at Visit 2, so in these models, depressive symptoms at Visits 2 through Visit 5 were modeled. The TENSE was completed at Visit 3, so in these models, depressive symptoms at Visits 3 through 5 were modeled. We also performed the following pre-planned contrasts: (1) between-group mean differences in depressive symptoms at Visit 5 at high and low levels of social stress (25th and 75th percentiles); (2) within-group changes in depressive symptoms at high and low levels of social stress from the visit in which stress was assessed to Visit 5; and (3) overall group means of depressive symptoms at high and low social stress (pooled across time points).

Models adjusted for the covariates used in our prior publications from this randomized, controlled trial – age, sagittal abdominal diameter, and sex. We additionally adjusted for baseline depressive symptoms because workload (p=.003), work discontentment (p<.0001), feeling overextended at work (p<.0001), insensitive interactions (p=.04), social overload (p=.001), lack of social recognition (p=.003), social tension (p<.0001), and social isolation (p<.0001) – but not other stress measures (ps>.08) – positively tracked with baseline

92

depressive symptoms; thus, we wanted to ensure that any observed moderation effect by stress was not just due to the fact that stressed participants had more room for improvement in depressive symptoms.

Two participants were lost to follow-up (n=1 in placebo, n=1 in 1.25 g/d) and three (n=1 in placebo, n=2 in 2.5 g/d) discontinued the intervention. Of these five participants, three did not have enough data to be included in models. Also, one participant was missing a sagittal abdominal diameter measurement and therefore was excluded from all models. Therefore, in models that include TENSE subscales, 134 participants were included. We decided to incorporate the TICS after some participants had completed Visit 2, so in these models, there were 130 people. Overall, 91% of the participants were overweight using the BMI cut point of 25 kg/m^2. Heightened systemic inflammation is a common underlying factor in both obesity and some cases of depression, so the relationships of interest may be especially strong among those who are overweight. Therefore, we conducted post-hoc analyses that excluded the 13 participants who were not overweight from all the primary models. Two-tailed tests of significance were conducted and all alpha levels were set at α = 0.05. Data was analyzed with SAS version 9.4 (Armonk, NY).

Results

Randomization Check

Treatment groups were different in terms of education ($\chi^2(4)$ =10.78, p=.029); upon further inspection, a lower percentage of individuals in the 2.5 g/d supplementation group identified as college graduates likely because a higher percentage of individuals in this group identified as attending graduate or professional school. However, supplementation groups did not differ on household annual income (p=.31). Importantly, groups did not differ on baseline depressive symptoms (p=.97), mood disorder history (p=.56), prevalence of clinically significant depressive symptoms (CES-D ≥ 16, p=.40), BMI (p=.21), sagittal abdominal diameter (p=.18), or

plasma omega-3 fatty acid levels ($p=.28$) (Table 4.1). Social isolation differed across groups ($F_{(2, 130)}=3.85$, $p=.024$), in that the 1.25 g/d group reported lower social isolation than the placebo ($p=.037$) and 2.5 g/d groups ($p=.010$), but groups did not differ on any other stress measure (ps>.19) (Table 4.2).

Descriptive Statistics

Participants were middle-aged ($M=51.04$, $SD=7.75$), overweight and obese (BMI $M=30.69$, $SD=4.33$), and physically healthy, given the strict exclusionary criteria. Most were female ($n=93$, 67%), White ($n=109$, 79%), not Hispanic or Latino ($n=132$, 96%), and married ($n=92$, 67%). In terms of socioeconomic status, most participants had earned a college degree (72%) and reported a household annual income above $50,000 (62%). At the screening visit, 38% had a history of a mood disorder or adjustment disorder with depressive features, and 12% had clinically significant depressive symptoms as indexed by the CES-D cut score.

Covariates

Those who had more depressive symptoms at baseline also had more depressive symptoms during the intervention ($B=0.51$, $SE=0.11$, $\chi^2 (1)=14.31$, $p=.0002$). Sagittal abdominal diameter ($p=.24$), age ($p=.64$), and sex ($p=.09$) were unrelated to depressive symptoms.

Primary Results

Social Stress and Supplementation Group

The pattern of results from the whole sample is captured Table S4.1. No social stress measure moderated omega-3 fatty acids' antidepressant effect differentially across visits (three-way interactions: ps>.10). After removing the non-significant three-way interaction term, omega-3 fatty acids' antidepressant effect averaged across Visits 3 through 5 depended on frequency of hostile interactions (two-way interaction: $\chi^2 (2)=7.08$, $p=.029$). Specifically, among those at the 75th percentile of hostile interactions, both the 2.5 g/d ($\chi^2 (1)=6.43$, $p=.011$) and the 1.25 g/d ($\chi^2 (1)=6.20$, $p=.013$) omega-3 fatty acid supplementation groups had lower depressive

symptoms than the placebo group; however, these between-group differences in depressive symptoms did not exist among those at the 25th percentile of hostile interactions (*p*s>.09). All two-way interactions with other social stress measures were non-significant (*p*s>.12).

Pre-planned contrasts showed that there were significant group differences at Visit 5 following the expected dose-response pattern: Among those at the 75th percentile of hostile interactions, the 2.5 g/d omega-3 supplementation tracked with lower depressive symptoms at Visit 5, compared to the placebo group (χ^2 (1)=4.21, *p*=.040), and the 1.25 g/d supplementation group had marginally lower depressive symptoms than the placebo group (χ^2 (1)=3.60, *p*=.058), but the two omega-3 fatty acid supplementation groups did not differ (*p*=.73). In contrast, there were no between-group differences in depressive symptoms at Visit 5 for those at the 25th percentile of hostile interactions (*p*s>.24) (Figure 4.1B). All other pre-planned contrasts for hostility as well as other social stress measures were non-significant (*p*s>.08).

Some social stress measures were directly related to depressive symptoms. Specifically, those who reported more hostile interactions (*B*=1.68, *SE*=0.68, χ^2 (1)=4.26, *p*=.039), insensitive interactions (*B*=2.14, *SE*=0.69, χ^2 (1)=6.90, *p*=.009), interfering interactions (*B*=2.84, *SE*=0.95, χ^2 (1)=4.37, *p*=.037), social tension (*B*=0.40, *SE*=0.15, χ^2 (1)=6.24, *p*=.013), social isolation (*B*=0.36, *SE*=0.12, χ^2 (1)=7.31, *p*=.007), and performance pressure (*B*=0.19, *SE*=0.06, χ^2 (1)=7.84, *p*=.005) had more depressive symptoms.

Social Stress and Plasma Levels of Omega-3 Fatty Acids

Hostility interacted with total plasma level of omega-3 fatty acids to predict depressive symptoms (χ^2 (1)=4.23, *p*=.040), in that higher levels of plasma omega-3 fatty acids only had an antidepressant effect among those at the 75th percentile of hostile interactions (*B*=-0.53, *SE*=0.21, χ^2 (1)=6.32, *p*=.012) but not among those at the 25th percentile of hostile interactions (*p*=.63). The antidepressant effect of plasma omega-3 fatty acids was not dependent on frequency of interactions characterized by insensitivity (*p*=.58), ridicule (*p*=.16), or interference

95

(p=.58), nor by feelings of social overload (p=.38), social tension (p=.13), social isolation

(p=.51), or performance pressure (p=.13). However, plasma omega-3 fatty acids' relationship

with depressive symptoms depended on lack of social recognition (χ^2 (1)=5.11, p=.024). That is,

higher levels of plasma omega-3 fatty acids predicted lower depressive symptoms only among

those at the 75th percentile of lacking social recognition (B=-0.42, SE=0.19, χ^2 (1)=5.06, p=.025)

but not among those at the 25th percentile of lacking social recognition (p=.99).

Work Stress and Omega-3 Fatty Acids

The work stress subscales did not moderate omega-3 fatty acids' antidepressant effect

differentially across visits (three-way interactions: ps>.36), or pooled across visits (two-way

interactions: ps>.39). All pre-planned contrasts were non-significant (ps>.08). Those who

reported feeling more overloaded at work (main effect: B=0.24, SE=0.08, χ^2 (1)=6.68, p=.010),

more discontent with work (main effect: B=0.35, SE=0.10, χ^2 (1)=9.52, p=.002), and more

overextended at work (main effect: B=0.64, SE=0.15, χ^2 (1)=8.91, p=.003) had more depressive

symptoms. Plasma omega-3 fatty acids' association with depressive symptoms did not depend

on feelings of work discontent (p=.79), feeling overextended at work (p=.52), or feeling

overloaded at work (p=.99).

Sensitivity Analyses among Overweight and Obese Participants

The pattern of results from the overweight subsample is depicted in Table S4.2. After

excluding normal weight individuals (BMI < 25 kg/m^2), a similar pattern of results emerged with

a few notable exceptions that tended to better support original hypotheses. The effect of omega-

3 fatty acid supplementation group on depressive symptoms did not vary by any social stress

measure differentially across visits (three-way interactions: ps>.06). After removing the non-

significant three-way interaction term, the association between omega-3 fatty acid

supplementation group and depressive symptoms depended on frequency of hostile interactions

(two-way interaction: χ^2 (2)=6.74, p=.034). The 2.5 g/d (χ^2 (1)=7.33, p=.007) and 1.25 g/d (χ^2

(1)=6.43, p=.011) doses of omega-3 fatty acids lowered depressive symptoms compared to placebo across visits among those at the 75th percentile of hostile interactions, but depressive symptoms did not differ across the two omega-3 fatty acid supplementation groups (p=.60), nor among those with less frequent hostility (ps>.25) (Figure 4.1A). Also, a novel finding emerged in this subsample: The association between omega-3 fatty acid supplementation group and depressive symptoms depended on performance pressure (two-way interaction: χ^2 (2)=6.54, p=.038). Among those at the 75th percentile of performance pressure, the 2.5 g/d group had lower depressive symptoms across visits than the placebo group (χ^2 (1)=4.83, p=.028) (Figure 4.2), but no other between-group contrast was significant (ps>.05). In this subsample, omega-3 fatty acid supplementation's relationship with depressive symptoms did not depend on social isolation (p=.35), social tension (p=.08), lack of social recognition (p=.13), social overload (p=.17), nor interactions that involved interference (p=.51), ridicule (p=.63), or insensitivity (p=.15).

Similar to the analyses that included healthy weight individuals, pre-planned contrasts revealed that at Visit 5, the 2.5 g/d (χ^2 (1)=4.74, p=.030) and 1.25 g/d (χ^2 (1)=4.10, p=.043) groups had lower depressive symptoms compared to the placebo group only among those at the 75th percentile of hostile interactions (Figure 4.1B). The pre-planned contrasts revealed two additional unique findings in this subsample: Among overweight and obese individuals with more frequent social tension, the 2.5 g/d (χ^2 (1)=5.07, p=.024) and 1.25 g/d (χ^2 (1)=3.95, p=.047) doses of omega-3 fatty acid lowered depressive symptoms across visits, compared to the placebo group, but there was no difference between omega-3 fatty acid supplementation groups (p=.85), and this dose-response relationship was not evident among overweight individuals with less frequent social tension (ps>.19) (Figure 4.3A). Similarly, among overweight individuals at the 75th percentile of lacking social recognition, the 2.5 g/d omega-3 fatty acid group had lower depressive symptoms across visits than the placebo group (χ^2 (1)=4.32,

p=.038) (Figure 4.4A), but none of the other between-group contrasts were significant (ps>.21). Also, no other pre-planned contrasts with other stress subscales were significant (ps>.05).

When looking only at overweight and obese participants, plasma omega-3 fatty acids' relationship with depressive symptoms did not depend on social tension (p=.054), performance pressure (p=.13), social isolation (p=.36), social overload (p=.27), nor frequency of interactions involving ridicule (p=.16), interference (p=.87), or insensitivity (p=.55). However, this relationship significantly dependent on lack of social recognition (χ^2 (1)=5.51, p=.019) (Figure 4.4B) and frequency of hostile interactions (χ^2 (1)=3.87, p=.049) (Figure 4.1C). Among overweight individuals at the 75th percentile of lacking social recognition, higher levels of plasma omega-3 fatty acids predicted lower depressive symptoms (B=-0.50, SE=0.21, χ^2 (1)=5.77, p=.016), yet this relationship was non-significant among overweight individuals at the 25th percentile of lacking social recognition (p=.81). A similar pattern emerged for frequency of hostile interactions: Only among overweight individuals at the 75th percentile of hostile interactions did higher levels of plasma omega-3 fatty acids predict lower depressive symptoms (B=-0.63, SE=0.24, χ^2 (1)=6.71, p=.010), but this relationship was non-significant among overweight individuals at the 25th percentile of hostile interactions (p=.92).

Among overweight and obese participants, omega-3 fatty acid supplementation's relationship with depressive symptoms did not depend on any work stress subscale differentially across time (three-way interactions: ps>.51) or pooled across visits (two-way interactions: ps>.18). Also, the relationship between plasma omega-3 fatty acids and depressive symptoms was not moderated by any work stress subscale (ps>.35).

Discussion

In these secondary analyses of a randomized, placebo-controlled trial of omega-3 fatty acid supplementation among overweight and obese, sedentary, and physically healthy middle-aged adults, omega-3 fatty acids' effect on depressive symptoms depended on participants' levels of social stress. In line with hypotheses, this effect was unique to social stress; there was no evidence that work stress moderated the relationship between omega-3 fatty acids and depressive symptoms. Among the social stress measures, hostile interactions with close others over the past month most consistently made a difference – even among the full sample that included the healthy weight individuals. This result aligns with our prior finding that those with frequent conflict-related social stress and heightened inflammatory responsivity to a lab-based social stressor had the greatest increases in depressive symptoms over time (Madison, Andridge, et al., 2021); conversely, the current study showed that those with frequent conflict benefited the most from omega-3 fatty acids' antidepressant effect. The observed relationships were dose-dependent, as expected, suggesting a causal relationship between omega-3 fatty acid supplementation and lower depressive symptoms, dependent on social stress levels. Notably, the findings for the supplementation groups were replicated when using plasma levels of omega-3 fatty acids. In fact, another social stress variable – lack of social recognition – moderated plasma omega-3 fatty acids' relationship with depressive symptoms, in that higher levels of plasma omega-3 fatty acids tracked with lower depressive symptoms only among those at the 75th percentile of lacking social recognition. Results were even more pronounced when examining the overweight and obese subsample. In this subsample, there was evidence that hostile interactions, social tension, performance pressure, and lack of social recognition all moderated omega-3 fatty acids' relationship with depressive symptoms.

In this study, work stress did not moderate omega-3 fatty acids' relationship with depressive symptoms. These null results align with prior work showing the unique biological and

99

psychological salience of social stress, compared to other types of stress. For example, a seminal meta-analytic finding showed that the effect of a lack of strong social relationships on mortality risk was comparable to well-known risk factors including smoking and alcohol use, and exceeded the influence of other risk factors like obesity (Holt-Lunstad et al., 2010). Indeed, the Social Signal Transduction Theory of Depression focuses specifically on conflict and exclusion as powerful and reliable predictors of proinflammatory signaling and ultimately depression (Slavich & Irwin, 2014). Although there are untestable evolutionary theories as to social stress's unique biological and psychological relevance, more work is needed to elucidate whether there are distinctive neurobiological pathways mediating social stress's effect. Another possibility is that social stress – especially repetitive conflict with a close other – hits closer to home, literally and figuratively, than other types of stress, like a nagging boss. Ideally, the home environment is a place of rest and recovery, but an individual in a conflictual relationship may lay in bed with the source of their stress – prohibiting rest and recovery. In a hostile marriage, a potential source of support morphs into major stressor. Also, the Socioemotional Selectivity Theory posits that a bad marriage would have even greater effects in middle- and older adulthood as social networks downsize and the spouse becomes the primary source of interaction (Carstensen et al., 1999). Thus, our sample's age demographic may play a role in our results.

The pattern of results also demonstrated that only certain types of social stress mattered for omega-3 fatty acids' relationship with depressive symptoms. Generally speaking, social stress subscales that focused on perceived quantity of social interactions – both too many (social overload) and too few (social isolation) – did not moderate omega-3 fatty acids' anti-depressant effect. In contrast, many of the social stress subscales that indexed quality of social interactions (i.e., hostility, tension, lack of recognition, performance pressure) helped to determine omega-3 fatty acids' association with depressive symptoms, particularly among the overweight and obese

100

subsample. Given this pattern of results, it is unclear why ridiculing, insensitive, and interfering interactions did not moderate omega-3 fatty acids' antidepressant effect.

Our study paradigm and findings represent a step forward in terms of personalized, preventative medicine. Firstly, these results show that it is important to consider individual difference moderators of a treatment or preventative intervention's effect, as failing to do so may yield an overall null effect (Kiecolt-Glaser et al., 2011, 2012). Secondly, our study provides insight into a potential *proactive* depression prevention strategy that is especially relevant in societies in which chronic inflammation and subthreshold depression is prevalent. Thirdly, because this preventative strategy targets inflammation, it may also lower risk for comorbid physical disease development. Combining prevention and treatment efforts and implementing interventions that target common etiological factors in co-occurring disorders (e.g., inflammation) are two facets of the recently proposed community-embedded model, which is designed to more adequately address widespread mental health burden (Puffer & Ayuku, 2022).

From a biological perspective, it makes sense that social stress's modulating effect was most evident among the overweight and obese subsample. Elevated systemic inflammation and depression are more common among individuals who are overweight and obese (Milaneschi et al., 2019). Specifically, meta-analytic evidence suggests that obese individuals are 32% more likely to have depression than their normal-weight peers (Pereira-Miranda et al., 2017). Adipose tissue actively secretes proinflammatory soluble mediators (Kern et al., 2001), conducive to the development of a low mood. Therefore, it makes sense that omega-3 fatty acids' antidepressant effect would be most notable among overweight individuals who experience frequent social stress, as they have two risk factors for elevated inflammation and depression. These findings are especially relevant in the U.S. given that current projections estimate that nearly half of U.S. adults will be obese by 2030, and that a plurality of women, non-Hispanic Black adults, and low-income individuals will be severely obese (Ward et al., 2019).

101

A major caveat is that these results emerged among a sample with mild depressive symptoms and low levels of social stress. Our results may be even more pronounced among a sample with a wider range of stress levels. Results should be replicated among more diverse samples, which may have higher levels of stress levels (e.g., minority stress, poverty). Indeed, our sample was primarily White and well-educated, but those with minoritized identities are more likely to suffer from chronic depressive symptoms that impair daily functioning, which healthcare providers may not readily identify due to differing symptom presentations (Bailey et al., 2019). Relatedly, it was not a clinical sample of depressed patients, so our findings do not speak to the treatment of clinical depression (e.g., major depressive disorder, persistent depressive disorder). Even so, these results have implications for prevention of depression recurrence, as 38% of the sample had a mood disorder or adjustment disorder history. Our findings are also relevant for the treatment of commonly occurring subthreshold depressive symptoms in the general population. Such symptoms are clinically important, as they predict poorer physical health and less work productivity (Beck et al., 2011; Lyness et al., 2006). In essence, results from this non-clinical sample are relevant to the general population, and omega-3 fatty acid supplementation may help to reduce widespread productivity and health loss due to mild depressive symptoms.

The current work helps to explain why we and others have failed to find a direct effect of omega-3 fatty acid supplementation on depressive symptoms (Kiecolt-Glaser et al., 2011, 2012). That is, omega-3 fatty acid supplementation may help to prevent depressive symptoms among those who experience frequent social stress and therefore are more at risk for inflammation-related depressive symptom increases. The current work is a corollary of the Social Signal Theory of Depression, for which we have already provided empirical support (Madison, Andridge, et al., 2021). We demonstrated that individuals who have greater inflammatory reactivity to a lab-based social stressor as well as more frequent social stress, but

102

not other kinds of stress, report greater depressive symptom increases over time (Madison et al., 2021). These findings parallel other results from our lab which showed that people who had frequent social stress, but not other types of stress, were more sensitive to the mood and pain effects of mild inflammatory increases (Madison et al., 2023). We also have demonstrated that four months of omega-3 fatty acid supplementation can reduce inflammatory reactivity to acute social stress (Madison, Belury, et al., 2021). In this study, we close the loop, showing that omega-3 fatty acids' antidepressant effect is evident among those who have more frequent social stress. In short, those who are more psychologically vulnerable to the effects of inflammation (i.e., the socially-stressed) may experience a greater anti-depressant effect of omega-3 as it mutes their inflammatory responses.

This work suggests that for those in the general population with frequent social stress, omega-3 fatty acid supplementation may help to ward off depression symptom increases, perhaps by lowering inflammatory responses to stress (Madison, Belury, et al., 2021). This focus is advantageous because stress is inevitable, and omega-3 supplementation simply targets the cellular response to stress. According to the Social Signal Transduction Theory, it may also be fruitful to target stress exposure frequency. Specifically, among those who experience frequent social stress, such as a hostile marriage, addressing the stress directly (e.g., via communication or conflict resolution skills, marriage therapy, or separation/divorce) may be a necessary step in treating or preventing depression. Indeed, from a clinical standpoint, it is unhelpful, undermining, and even negligent to recommend an anti-inflammatory dietary supplement as a depression prevention strategy for someone in an abusive relationship, for example. Nevertheless, these findings show how an anti-inflammatory dietary supplement can help to buffer the mental health impact of commonplace social stress, which at times may be unavoidable.

Strengths and Limitations

Besides the demographic features of our sample discussed above, other important features of our sample include that they were sedentary, overweight, and middle-aged. These factors likely accentuated our results, and our findings may not replicate among a more active, healthy weight, and younger sample. Also, our stress measures relied on self-report data – another limitation due to the biases inherent in self-report. One idea for future work would be to observe a social interaction (e.g., a marital conflict) at the screening visit, code behavior (e.g., hostility), and then randomize participants with high and low levels of hostility to supplementation groups or placebo. It is also worth mentioning that the TICS and TENSE were administered at different visits, and this along with our limited statistical power precluded head-to-head comparison. Another limitation of this work is that, as an exploratory study, there were many statistical tests and we tested a greater number of social stress subscales than work stress subscales. Our significant results would not survive even the least conservative multiple test correction. That said, our *a priori* hypotheses focused on conflict-related social stress; because it was an exploratory study, we tested non-social stress as a comparison, but we did not expect to find effects. Although results need to be replicated before informing clinical practice, our pattern of results is notable given that it aligned with our theory-driven hypotheses as well as our prior results showing that conflict-related social stress can uniquely sensitize people to inflammation's psychological effects (Madison, Andridge, et al., 2021; Madison et al., 2023).

Strengths of our study include the three-arm, randomized, placebo-controlled design that tested two different doses of omega-3 fatty acid supplementation. Also, we measured plasma levels of omega-3 fatty acids to bolster evidence that increases in circulating omega-3 fatty acid levels were the mechanism of action. We largely replicated our results when substituting plasma levels of omega-3 fatty acids for supplementation group.

Clinical Implications

  One-third to one-half of depressed people have elevated inflammation (Raison & Miller, 2011; Rethorst et al., 2014). The relationship is bidirectional (Mac Giollabhui et al., 2020), promoting a vicious cycle of poor mental and physical health. This phenomenon helps to explain why depression commonly co-occurs with inflammatory diseases (Marrie et al., 2017) and why one-third to one-half of depression cases are resistant to traditional antidepressant treatments, which do not specifically target inflammation (e.g., selective serotonin reuptake inhibitors) (Nemeroff, 2007). The current study tests a safe, low-cost anti-inflammatory dietary supplement – omega-3 fatty acids – which may help to prevent the onset of inflammation-associated, difficult-to-treat cases of depression.

Chapter 4 References

Appleton, K. M., Voyias, P. D., Sallis, H. M., Dawson, S., Ness, A. R., Churchill, R., & Perry, R.
(2021). Omega-3 fatty acids for depression in adults. *Cochrane Database of Systematic
Reviews*, *11*.

Bailey, R. K., Mokonogho, J., & Kumar, A. (2019). Racial and ethnic differences in depression:
Current perspectives. *Neuropsychiatric Disease and Treatment*, 603–609.

Ballinger, G. A. (2004). Using generalized estimating equations for longitudinal data analysis.
Organizational Research Methods, *7*(2), 127–150.

Beck, A., Crain, A. L., Solberg, L. I., Unützer, J., Glasgow, R. E., Maciosek, M. V., & Whitebird,
R. (2011). Severity of depression and magnitude of productivity loss. *The Annals of
Family Medicine*, *9*(4), 305–311.

Belury, M. A., & Kempa-Steczko, A. (1997). Conjugated linoleic acid modulates hepatic lipid
composition in mice. *Lipids*, *32*(2), 199–204.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification.
Canadian Journal of Biochemistry and Physiology, *37*(8), 911–917.

Bonafede, M. M., Gandra, S. R., Watson, C., Princic, N., & Fox, K. M. (2012). Cost per treated
patient for etanercept, adalimumab, and infliximab across adult indications: A claims
analysis. *Advances in Therapy*, *29*(3), 234–248.

Carstensen, L. L., Isaacowitz, D. M., & Charles, S. T. (1999). Taking time seriously: A theory of
socioemotional selectivity. *American Psychologist*, *54*(3), Article 3.

Cave, C., Hein, N., Smith, L. M., Anderson-Berry, A., Richter, C. K., Bisselou, K. S., Appiah, A.
K., Kris-Etherton, P., Skulas-Ray, A. C., & Thompson, M. (2020). Omega-3 long-chain
polyunsaturated fatty acids intake by ethnicity, income, and education level in the United
States: NHANES 2003–2014. *Nutrients*, *12*(7), 2045.

Cuijpers, P., Smit, F., & Van Straten, A. (2007). Psychological treatments of subthreshold

depression: A meta-analytic review. *Acta Psychiatrica Scandinavica*, *115*(6), 434–441.

Holt-Lunstad, J., Smith, T. B., & Layton, J. B. (2010). Social relationships and mortality risk: A

meta-analytic review. *PLoS Medicine*, *7*(7), e1000316.

Kern, P. A., Ranganathan, S., Li, C., Wood, L., & Ranganathan, G. (2001). Adipose tissue tumor

necrosis factor and interleukin-6 expression in human obesity and insulin resistance.

American Journal of Physiology-Endocrinology and Metabolism, 280, E745-E751.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., & Glaser, R. (2011). Omega-

3 supplementation lowers inflammation and anxiety in medical students: A randomized

controlled trial. *Brain, Behavior, and Immunity*, *25*(8), Article 8.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., Hwang, B. S., & Glaser, R.

(2012). Omega-3 supplementation lowers inflammation in healthy middle-aged and older

adults: A randomized controlled trial. *Brain, Behavior, and Immunity*, *26*(6), 988–995.

Kiecolt-Glaser, J. K., Epel, E. S., Belury, M. A., Andridge, R., Lin, J., Glaser, R., Malarkey, W.

B., Hwang, B. S., & Blackburn, E. (2013). Omega-3 fatty acids, oxidative stress, and

leukocyte telomere length: A randomized controlled trial. *Brain, Behavior, and Immunity*,

28, 16–24.

Liao, Y., Xie, B., Zhang, H., He, Q., Guo, L., Subramaniapillai, M., Fan, B., Lu, C., & Mclntyer,

R. (2019). Efficacy of omega-3 PUFAs in depression: A meta-analysis. *Translational

Psychiatry*, *9*(1), 1–9.

Lyness, J. M., Heo, M., Datto, C. J., Ten Have, T. R., Katz, I. R., Drayer, R., Reynolds III, C. F.,

Alexopoulos, G. S., & Bruce, M. L. (2006). Outcomes of minor and subsyndromal

depression among elderly patients in primary care settings. *Annals of Internal Medicine*,

144(7), 496–504.

Mac Giollabhui, N., Ng, T. H., Ellman, L. M., & Alloy, L. B. (2020). The longitudinal associations of inflammatory biomarkers and depression revisited: Systematic review, meta-analysis, and meta-regression. *Molecular Psychiatry*, 1–13.

Madison, A. A., Andridge, R., Shrout, M. R., Renna, M. E., Bennett, J. M., Jaremka, L. M., Fagundes, C. P., Belury, M. A., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2021). Frequent interpersonal stress and inflammatory reactivity predict depressive symptom increases: Two tests of the social signal transduction theory of depression. *Psychological Science*, *33*(1), 152–164.

Madison, A. A., Belury, M. A., Andridge, R., Renna, M. E., Shrout, M. R., Malarkey, W. B., Lin, J., Epel, E. S., & Kiecolt-Glaser, J. K. (2021). Omega-3 supplementation and stress reactivity of cellular aging biomarkers: An ancillary substudy of a randomized, controlled trial in midlife adults. *Molecular Psychiatry*, 1–9.

Madison, A. A., Renna, M. E., Andridge, R., Peng, J., Sheridan, J., Lustberg, M., Ramaswamy, B., Wesolowski, R., VanDeusen, J. B., Williams, N. O., Sardesai, S. D., Noonan, A., Reinbolt, R. E., Stover, D., Cherian, M., Malarkey, W. B., & Kiecolt-Glaser, J. (2023). Conflicts hurt: Social stress predicts elevated pain and sadness following mild inflammatory increases. *Pain*, 10-1097.

Marrie, R. A., Walld, R., Bolton, J. M., Sareen, J., Walker, J. R., Patten, S. B., Singer, A., Lix, L. M., Hitchon, C. A., & El-Gabalawy, R. (2017). Increased incidence of psychiatric disorders in immune-mediated inflammatory disease. *Journal of Psychosomatic Research*, *101*, 17–23.

Marsland, A. L., Walsh, C., Lockwood, K., & John-Henderson, N. A. (2017). The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *64*, 208–219.

Milaneschi, Y., Simmons, W. K., van Rossum, E. F., & Penninx, B. W. (2019). Depression and obesity: Evidence of shared biological mechanisms. *Molecular Psychiatry*, *24*(1), 18–33.

Mocking, R., Harmsen, I., Assies, J., Koeter, M., Ruhé, H., & Schene, A. (2016). Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. *Translational Psychiatry*, *6*(3), e756–e756.

Nemeroff, C. B. (2007). Prevalence and management of treatment-resistant depression. *Journal of Clinical Psychiatry*, *68*(8), 17.

Ong, K. L., Allison, M. A., Cheung, B. M., Wu, B. J., Barter, P. J., & Rye, K.-A. (2013). Trends in C-reactive protein levels in US adults from 1999 to 2010. *American Journal of Epidemiology*, *177*(12), 1430–1442.

Papanikolaou, Y., Brooks, J., Reider, C., & Fulgoni, V. L. (2014). US adults are not meeting recommended levels for fish and omega-3 fatty acid intake: Results of an analysis using observational data from NHANES 2003–2008. *Nutrition Journal*, *13*(1), 31.

Pariante, C. M. (2021). Increased Inflammation in Depression: A Little in All, or a Lot in a Few? *American Journal of Psychiatry*, *178*(12), 1077–1079.

Pereira-Miranda, E., Costa, P. R., Queiroz, V. A., Pereira-Santos, M., & Santana, M. L. (2017). Overweight and obesity associated with higher depression prevalence in adults: A systematic review and meta-analysis. *Journal of the American College of Nutrition*, *36*(3), 223–233.

Puffer, E. S., & Ayuku, D. (2022). A Community-Embedded Implementation Model for Mental-Health Interventions: Reaching the Hardest to Reach. *Perspectives on Psychological Science*, 17456916211049362.

Radloff, L. S. (1977). The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement*, *1*(3), 385–401.

Raison, C. L., & Miller, A. H. (2011). Is depression an inflammatory disorder? *Current Psychiatry Reports*, *13*(6), 467–475.

Raison, C. L., Rutherford, R. E., Woolwine, B. J., Shuo, C., Schettler, P., Drake, D. F., Haroon, E., & Miller, A. H. (2013). A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: The role of baseline inflammatory biomarkers. *JAMA Psychiatry*, *70*(1), Article 1.

Rethorst, C. D., Bernstein, I., & Trivedi, M. H. (2014). Inflammation, obesity and metabolic syndrome in depression: Analysis of the 2009–2010 National Health and Nutrition Survey (NHANES). *The Journal of Clinical Psychiatry*, *75*(12), e1428.

Ruehlman, L. S., & Karoly, P. (1991). With a little flak from my friends: Development and preliminary validation of the Test of Negative Social Exchange (TENSE). *Psychological Assessment: A Journal of Consulting and Clinical Psychology*, *3*(1), 97.

Sánchez-Villegas, A., Martínez-González, M. A., Estruch, R., Salas-Salvadó, J., Corella, D., Covas, M. I., Arós, F., Romaguera, D., Gómez-Gracia, E., & Lapetra, J. (2013). Mediterranean dietary pattern and depression: The PREDIMED randomized trial. *BMC Medicine*, *11*(1), 1–12.

Schulz, P., & Schlotz, W. (2002). Das Trierer Inventar zur Erfassung von chronischem Stress— Version 2 (TICS 2). *Trierer Psychologische Berichte*, *29*.

Shafiei, F., Salari-Moghaddam, A., Larijani, B., & Esmaillzadeh, A. (2019). Adherence to the Mediterranean diet and risk of depression: A systematic review and updated meta-analysis of observational studies. *Nutrition Reviews*, *77*(4), 230–239.

Shantha, N. C., Decker, E. A., & Hennig, B. (1993). Comparison of methylation methods for the quantitation of conjugated linoleic acid isomers. *Journal of AOAC International*, *76*(3), 644–649.

Slavich, G. M., & Irwin, M. R. (2014). From stress to inflammation and major depressive

disorder: A social signal transduction theory of depression. *Psychological Bulletin*,

140(3), Article 3.

Vannice, G., & Rasmussen, H. (2014). Position of the academy of nutrition and dietetics: Dietary

fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics*, *114*(1),

136–153.

Ward, Z. J., Bleich, S. N., Cradock, A. L., Barrett, J. L., Giles, C. M., Flax, C., Long, M. W., &

Gortmaker, S. L. (2019). Projected US state-level prevalence of adult obesity and severe

obesity. *New England Journal of Medicine*, *381*(25), 2440–2450.

Weissman, M. M., Sholomskas, D., Pottenger, M., Prusoff, B. A., & Locke, B. Z. (1977).

Assessing depressive symptoms in five psychiatric populations: A validation study.

American Journal of Epidemiology, *106*(3), 203–214.

Zeger, S. L., & Liang, K.-Y. (1986). Longitudinal data analysis for discrete and continuous

outcomes. *Biometrics*, 121–130.

Table 4.1. Baseline characteristics.

		N	Placebo M(SD) or n(%)	1.25 g/d M(SD) or n(%)	2.5 g/d M(SD) or n(%)
Age (years)		138	51.1 (8.6)	51.1 (8.0)	51.0 (6.7)
Female		138	36 (78%)	28 (61%)	29 (63%)
Race		138			
	White		33 (72%)	39 (85%)	37 (80%)
	Black		9 (20%)	5 (11%)	8 (17%)
	Asian		2 (4%)	1 (2%)	1 (2%)
	Other		2 (4%)	1 (2%)	0 (0%)
BMI (kg/m^2)		137	30.0 (4.0)	31.6 (4.8)	30.6 (4.1)
Sagittal abdominal diameter (cm)		137	22.8 (3.2)	23.9 (3.4)	22.9 (2.9)
CES-D continuous score		138	7.7 (9.1)	7.7 (8.7)	7.4 (6.4)
CES-D cut score (% above)		138	6(13%)	7(15%)	3(7%)
Mood disorder history (% yes)		123	16(39%)	17(40%)	20(50%)
Plasma omega-3 (% of total fatty acids)		132	3.7 (0.8)	3.9 (0.8)	4.0 (1.1)
Education*		138			
	High school graduate or some college		13 (28%)	11 (24%)	15 (33%)
	College graduate		22 (48%)	21 (46%)	9 (19%)
	Graduate school		11 (24%)	14 (30%)	22 (48%)
Household income		138			
	<$50,000		19 (41%)	15 (33%)	11 (24%)
	$50,000 - $100,000		17 (37%)	19 (41%)	25 (54%)
	>$100,000		8 (17%)	10 (22%)	6 (13%)

Prefer not to answer	2 (4%)	2 (4%)	4 (9%)

*$p<.05$ chi-square or anova comparison across groups; g/d = grams per day; CES-D = Center for Epidemiological Studies Depression Scale

Table 4.2. Means and Standard Deviations of Stress Variables

	Placebo		1.25 g/d		2.5 g/d	
	n	*M(SD)*	*n*	*M(SD)*	*n*	*M(SD)*
Hostile interactions (TENSE)	45	.78(.93)	46	.96(.86)	44	.85(.79)
Ridicule interactions (TENSE)	45	.31(.68)	46	.28(.48)	44	.27(.50)
Insensitive interactions (TENSE)	45	.84(.99)	46	.75(.78)	44	.77(.77)
Interfering interactions (TENSE)	45	.61(.88)	46	.48(.54)	44	.45(.44)
Social overload (TICS)	44	9.50(4.70)	44	9.84(4.40)	43	9.65(5.18)
Social tension (TICS)	44	4.77(3.48)	44	5.34 (3.37)	43	5.51(3.22)
Social isolation (TICS)*	44	7.48(4.67)	44	5.52(3.83)	43	7.95(4.48)
Lack of social recognition (TICS)	44	4.80(3.22)	44	4.71(3.18)	43	5.02(2.83)
Work overload (TICS)	44	11.93(7.29)	44	12.07(6.55)	43	12.74(6.04)
Work discontent (TICS)	44	9.77(5.11)	44	8.80(4.87)	43	9.95(4.64)
Performance pressure (TICS)	44	13.48(6.63)	44	15.02(6.28)	43	15.81(6.30)
Overextended at work (TICS)	44	5.89(3.87)	44	4.93(3.93)	43	5.58(2.81)

g/d = grams per day; TENSE = Test of Negative Social Exchange, measured at Visit 3; TICS = Trier Inventory of Chronic Stressors, measured at Visit 2; *$p<.05$ mean difference across groups

Figure Captions

Figure 4.1A-C. Omega-3 fatty acids' relationship with depressive symptoms depended on frequency of hostile interactions. Among the overweight and obese subsample, frequency of hostile interactions moderated omega-3 fatty acid supplementation's effect on depressive symptoms pooled across Visits 3 to 5 (p=.034). Both the 2.5 grams per day (g/d) (p=.007) and 1.25 g/d (p=.011) doses lowed depressive symptoms compared to placebo across visits among those with more frequent hostile interactions (1A). Pre-planned contrasts showed that after four months of omega-3 fatty acid supplementation, the 2.5 g/d (p=.030) and 1.25 g/d (p=.043) doses lowed depressive symptoms, compared to placebo, among those with more frequent hostile interactions (1B). Plasma omega-3 fatty acid levels corroborated these findings, in that among those with more frequent hostile interactions, higher levels of plasma omega-3 fatty acid were associated with lower depressive symptoms across visits (p=.049) (1C).

Figure 4.2. Performance pressure moderated omega-3 fatty acid supplementation's effect on depressive symptoms. The association between omega-3 fatty acid supplementation group and depressive symptoms depended on performance pressure among the overweight and obese subsample (p=.038). Among those who reported more frequent performance pressure, those in the 2.5 g/d group had lower depressive symptoms across visits than those in the placebo group (p=.028).

Figure 4.3. Omega-3 fatty acids' association with depressive symptoms depended on social tension. Among the overweight and obese subsample, pre-planned contrasts revealed that the 2.5 g/d (p=.024) and 1.25 g/d (p=.047) doses of omega-3 fatty acids lowered depressive symptoms across visits, but only among those with more frequent social tension.

Figure 4.4A-B. Lack of social recognition moderated omega-3 fatty acids' effect on depressive symptoms. Pre-planned contrasts among the overweight and obese subsample revealed that the 2.5 g/d omega-3 fatty acid group had lower depressive symptoms across visits than the placebo group, but only among those who more frequently lacked social recognition (p=.038) (4A). Also, the relationship between plasma omega-3 fatty acids and depressive symptoms depended on lack of social recognition (p=.019). Specifically, among those who frequently lacked social recognition, higher levels of plasma omega-3 fatty acids tracked with lower depressive symptoms (p=.016) (4B).

Figure 4.1A-C. Omega-3 fatty acids' relationship with depressive symptoms depended on frequency of hostile interactions.
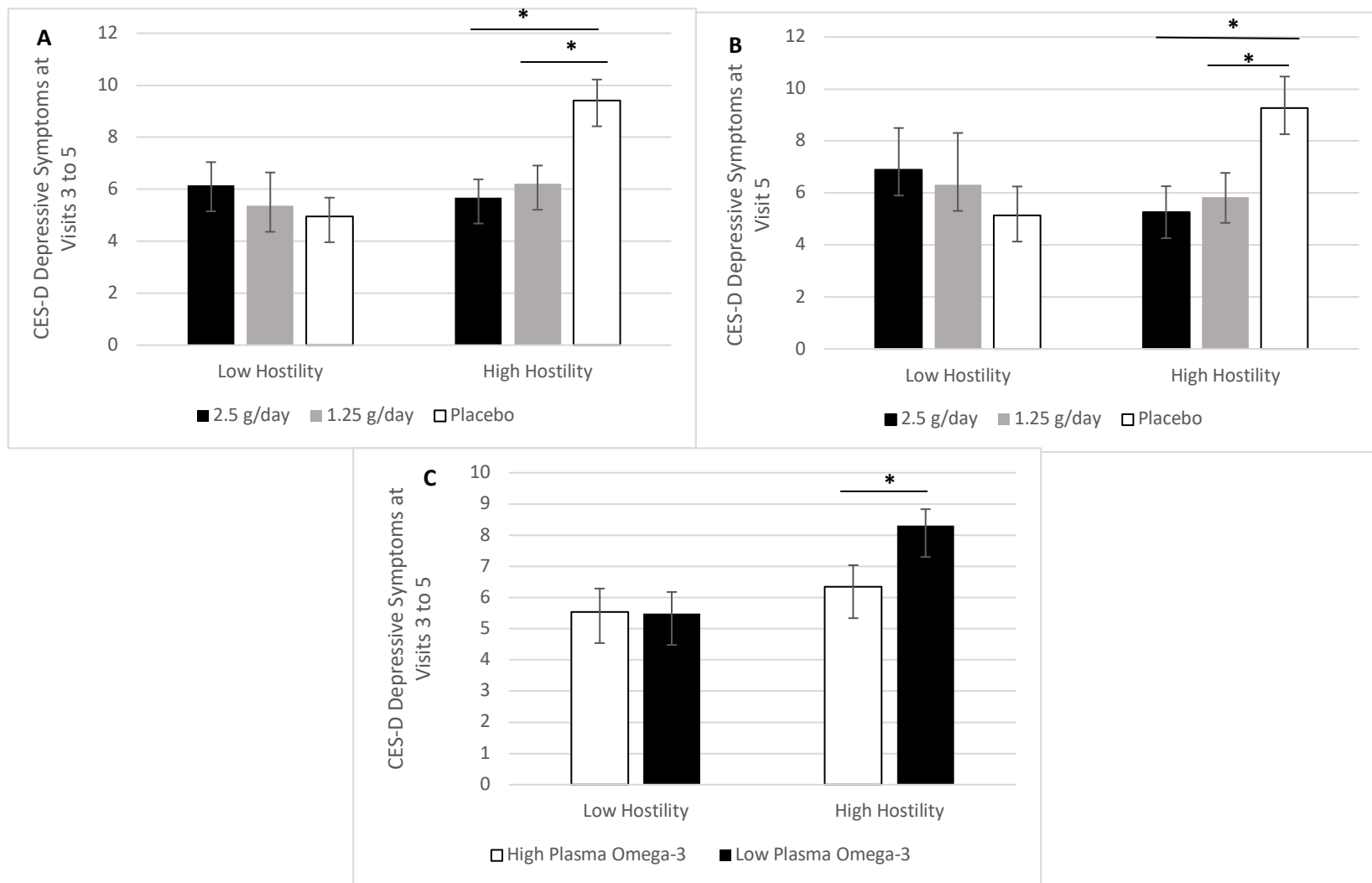
Figure 4.2. Performance pressure moderated omega-3 fatty acid supplementation's effect on depressive symptoms.
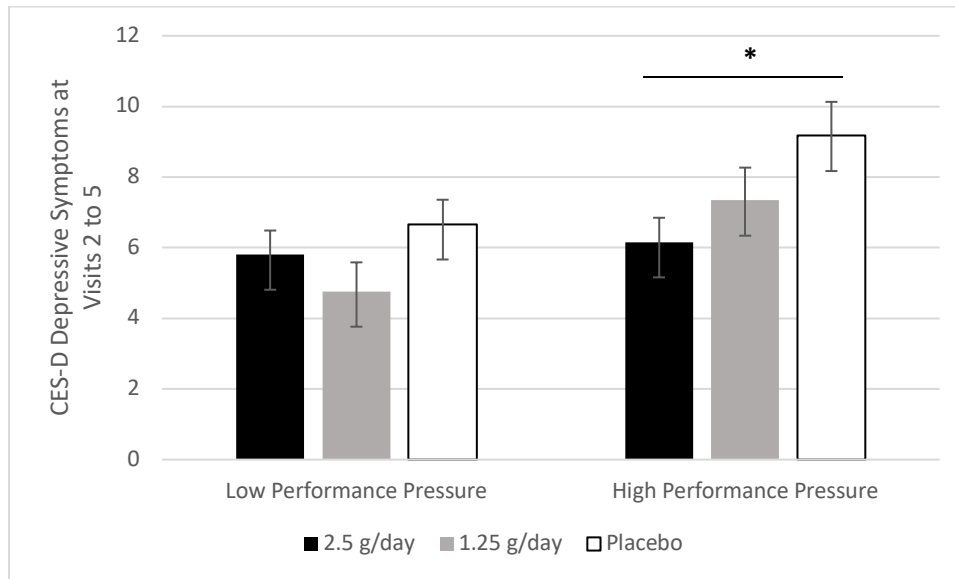
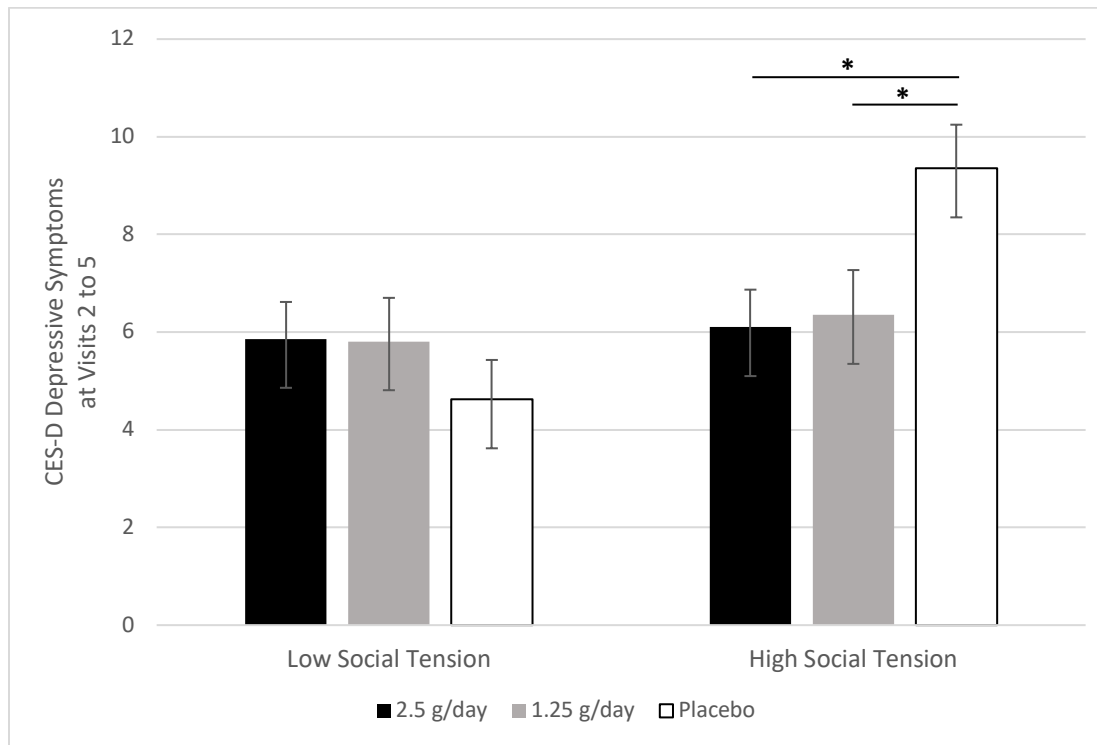Figure 4.3. Omega-3 fatty acids' association with depressive symptoms depended on social tension.

Figure 4.4A-B. Lack of social recognition moderated omega-3 fatty acids' effect on depressive symptoms.
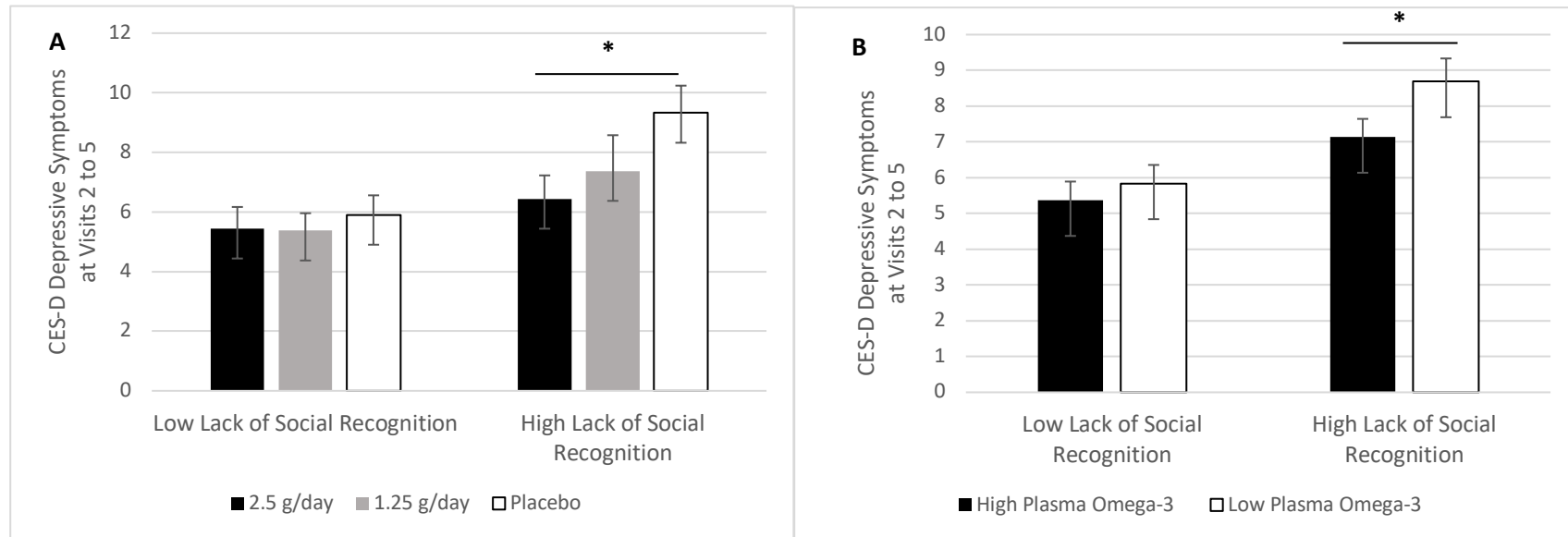
Table S4.1. Pattern of significant and non-significant results across the entire sample.

Stress Measures	Group*Visit*Stress	Group*Stress	Pre-Planned Contrast: Between-Group Differences for High and Low stress at Visit 5	Pre-Planned Contrast: Within-Group Change Across Visits at High and Low Stress	Pre-Planned Contrast: Between-Group Differences for High and Low Stress Pooled Across Visits	Plasma Omega-3*Stress
Hostile Interactions	n.s.	*	*	n.s.	n.s.	*
Ridicule Interactions	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Insensitive Interactions	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interfering Interactions	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Social Tension	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Social Overload	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Performance Pressure	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lack of Social Recognition	n.s.	n.s.	n.s.	n.s.	n.s.	*
Social Isolation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Work Discontent	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Work Overload	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Overextended at Work	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*$p<.05$

Table S4.2. Pattern of significant and non-significant results among overweight subsample.

Stress Measures	Group*Visit*Stress	Group*Stress	Pre-Planned Contrast: Between-Group Differences for High and Low Stress at Visit 5	Pre-Planned Contrast: Within-Group Change Across Visits at High and Low Stress	Pre-Planned Contrast: Between-Group Differences for High and Low Stress Pooled Across Visits	Plasma Omega-3*Stress
Hostile Interactions	n.s.	*	*	n.s.	n.s.	*
Ridicule Interactions	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Insensitive Interactions	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interfering Interactions	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Social Tension	n.s.	n.s.	n.s.	n.s.	*	marginal
Social Overload	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Performance Pressure	n.s.	*	n.s.	n.s.	n.s.	n.s.
Lack of Social Recognition	n.s.	n.s.	n.s.	n.s.	*	*
Social Isolation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Work Discontent	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Work Overload	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Overextended at Work	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*$p<.05$

Chapter 5: Discussion

Summary of Findings

This body of work includes a theory-driven examination of etiological factors underlying depressive symptom worsening, as well as an efficacy trial of a preventative intervention that targets these factors. Specifically, Chapter 2 provides empirical support for the Social Signal Transduction Theory of Depression, showing in two samples that those with heightened inflammatory responsivity to a lab-based social stressor combined with frequent social stress experience greater depressive symptom worsening over time (Madison et al., 2021). These results emerged in both a cancer and non-cancer sample, as well as across two distinct laboratory stress paradigms – a marital conflict and a speech stressor. The latter point suggests that inflammatory responsivity to stressors with close others, as well as social-evaluative stress with complete strangers, may both be relevant to depression risk. Also, this study demonstrated the unique biological and psychological relevance of social stress, as work stress did not influence the relationship between inflammatory responsivity and depressive symptom worsening.

The second study, presented in Chapter 3, targets one of these risk factors for depressive symptom worsening – exaggerated inflammatory responding to social stress – with an inexpensive, over-the-counter anti-inflammatory dietary supplement: Omega-3 (Madison et al., 2021). This study featured the same lab-based speech stressor included in Chapter 2, which participants completed before and after four months of omega-3 supplementation. Compared to placebo, the 2.5 g/day group had 33% lower geometric mean levels of the anti-inflammatory cytokine IL-6 throughout the stressor. In addition, both the 2.5 and 1.25 g/day doses prevented a 26% post-stress drop in the anti-inflammatory cytokine IL-10. In essence, omega-3 supplementation promoted a pattern of physiological stress responding that may help to prevent

depressive symptom increases over time. Even so, prior work is mixed regarding omega-3 supplementation's anti-depressant effect – the focus of Chapter 4.

Meta-analytic evidence suggests that omega-3 supplementation may benefit some individuals more so than others when it comes to depression alleviation (Appleton et al., 2021). For example, some studies in which participants had mild depressive symptoms reported null effects (e.g., Kiecolt-Glaser et al., 2011, 2012). Indeed, omega-3's antidepressant effect may be more apparent, uniform, and robust among those with severe depressive symptoms. Chapter 4 showcases work testing whether omega-3 supplementation may help to prevent depressive symptom worsening among a certain at-risk subgroup – those who experience frequent social stress. Given that omega-3 reduced inflammatory responsivity to social stress (Chapter 3), which is one risk factor for depressive symptom worsening (Chapter 2), it follows as a corollary of the Social Signal Transduction Theory of Depression, that those with frequent social stress might experience the greatest depression-prevention benefit from omega-3 supplementation. Hypotheses were largely supported, with the most robust effects emerging among the overweight subsample – for whom inflammation and depression are more common (Pereira-Miranda et al., 2017; Rethorst et al., 2014). That is, among overweight participants who reported more frequent hostile interactions, social tension, performance pressure, and lack of social recognition, omega-3 supplementation reduced depressive symptoms – an effect that was not evident among the less socially-stressed or among those who reported any frequency of work stress. As additional evidence, social stress but not work stress also modulated the relationship between plasma omega-3 and depressive symptoms, indicating that circulating omega-3 was indeed the anti-depressant mechanism of action. Therefore, social stress's unique relationship with inflammation and depression was apparent yet again in this study.

Social Stress's Unique Role

Conflict-related social stress (frequent hostile interactions) most consistently predicted depressive symptom increases (Chapter 2) and modulated omega-3's relationship with depressive symptoms (Chapter 4) – regardless of who was included in analyses. For example, Chapter 4 showed that among the full sample and the overweight subsample, frequency of hostile interactions in the past month determined omega-3's relationship with depressive symptoms. This result corresponded with findings among the first sample in Chapter 2: Conflict-related social stress predicted depressive symptom worsening among those with heightened inflammatory responsivity to social stress. In Chapter 4, other social stress subscales that indexed the quality, rather than quantity, of social interactions also modulated omega-3's relationship with depressive symptoms. Specifically, in additional to hostile interactions, performance pressure, social tension, and lack of social recognition, but not social overload or social isolation, shaped omega-3 supplementation's impact on depressive symptoms. These results slightly diverge from those among the second sample in Chapter 2. In this sample, sense of social belonging and loneliness predicted depressive symptom increases among those with exaggerated inflammatory responsivity to social stress. That is, in this sample, perceived quantity of social interactions was integral to inflammation-related depressive symptoms. Further work should investigate whether perceived quantity of social interactions is relevant to inflammation-related depressive symptoms.

Zooming out from the type of social stress, Chapters 2 and 4 both demonstrated the unique biological and psychological salience of social stress, compared to other types of stress – like general life stress or work stress. That is, in Chapter 2, work stress and general life stress did not interact with inflammatory responses to a lab stressor to predict depressive symptom increases. Yet, it is notable that the lab stressor was social in nature – one limitation; it remains to be seen whether frequency of work stress interacts with magnitude of inflammatory

responses to a non-social stressor to predict depressive symptom trajectories. Additionally,

Chapter 4 showed that work stress did not modulate omega-3's relationship with depressive

symptoms. Although they show social stress's importance to inflammation-related depression,

these results do not shed light onto why social stress outranked work stress and general life

stress in these models. One possibility is that social tasks, or at least those commonly used in

lab paradigms, inherently included a greater degree of uncertainty than non-social tasks (Berkay

& Jenkins, 2022). Another possibility: Social stressors may be more personally relevant and

simply more stressful than non-social stressors. For example, a 20-minute conflict with a spouse

over a highly contentious topic in the marriage is more salient and tailored to the individual than

an impersonal yet demanding cognitive test. It is also possible that social stress triggers activity

in different neuroimmune pathways, compared to non-social stress, but a specific social

pathway has yet to emerge in the literature. Yet it is notable that across samples, timeframes,

and paradigms, we have consistently showed that the socially-stressed are more vulnerable to

inflammation's mood effects (Madison, Andridge, et al., 2021; Madison et al., 2022). Future

work should explore whether there are unique biological or psychological features of the

socially-stressed that help to explain why they experience lower mood in response to both acute

and chronic inflammation.

Overarching Framework of This Work

The framework for this body of work is novel and deserves further comment because it

has the potential to address the growing public health burden of psychiatric disorders and

chronic inflammatory diseases, which often co-occur. The components of this framework,

discussed point-by-point below, are: (1) Starting with a theory-driven examination of etiological

factors, which then flowed to testing a relevant intervention to address those factors; (2)

Targeting etiological factors that are common to comorbid physical diseases and psychiatric

disorders; (3) Identifying pre-existing individual differences that modulate a treatment or

preventative intervention's effect; and (4) Studying relatively healthy samples with subthreshold symptomology to test proactive, preventative interventions.

1. From Etiology to Intervention

Although it is intuitive for etiology to undergird intervention development, many antidepressants and antipsychotics were initially developed without understanding the underlying molecular mechanisms (Hyman, 2013), and serendipity was a major factor in the discovery of many psychotropic drugs (Braslow & Marder, 2019). Even though there is now a better understanding of molecular mechanisms (e.g., neurotransmitter reuptake), it is not necessarily the case that psychotropic drugs reduce symptomology via this mechanism. For example, serotonin reuptake inhibitors may not actually lower depressive symptoms by increasing the availability of serotonin in the synapse. If the availability of serotonin in the synapse were the mechanism of action, the antidepressant effects would emerge within hours or days, rather than weeks or months. Indeed, the notion that serotonin plays a role in depression is still highly prevalent even though there is no consistent evidence to support an association between serotonin and depression (Moncrieff et al., 2022). A lack of understanding of the mechanism of action yields many unanswered questions, such as whether antidepressants are iatrogenic – ultimately elevating risk for relapse when discontinued (Hollon, 2020). In short, nebulous connections between etiology and treatment plague medication-related depression treatment and hinder its efficacy.

A lack of etiologically-informed intervention development also plagued psychology in the early days. For instance, Freud's theories, which formed the foundation for psychoanalysis, were not empirically-based, and because they attempted to explain rather than predict behavior, they were ultimately unfalsifiable and therefore unsupportable. However, more modern psychological interventions typically flowed from

126

a theory- and data-driven understanding of psychosocial, cognitive, behavioral, and affective etiological factors (e.g., thought distortions; Beck, 1963). The gold-standard psychotherapy to treat depression, cognitive-behavioral therapy, is indeed highly effective, and yet there are still many patients for whom it is less likely to work (e.g., men, those with comorbidities) (Johnsen & Friborg, 2015; Newton-Howes et al., 2014). Cognitive-behavioral therapy has a number needed to treat of 2.6 (Cuijpers et al., 2013). One critical consideration is that there are many different presentations of depression (Fried & Nesse, 2015), and this heterogeneity may represent different etiologies, which may dictate treatment efficacy (Boschloo et al., 2019).

The above discussion highlights that intervention development in the fields of psychiatry and psychology should start with etiology, and account for the possibility that there may be several different etiological pathways for a single disorder (i.e., equifinality). A failure to match treatment to etiology likely contributes to the high rate of so-called treatment-resistant depression (TRD: 30-50% of cases; Nemeroff, 2007). TRD itself is a misnomer because it does not consider psychotherapy treatment (Schroder et al., 2022), even though meta-analytic evidence demonstrates that psychotherapy can effectively treat it (Li et al., 2018). Also, this terminology suggests that the individual's depression is the problem, rather than the field's inadequate approach to treatment. Even so, the TRD categorization represents the widespread suffering resulting from inadequate treatment-matching.

The current body of work addresses these issues. Firstly, it tested a theory regarding the psychophysiological etiology of depression (The Social Signal Transduction Theory of Depression; Chapter 2). Notably, it did so among a non-clinical sample, prospectively following participants over time to assess depressive symptom trajectories, which is ideal for etiology research. These data supported the theory's

premise that heightened inflammatory responsivity to social stress combined with repetitive social stress exposure predicts depressive symptom worsening. The next step was to test an intervention targeting one of these etiological factors (i.e., omega-3 supplementation to reduce inflammatory responsivity to acute social stress; Chapter 3). The final step was to test whether it reduced depressive symptoms among those who experienced frequent social stress (Chapter 4). In essence, this body of work presents a theory-driven exploration of a specific etiological factor, and a strategy to modulate it to prevent depression among an at-risk sample.

2. Etiological Factors that Drive Comorbidity Development

Acute inflammation, during sickness or following a vaccine, can trigger transient depressive-like symptoms, including appetite changes, social withdrawal, and low mood (Lasselin et al., 2020). Similarly, longer-standing inflammation is associated with more chronic depressive symptoms (Mac Giollabhui et al., 2020). Indeed, naturalistic experiments, such as proinflammatory treatments for cancer patients (Capuron et al., 2004; Udina et al., 2012) as well as anti-inflammatory treatments for autoimmune diseases (e.g. Tiosano et al., 2020), provide evidence of a causal relationship between inflammation and depression. Thus, depression has joined the long list of negative outcomes resulting from chronic, systemic inflammation (Franceschi & Campisi, 2014; Furman et al., 2019). Indeed, chronic inflammation is a well-known hazard for cardiovascular disease (Kaptoge et al., 2014), cancer incidence and prognosis (Michels et al., 2021; Yang et al., 2018), type 2 diabetes (Wang et al., 2013), and infertility (Halis & Arici, 2004). Depression often co-occurs with these diseases and conditions (Gold et al., 2020), and chronic inflammation may be the common culprit – as well as a worthwhile treatment target. It is possible that exaggerated inflammatory responses to everyday hassles and stressors may set the stage for chronic inflammation, especially in

the context of frequent or repetitive stressors, which can prevent full recovery (Madison, 2021). Therefore, reducing inflammatory responses to such stressors may help to stave off not only depression but other potential medical comorbidities that commonly occur with depression. To effectively allocate limited healthcare resources, it is beneficial to identify and target etiological factors like inflammation that fuel a constellation of negative health outcomes.

3. Individual Difference Moderators

The current work illustrates the value in investigating theory-driven individual-difference moderators of a treatment or preventative intervention's effect – the bedrock of personalized medicine. Indeed, among the whole sample featured in Chapter 4, omega-3 did not have an anti-depressant effect (Kiecolt-Glaser et al., 2012); this effect was only apparent among the socially-stressed. This approach is important for two reasons: (1) Many etiological pathways can lead to the same disorder, as discussed above; and (2) Some individuals may be more psychological vulnerable to certain etiological factors. To use the inflammatory subtype of depression as an example, inflammatory markers are clinically elevated in up to half of depressed patients (Rethorst et al., 2014); yet, even subthreshold levels of inflammation may drive depression in particularly sensitive individuals (Pariante, 2021), or other etiological factors may be to blame for depression even in cases in which inflammation is clinically elevated. Recent work has uncovered a variety of individual difference factors that modulate psychological vulnerability to inflammation. For example, women with more state anxiety, perceived stress, negative affect, or sleep disturbances had higher depressive symptoms in the presence of elevated inflammation (Manigault et al., 2021). Individual differences in psychological sensitivity to inflammation is also evident following acute inflammatory stimuli (Lasselin, 2021; Madison et al., 2022). The current work shows that those who

experienced frequent social stress were more susceptible to inflammation-related

depressive symptom worsening, and, therefore, they also benefitted more from omega-

3's antidepressant effect. In line with a personalized medicine approach, this work shows

the importance of testing theory-driven *a priori*-specified individual differences factors as

moderators of treatment effectiveness.

4. Proactive Prevention

The public health burden of depression has surpassed treatment availability in

the U.S. Even before the COVID-19 pandemic, the World Health Organization found that

depression was the leading cause of disability worldwide, as the prevalence had soared

by almost 20% between 2005 and 2015, such that over 300 million people were

estimated to currently suffer with it (World Health Organization, 2017). During the

COVID-19 pandemic, the prevalence of major depressive disorder again rose sharply –

by about 28% -- worldwide, with an additional 53.2 million cases globally that

disproportionately affecting women and young people (Santomauro et al., 2021).

Therefore, in 2020, major depressive disorder caused almost 50 million disability-

adjusted life years, or years lost due to ill-health – a measure of disease burden

(Santomauro et al., 2021). Most depressed people do not receive any treatment. Only

one in five people with major depressive disorder in high-income countries and one in 27

people in low-income countries received minimally adequate treatment, even though a

majority acknowledged their need for treatment (Thornicroft et al., 2017). Lack of

treatment is concerning, as meta-analytic evidence pooled from the waitlist control arms

of 16 randomized trials suggests that only 8% to 18% of people with untreated

depression spontaneously remit within 12 weeks (Mekonen et al., 2021). Depression's

significant public health burden, combined with a lack of accessible evidence-based

treatments, points to the need for widespread preventative interventions.

Chapters 3 and 4 focused on a safe and easily accessible dietary supplement –

omega-3 – as a strategy to reduce inflammatory responses to social stress and prevent

depressive symptoms among the socially-stressed. In addition to potentially staving off

clinical depression onset or recurrence, another meaningful outcome is subthreshold

depressive symptom reduction because even mild symptoms can impinge on quality of

life, physical health, and productivity (Beck et al., 2011; Cronin-Stubbs et al., 2000;

Lyness et al., 2006; Wagner et al., 2000). Widespread omega-3 supplementation in the

U.S., where the general population consumes only about 7% of the recommended daily

amount, on average (Papanikolaou et al., 2014; Vannice & Rasmussen, 2014), may be

an effective and safe depression prevention strategy that could, in turn, improve physical

health and productivity.

Limitations and Future Directions

Aside from Study 2 in Chapter 1, which was a breast cancer survivor sample, these

studies were conducted with relatively healthy, well-educated, and mostly White adults living in

the U.S. It is unclear if these results would replicate in a more diverse sample, so further work is

needed in this domain. Accordingly, stress levels were also relatively low across samples, and

observed effects may be even more evident in a sample with wider ranges of stress. In fact,

observed effects may be even larger in a diverse sample in which minority stress and

discrimination – insidious, chronic forms of social stress targeted at one's identity – are more

prevalent. Another limitation is that the laboratory stress paradigms included in these studies

were social in nature, and it is unclear if degree of inflammatory responsivity to other types of

stressors (e.g., a cognitive test) responds to omega-3 supplementation or predicts depressive

symptom increases. However, Chapters 2 and 4 showed that social stress, contrasted to work

stress, distinctively predicted depressive symptoms and modulated omega-3's effect on

depressive symptoms. Further work is needed to ascertain why social stress may be particularly

relevant to inflammation-associated depressive symptoms. Also, this work only tested one potential anti-inflammatory strategy to prevent inflammation-related depression (i.e., omega-3 supplementation), and future work should identify whether other anti-inflammatory strategies (e.g., Mediterranean diet, meditation, exercise, vagus nerve stimulation) can have similar effects.

This line of work focused on treating one specific aspect of the Social Signal Transduction Theory of Depression – elevated inflammatory responses to social stress. This theory also suggests that reducing the frequency of social stress may also lower depressive symptoms. Indeed, in Chapters 2 and 4, there were fairly consistent main effects for social stress in that regardless of inflammatory response magnitude or levels of omega-3, more frequent social stress predicted greater depressive symptoms. These results accord with the common-sense notion that lessening the frequency of social stress exposure would reduce depressive symptoms. Indeed, in outlining his Social Safety Theory, Slavich articulated mechanisms through which various psychotherapeutic interventions might reduce social threat and increase social safety (Slavich, 2020). For example, the loving-kindness meditation in the mindfulness-based stress reduction protocol might enhance compassion and feelings of kindness toward familiar and unfamiliar others. Even so, it is not possible to circumvent all social stress, and therefore reducing inflammatory responsivity to social stress is an advantageous complementary treatment goal.

Conclusion

In line with personalized, proactive, and preventative medicine, this body of work has shown a psycho-immunological pathway to depression, ultimately finding support for the Social Signal Transduction Theory of Depression. The results suggest that frequent conflict-related social stress exposure, combined with heightened inflammatory responses to social stress, may contribute to depressive symptom worsening over time. Additionally, by reducing inflammatory

132

responses to social stress, omega-3 may lower depressive symptoms among those who are

socially stressed. These findings demonstrate the role of social stress-induced inflammation in

subthreshold depressive symptoms, as well as the efficacy of a common anti-inflammatory

dietary supplement in decreasing subthreshold symptoms among those particularly at-risk for

inflammation-related depression.

Chapter 5 References

Appleton, K. M., Voyias, P. D., Sallis, H. M., Dawson, S., Ness, A. R., Churchill, R., & Perry, R.

    (2021). Omega-3 fatty acids for depression in adults. *Cochrane Database of Systematic*

    Reviews, *11*.

Beck, A., Crain, A. L., Solberg, L. I., Unützer, J., Glasgow, R. E., Maciosek, M. V., & Whitebird,

    R. (2011). Severity of depression and magnitude of productivity loss. *The Annals of*

    Family Medicine, *9*(4), 305–311.

Beck, A. T. (1963). Thinking and depression: I. Idiosyncratic content and cognitive distortions.

    Archives of General Psychiatry, *9*(4), 324–333.

Berkay, D., & Jenkins, A. C. (2022). A Role for Uncertainty in the Neural Distinction Between

    Social and Nonsocial Thought. *Perspectives on Psychological Science*,

    17456916221112076.

Boschloo, L., Bekhuis, E., Weitz, E. S., Reijnders, M., DeRubeis, R. J., Dimidjian, S., Dunner, D.

    L., Dunlop, B. W., Hegerl, U., & Hollon, S. D. (2019). The symptom-specific efficacy of

    antidepressant medication vs. Cognitive behavioral therapy in the treatment of

    depression: Results from an individual patient data meta-analysis. *World Psychiatry*,

    18(2), 183–191.

Braslow, J. T., & Marder, S. R. (2019). History of Psychopharmacology. *Annual Review of*

    Clinical Psychology, *15*(1), 25–50.

Capuron, L., Ravaud, A., Miller, A. H., & Dantzer, R. (2004). Baseline mood and psychosocial

    characteristics of patients developing depressive symptoms during interleukin-2 and/or

    interferon-alpha cancer therapy. *Brain, Behavior, and Immunity*, *18*(3), 205–213.

Cronin-Stubbs, D., De Leon, C. F. M., Beckett, L. A., Field, T. S., Glynn, R. J., & Evans, D. A.

    (2000). Six-year effect of depressive symptoms on the course of physical disability in

    community-living older adults. *Archives of Internal Medicine*, *160*(20), 3074–3080.

Cuijpers, P., Berking, M., Andersson, G., Quigley, L., Kleiboer, A., & Dobson, K. S. (2013). A

   meta-analysis of cognitive-behavioural therapy for adult depression, alone and in

   comparison with other treatments. *The Canadian Journal of Psychiatry*, *58*(7), 376–385.

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential

   contribution to age-associated diseases. *Journals of Gerontology Series A: Biomedical

   Sciences and Medical Sciences*, *69*(Suppl_1), S4–S9.

Fried, E. I., & Nesse, R. M. (2015). Depression is not a consistent syndrome: An investigation of

   unique symptom patterns in the STAR* D study. *Journal of Affective Disorders*, *172*, 96–

   102.

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L.,

   Gilroy, D. W., Fasano, A., & Miller, G. W. (2019). Chronic inflammation in the etiology of

   disease across the life span. *Nature Medicine*, *25*(12), 1822–1832.

Gold, S. M., Köhler-Forsberg, O., Moss-Morris, R., Mehnert, A., Miranda, J. J., Bullinger, M.,

   Steptoe, A., Whooley, M. A., & Otte, C. (2020). Comorbid depression in medical

   diseases. *Nature Reviews Disease Primers*, *6*(1), 1–22.

Halis, G., & Arici, A. (2004). Endometriosis and inflammation in infertility. *Annals of the New

   York Academy of Sciences*, *1034*(1), 300–315.

Hollon, S. D. (2020). Is cognitive therapy enduring or antidepressant medications iatrogenic?

   Depression as an evolved adaptation. *American Psychologist*, *75*(9), 1207.

Hyman, S. E. (2013). *Psychiatric drug development: Diagnosing a crisis*. *2013*.

Johnsen, T. J., & Friborg, O. (2015). The effects of cognitive behavioral therapy as an anti-

   depressive treatment is falling: A meta-analysis. *Psychological Bulletin*, *141*(4), 747.

Kaptoge, S., Seshasai, S. R. K., Gao, P., Freitag, D. F., Butterworth, A. S., Borglykke, A., Di

   Angelantonio, E., Gudnason, V., Rumley, A., & Lowe, G. D. (2014). Inflammatory

cytokines and risk of coronary heart disease: New prospective study and updated meta-analysis. *European Heart Journal*, *35*(9), 578–589.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., & Glaser, R. (2011). Omega-3 supplementation lowers inflammation and anxiety in medical students: A randomized controlled trial. *Brain, Behavior, and Immunity*, *25*(8), Article 8.

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., Hwang, B. S., & Glaser, R. (2012). Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: A randomized controlled trial. *Brain, Behavior, and Immunity*, *26*(6), 988–995.

Lasselin, J. (2021). Back to the future of psychoneuroimmunology: Studying inflammation-induced sickness behavior. *Brain, Behavior, & Immunity-Health*, *18*, 100379.

Lasselin, J., Lekander, M., Benson, S., Schedlowski, M., & Engler, H. (2020). Sick for science: Experimental endotoxemia as a translational tool to develop and test new therapies for inflammation-associated depression. *Molecular Psychiatry*, 1–12.

Li, J.-M., Zhang, Y., Su, W.-J., Liu, L.-L., Gong, H., Peng, W., & Jiang, C.-L. (2018). Cognitive behavioral therapy for treatment-resistant depression: A systematic review and meta-analysis. *Psychiatry Research*, *268*, 243–250.

Lyness, J. M., Heo, M., Datto, C. J., Ten Have, T. R., Katz, I. R., Drayer, R., Reynolds III, C. F., Alexopoulos, G. S., & Bruce, M. L. (2006). Outcomes of minor and subsyndromal depression among elderly patients in primary care settings. *Annals of Internal Medicine*, *144*(7), 496–504.

Mac Giollabhui, N., Ng, T. H., Ellman, L. M., & Alloy, L. B. (2020). The longitudinal associations of inflammatory biomarkers and depression revisited: Systematic review, meta-analysis, and meta-regression. *Molecular Psychiatry*, 1–13.

Madison, A. A. (2021). Boosting stress resilience using flexibility as a framework to reduce depression risk. *Brain, Behavior, & Immunity-Health*, 100357.

Madison, A. A., Andridge, R., Shrout, M. R., Renna, M. E., Bennett, J. M., Jaremka, L. M.,
Fagundes, C. P., Belury, M. A., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2021). Frequent
interpersonal stress and inflammatory reactivity predict depressive symptom increases:
Two tests of the social signal transduction theory of depression. *Psychological Science*,
33(1), 152–164.

Madison, A. A., Belury, M. A., Andridge, R., Renna, M. E., Shrout, M. R., Malarkey, W. B., Lin,
J., Epel, E. S., & Kiecolt-Glaser, J. K. (2021). Omega-3 supplementation and stress
reactivity of cellular aging biomarkers: An ancillary substudy of a randomized, controlled
trial in midlife adults. *Molecular Psychiatry*, 1–9.

Madison, A. A., Renna, M., Andridge, R., Peng, J., Shrout, M. R., Sheridan, J., Lustberg, M.,
Ramaswamy, B., Wesolowski, R., & Williams, N. O. (2022). Conflicts hurt: Social stress
predicts elevated pain and sadness after mild inflammatory increases. *Pain*, 10–1097.

Manigault, A. W., Ganz, P. A., Irwin, M. R., Cole, S. W., Kuhlman, K. R., & Bower, J. E. (2021).
Moderators of inflammation-related depression: A prospective study of breast cancer
survivors. *Translational Psychiatry*, *11*(1), 1–9.

Mekonen, T., Ford, S., Chan, G. C., Hides, L., Connor, J. P., & Leung, J. (2021). What is the
short-term remission rate for people with untreated depression? A systematic review and
meta-analysis. *Journal of Affective Disorders*, 296, 17-25.

Michels, N., van Aart, C., Morisse, J., Mullee, A., & Huybrechts, I. (2021). Chronic inflammation
towards cancer incidence: A systematic review and meta-analysis of epidemiological
studies. *Critical Reviews in Oncology/Hematology*, *157*, 103177.

Moncrieff, J., Cooper, R. E., Stockmann, T., Amendola, S., Hengartner, M. P., & Horowitz, M. A.
(2022). The serotonin theory of depression: A systematic umbrella review of the
evidence. *Molecular Psychiatry*, 1–14.

Nemeroff, C. B. (2007). Prevalence and management of treatment-resistant depression. *Journal of Clinical Psychiatry*, *68*(8), 17.

Newton-Howes, G., Tyrer, P., Johnson, T., Mulder, R., Kool, S., Dekker, J., & Schoevers, R. (2014). Influence of personality on the outcome of treatment in depression: Systematic review and meta-analysis. *Journal of Personality Disorders*, *28*(4), 577.

Papanikolaou, Y., Brooks, J., Reider, C., & Fulgoni, V. L. (2014). US adults are not meeting recommended levels for fish and omega-3 fatty acid intake: Results of an analysis using observational data from NHANES 2003–2008. *Nutrition Journal*, *13*(1), 31.

Pariante, C. M. (2021). Increased Inflammation in Depression: A Little in All, or a Lot in a Few? *American Journal of Psychiatry*, *178*(12), 1077–1079.

Pereira-Miranda, E., Costa, P. R., Queiroz, V. A., Pereira-Santos, M., & Santana, M. L. (2017). Overweight and obesity associated with higher depression prevalence in adults: A systematic review and meta-analysis. *Journal of the American College of Nutrition*, *36*(3), 223–233.

Rethorst, C. D., Bernstein, I., & Trivedi, M. H. (2014). Inflammation, obesity and metabolic syndrome in depression: Analysis of the 2009–2010 National Health and Nutrition Survey (NHANES). *The Journal of Clinical Psychiatry*, *75*(12), e1428.

Santomauro, D. F., Herrera, A. M. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M., Abbafati, C., Adolph, C., Amlag, J. O., & Aravkin, A. Y. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *The Lancet*, *398*(10312), 1700–1712.

Schroder, H. S., Patterson, E. H., & Hirshbein, L. (2022). Treatment-resistant depression reconsidered. *SSM-Mental Health*, *2*, 100081.

Slavich, G. M. (2020). Social safety theory: A biologically based evolutionary perspective on life stress, health, and behavior. *Annual Review of Clinical Psychology*, *16*, 265.

Thornicroft, G., Chatterji, S., Evans-Lacko, S., Gruber, M., Sampson, N., Aguilar-Gaxiola, S., Al-Hamzawi, A., Alonso, J., Andrade, L., & Borges, G. (2017). Undertreatment of people with major depressive disorder in 21 countries. *The British Journal of Psychiatry*, *210*(2), 119–124.

Tiosano, S., Yavne, Y., Watad, A., Langevitz, P., Lidar, M., Feld, J., Tishler, M., Aamar, S., Elkayam, O., & Balbir-Gurman, A. (2020). The impact of tocilizumab on anxiety and depression in patients with rheumatoid arthritis. *European Journal of Clinical Investigation*, *50*(9), e13268.

Udina, M., Castellví, P., Moreno-España, J., Navinés, R., Valdés, M., Forns, X., Langohr, K., Solà, R., Vieta, E., & Martín-Santos, R. (2012). Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. *The Journal of Clinical Psychiatry,* 73(8), 9260.

Vannice, G., & Rasmussen, H. (2014). Position of the academy of nutrition and dietetics: Dietary fatty acids for healthy adults. *Journal of the Academy of Nutrition and Dietetics*, *114*(1), 136–153.

Wagner, H. R., Burns, B., Broadhead, W., Yarnall, K., Sigmon, A., & Gaynes, B. (2000). Minor depression in family practice: Functional morbidity, co-morbidity, service utilization and outcomes. *Psychological Medicine*, *30*(6), 1377–1390.

Wang, X., Bao, W., Liu, J., OuYang, Y.-Y., Wang, D., Rong, S., Xiao, X., Shan, Z.-L., Zhang, Y., & Yao, P. (2013). Inflammatory markers and risk of type 2 diabetes: A systematic review and meta-analysis. *Diabetes Care*, *36*(1), 166–175.

World Health Organization. (2017). *Depression and other common mental disorders: Global health estimates*. World Health Organization.

Yang, R., Chang, Q., Meng, X., Gao, N., & Wang, W. (2018). Prognostic value of systemic immune-inflammation index in cancer: A meta-analysis. *Journal of Cancer*, *9*(18), 3295.