Childhood Adversity and Systemic Inflammation in Preschool-Aged Children:

The Role of Family Cohesion

By

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Submitted to the graduate degree program in Clinical Child Psychology in the Departments of Psychology and Applied Behavioral Science and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Abstract

Systemic inflammation is a critical physiological mechanism that appears to link exposure to early childhood adversity to later disease. However, some children exposed to adversity have low levels of inflammatory proteins and do not go on to develop health problems. Thus, understanding what factors contribute to less inflammation in some (but not in others) is key to environmental effects on youth health. Family cohesion is one potential contributor to the differences in inflammation levels among adversity-exposed children. This study aimed to test the association between adversity and systemic inflammation, and the role of family cohesion as a moderator of this relation in 145 3- to 6-year-old children recruited from Head Start and the state Department of Social Services. Parents completed the Childhood Experiences Measure and the Cohesion subscale of the Family Environment Scale. Biomarkers linked to systemic inflammation (i.e., IL-6, IL-1β, IL-8, TNF-α, and CRP) were collected via saliva. Using path modeling, the results indicated that increases in adversity exposure were associated with increases in inflammation; adversity explained 27% of the variance in inflammation. The model testing family cohesion as a moderator was nonsignificant. Although family cohesion did not serve as a buffer as expected, dosage and frequency of adversity emerged as important factors influencing systemic inflammation in young children. These findings may suggest a need for a sharpened awareness of early adversity's impact on biology among professionals who work with families exposed to adversity. Otherwise, the presence of these potential future disease indicators may go unnoticed in young children.

Acknowledgements

I would first like to acknowledge the parents and preschoolers who shared their time, life experiences, and saliva; without them this work would not have been possible. I only hope that my research, now and in the future, can benefit families like them. I am also extremely appreciative of the PAIR project's numerous community partners, not only for their resources but for their ongoing belief in our research mission. I want to extend a big thank you to the entire PAIR project team: you all did so much work to make this dissertation come to life, not the least of which was tirelessly hauling a cooler filled with dry ice across Kansas and Missouri, and gracefully handling lots and lots of spit. And of course, I would like to acknowledge my mentor, Dr. Yo Jackson, and thank her deeply for her encouragement and guidance on this project. Throughout my time in graduate school she has provided me with invaluable training in conducting adversity and resilience research and has undoubtably helped shape the path of my career. I will always be grateful.

I am extraordinarily thankful for all the faculty in the KU Clinical Child Psychology Program who provided me with outstanding training. I am still in disbelief that I got to be a part of this community. I especially want to thank my committee members, Drs. Christopher Cushing, Paula Fite, Michael Roberts, Ric Steele, and Amy Mendenhall. I am grateful to have known Dr. Andrew Zinn, even for a short time. He will be missed. I would also like to thank my clinical and research mentors during my final year of training at Brown University, particularly Drs. Audrey Tyrka, Stephanie Parade, and Ron Seifer, for their enthusiasm towards me and my research, and guidance in helping me expand my expertise to the biological realm.

This journey would not have been possible without my friends: the old friends who simultaneously allowed me glimpses of life outside of graduate school while providing me with

unfailing support along the way, and the new friends who walked this graduate school path alongside me, who knew the challenges intimately, and believed wholeheartedly that I could do it anyway. Thank you all for not letting me give up and inspiring me with your own love for science and children.

I often say that my husband John should get an honorary PhD for all the work he has done to help me achieve mine. It's difficult to decide what I have valued most, from the delicious meals to moving across the country to talking for hours about challenging research problems. Perhaps for accepting all the bumps in the road with equanimity and kindness. You knew I could do this, even when I didn't. I can never thank you enough.

I offer my most sincere gratitude to my parents – thank you especially for your warmth, comfort, humor, good advice, and for filling laundry baskets up with books at the library. You opened many doors for me. I thank my sister Chelsea, whose trust in my knowledge and abilities has given me great confidence. You were my first partner in imagination. Finally, I am extraordinarily thankful for my grandparents, who are without a doubt my most enthusiastic supporters. I dedicate this dissertation to my grandmother Florence Huffhines, who always understood me. I'm eternally grateful to share your spark.

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Childhood adversity significantly increases the risk of developing physical health problems over the lifespan (Bucci, Marques, Oh, & Harris, 2016; Flaherty et al., 2009; Wegman & Stetler, 2009). For example, results from the seminal Adverse Childhood Experiences (ACEs) Study showed that the more categories of adversity one was exposed to, the greater the risk in adulthood of heart disease, cancer, chronic lung disease, and liver disease, among others (Felitti et al., 1998). Recent research has implicated systemic inflammation (i.e., chronic inflammation throughout the body) as the link between childhood adversity and later disease (Miller, Chen, & Parker, 2011; Nusslock & Miller, 2016). The idea that adversity heightens risk for disease via systemic inflammation has been fueled by the results of studies showing that, among adults exposed to adversity in childhood, key inflammatory proteins such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) are high (see Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016; Coelho, Viola, Walss-Bass, Brietzke, & Grassi-Oliveira, 2014, for reviews). Elevations in IL-6, TNF-α and CRP, as well as interleukin-8 (IL-8) and interleukin-1 beta (IL-1β), are in turn related to metabolic syndrome, heart disease and stroke, autoimmune conditions, some cancers, and premature aging (Chung et al., 2009).

Although fewer studies have examined the association between adversity and systemic inflammation in youth, there is a trend toward a positive association (see Slopen, Koenen, & Kubzansky, 2012, for review). High levels of both IL-6 and CRP, as well as several other inflammatory proteins, have been found in children and adolescents exposed to adversity, suggesting that the trajectory towards disease could begin in childhood (Cicchetti, Handley, &

Rogosch, 2015; Danese et al., 2011; Miller & Cole, 2012; Slopen, Kubzansky, McLaughlin, & Koenen, 2013).

Not all youth exposed to adversity, however, have high levels of inflammatory proteins. For example, within a sample of primarily Latino adolescents exposed to adversity, 46 adolescents had CRP levels in the low-risk for disease category (CRP < 1 mg/L), the levels for 13 adolescents fell into the intermediate-risk for disease category (CRP = 1-3 mg/L), and only seven adolescents had levels in the high-risk for disease category (CRP ≥ 3 mg/L; Fuligni et al., 2009). Similarly, in a sample of 3- to 6-year-olds exposed to adversity, CRP levels varied widely and ranged from the low-risk for disease category (CRP < 1 mg/L) to the high-risk for disease category (CRP ≥ 3 mg/L; Bernard, Hostinar, & Dozier, 2019). What is unclear, then, is why some youth exposed to adversity show systemic inflammation while others do not. Evidence points to family characteristics, such as family cohesion, as a potential explanation for differences in systemic inflammation levels among adversity-exposed youth. That is, adolescents with cohesive families have lower levels of inflammatory proteins than adolescents with non-cohesive families (Miller & Chen, 2010; Miller, Rohleder, & Cole, 2009), and the same appears to be true for young children as well (Bernard et al., 2019). Thus, family cohesion may buffer the effect of adversity on physiology.

This cross-sectional study tested whether family cohesion moderates the association between adversity and systemic inflammation measured via five inflammatory proteins in 3- to 6-year-olds exposed to significant adversity. Systemic inflammation in young children has rarely been studied, yet this age group is critical for establishing when evidence of immune dysregulation first begins to emerge in relation to adversity, allowing for greater understanding of the disease development process (Hostinar, Nusslock, & Miller, 2018). Examining how

adversity and family cohesion operate together may help to explain differences in inflammatory protein levels among children, and ultimately inform the field about how possible precursors to serious physical health problems like heart disease and cancer stem from childhood exposure to adversity.

Theoretical Background: Physiological Consequences of Adversity

Childhood is a period of considerable plasticity for the immune system (Miller et al., 2011; Brodin et al., 2015). That is, in utero and in early childhood, the immune system is especially sensitive to environmental exposures (Lupien, McEwen, Gunnar & Heim, 2009). Lifelong patterns of immune responding have historically been thought to result from early exposure to pathogens, allergens, and irritants (Finch & Crimmins, 2004; Prescott, 2006). Physical agents, however, are not the only factors influencing immune response. Exposure to psychosocial factors, including adversity, also affects the immune systems of children (Coe & Laudenslager, 2007).

Miller et al. (2011) proposed a conceptual model which was expanded on by Nusslock and Miller (2016), and Hostinar et al. (2018) explaining why many persons exposed to adversity in childhood have an enhanced pro-inflammatory response, systemic inflammation, and subsequent physical health problems (i.e., the biological embedding of childhood adversity model and the neuroimmune network hypothesis). Childhood adversity is an umbrella term encompassing child maltreatment (i.e., physical, sexual, and psychological/ emotional abuse; physical, medical, and educational neglect; and failure to supervise) and potentially traumatic life events (PTLEs; e.g., parental separation or divorce, parental incarceration, death of a family member, exposure to community violence, and others; Leeb, Paulozzi, Melanson, Simon, & Arias, 2008; Wheaton, 1994).

Exposure to childhood adversity results in a cascade of changes in the nervous, cardiovascular, endocrine, and immune systems (Shonkoff et al., 2012). Following an acute adverse event (e.g., being threatened with a gun, watching a caregiver be arrested) the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenocortical (HPA) axis release hormones that help direct energy to tissues such as the skeletal muscles and brain (i.e., those involved in the fight, flight, or freeze response; Hostinar, Sullivan, & Gunnar, 2014). In addition, immune cells exit lymphatic tissue and the spleen, where they reside, and enter the bloodstream, which raises the number of immune cells circulating in the body (Sompayrac, 2015). Ultimately this process is designed to protect the body from potential injury and facilitate healing quickly if injury occurs (Sompayrac, 2015). The body cannot differentiate between a physical threat that may result in injury and thus requires an immune system response (e.g., being threatened with a gun) and an emotional threat that does not require an immune system response (e.g., watching a caregiver be arrested), thus both types of threats result in an acute immune response. Moreover, this acute immune response which may have once been helpful in the face of physical confrontation (e.g., being physically assaulted) is maladaptive if it is repeatedly or continuously activated, such as is the experience of many children exposed to adversity.

Acute adverse events typically do not have a lasting impact on physiology (Cohen & Willis, 1985; Dickerson & Kemeny, 2004; Segerstrom & Miller, 2004). Rather, the physiological impact of adversity likely results from the degree to which adversity exposure is chronic (Miller et al., 2011). Chronic adversity is defined as an experience wherein abuse, neglect, or PTLEs do not have a specific ending—that is they reoccur over a short period of time. This is often operationalized as a high dosage or frequency of events (Miller, Chen, & Zhou, 2007). Exposure to chronic adversity sensitizes immune cells, causing them to produce an exaggerated response to

stress, which in turn results in high levels of proinflammatory cytokines (e.g., TNF-α, IL-6) circulating in the bloodstream (Glaser & Kiecolt-Glaser, 2005). Exposure to chronic adversity also causes immune cells to be less sensitive to glucocorticoids, a class of steroid hormones, which are important for stopping inflammation (e.g., cortisol; Cohen et al., 2012). Although insensitivity to glucocorticoids is adaptive when faced with an acute threat, it is harmful when the threat is ongoing, and ultimately contributes to the systemic inflammation associated with serious physical health problems (Glaser & Kiecolt-Glaser, 2005; Miller et al., 2011; Nusslock & Miller, 2016). When individuals are exposed to chronic adversity during a sensitive developmental period, that is, when immune function is highly malleable/plastic, as it is in early childhood, this pattern of over-reactive immune response may become stable over the life course, inflammation may become systemic, and risk for inflammation-related diseases significantly increases.

Adversity and Systemic Inflammation in Youth

A small but growing body of literature has explored the link between adversity and systemic inflammation in youth. Slopen et al. (2013) conducted a large prospective longitudinal study in which they examined adverse events at seven time points prior to age 8 and systemic inflammation at ages 10 (CRP, IL-6) and 15 (CRP). A total adverse events score for each time point was calculated by summing the perceived severity score of five events (i.e., being taken into foster care, being physically hurt by someone, being sexually abused, being separated from mother, and being separated from father). A cumulative adverse events score was also created by summing the total adverse events score across the seven time points, thus capturing a variant of chronicity. The cumulative adverse events score was associated with both IL-6 and CRP at age 10, and CRP at age 15 after adjusting for CRP at age 10. These findings suggest that chronicity

of adversity is a significant predictor of systemic inflammation in children and adolescents. Cicchetti et al. (2015) also investigated adversity and systemic inflammation within a large sample of maltreated and non-maltreated children ages 8 to 12. The authors did not, however, measure chronicity or severity of maltreatment, or clarify types of maltreatment experienced by their sample. Findings showed that children who experienced maltreatment in early and middle childhood had significantly higher levels of CRP compared to non-maltreated children.

Danese et al. (2011) provided further evidence for the association between adversity (albeit chronicity and severity were unknown) and CRP. Children's exposure to physical abuse was prospectively assessed during their first 10 years of life via parent interview, and child depressive symptoms and CRP levels were measured at age 12. Results from this study revealed that children who were physically abused *and* depressed had higher CRP levels compared with three groups of control children: those who were physically abused only, those who were depressed only, and those who were neither physically abused nor depressed. Miller and Cole (2012) similarly found that the coupling of adversity exposure and depression predicted systemic inflammation beyond adversity or depression alone. Specifically, adolescent girls ages 15 to 19 at high risk for depression via family history were assessed over 2.5 years. At visits when adolescents had recently experienced a depressive episode, they showed higher circulating IL-6 relative to visits without a recent depressive episode. The magnitude of these changes varied in proportion to lifetime adversity exposure (i.e., birth to a teenage mother, parental divorce or death, separation from parent lasting more than one year, low household education, and renting the family's residence rather than owning). To the extent that they had experienced more of these adverse events over the course of their lives, adolescents showed progressively larger IL-6 and CRP increases upon transitioning from healthy to depressed states. When youth were not

depressed, adversity was unrelated to inflammatory protein concentrations. Moreover, when youth without adversity transitioned to depression, their inflammatory protein concentrations declined. Each of these four studies provides evidence for the link between adversity and systemic inflammation in youth, and preliminary evidence for several significant moderators (i.e., chronicity, timing, and depression).

An additional study with adolescents (Fuligni et al., 2009) demonstrated more complicated findings. A small sample of Latino adolescents in 12th grade reported on 23 PTLEs (e.g., parent lost a job, death of a family member, serious illness) that occurred in the past 12 months, and daily interpersonal stress (e.g., parents had an argument, argued with mother, argued with father, argued with other family member, argued with a friend, argued with or punished by a teacher, harassed or picked on by a student at school) over the last 14 days. A summary variable was created that indicated the percentage of days on which any one of the 10 daily interpersonal stress events occurred to the adolescent. Adolescents who reported more frequent daily interpersonal stress had significantly higher levels of CRP, above and beyond PTLEs. This finding is unexpected given the evidence that stressors of acute duration do not generally make lasting imprints on physiology (Dickerson & Kemeny, 2004; Segerstrom & Miller, 2004). It is not clear, however, whether the daily stressors were truly acute, or indicative of more chronic interpersonal/relational problems, given that many of the items assessed family environment and there was no method to ensure that these daily stressors did not precede or continue beyond the 14 days. It may be that family environment explained more of the variance in CRP than did PTLEs. Theory and empirical evidence suggest that adversity and aspects of the family environment, such as cohesion, may work together to impact systemic inflammation (Hostinar et al., 2018).

In addition to systemic inflammation measured via IL-6 and CRP, two studies in older children and adolescents examined other inflammatory proteins. One study investigated IL-6, IL-1β, and IL-8 in a sample of 8- to 17-year-olds in a psychiatric inpatient unit and their community controls (Gariup, Gonzalez, Lázaro, Torres, Serra-Pagès, & Morer, 2015). This study found that IL-1β and IL-8 were positively correlated with both parent-reported and child-reported PTLEs in both groups, while accounting for psychiatric diagnoses. This result suggests that adversity exposure more so than mental health condition may impact levels of inflammatory proteins. Although this is contrary to findings of Danese and colleagues (2011) and Miller and Cole (2012) suggesting that depression is critical to the adversity/inflammation relation, the Gariup et al. (2015) sample included younger children, and it may be that adversity alone prior to adolescence impacts systemic inflammation and mental health disorders develop later, possibly due to adversity exposure, and then also contribute variance to systemic inflammation. In addition, Bücker et al. (2015) measured IL-6, IL-1β, IL-8, and TNF-α in a sample of 8- to 11-year-olds and found that TNF-α levels were higher in children who had been referred to child protective services (CPS) or were living in a foster home compared to children recruited from a community health center; IL-6, IL-1β, IL-8 were not statistically different between groups.

Three studies to date—two with preschool-aged children and one with infants—have examined associations between adversity and systemic inflammation in young children. Tyrka, Parade, Valentine, Eslinger, and Seifer's (2015) sample included 40 children ages 3 to 5, 18 of whom were identified as maltreated via child protective services record review, and 22 controls. Caregivers were further interviewed about potentially traumatic life events their child had experienced (e.g., death of a caregiver, car accident, witnessing violence, accidental burning, hospitalization). Maltreatment status was not significantly associated with CRP or IL-1β. The

number of PTLEs in the child's lifetime was positively associated with IL-1β, but not CRP. The lack of association between PTLEs and CRP contrasts with studies of older children and adolescents, thus it may be that elevation in CRP is not consistently evident until later in childhood. The significant association between number of PTLEs and IL-1β, however, shows some evidence for the relation between dosage of adversity and systemic inflammation even in very young children.

Bernard and colleagues (2019) examined CRP levels in a sample of 35 children receiving biological family preservation services from CPS and 29 community controls, ages 3 to 6. CRP levels were not significantly different between the two groups. However, CPS-referred children who demonstrated an insecure or disorganized attachment style with a caregiver had significantly higher CRP levels than both children in the control group and CPS-referred children with secure attachment styles. CPS-referred children with secure attachment styles had similar CRP levels as children in the control group.

Finally, one research group has examined early life adversity and CRP in 17-month old infants recruited through the Women, Infants, and Children program. In one study of this sample, both higher socioeconomic disadvantage and higher maternal psychosocial stress (i.e., parenting stress) was associated with elevations in infant CRP (David, Measelle, Ostlund, & Ablow, 2017). In another study of this sample, infants classified as having a disorganized attachment style had higher CRP levels than infants classified as having an insecure attachment style or a secure attachment style (Measelle, David, & Ablow, 2017). Measelle and Ablow (2018) further examined proinflammatory cytokines in this sample (i.e., IL-1β, IL-6, IL-8, and TNF-α). An inflammatory load score was computed by summing the standardized values of each of these cytokines and CRP. Further, standardized scores on the Parent Stress Inventory-Short Form and

the Recent Life Events Scale were averaged to create a composite family stress variable. Results indicated that higher levels of family stress were associated with a higher inflammatory load score in infants. Together, these findings suggest that exposure to adversity and problematic family relationships are associated with inflammation in even extremely young children.

Family Cohesion as a Moderator of Adversity and Systemic Inflammation

Although an emerging body of evidence points to an association between childhood adversity and systemic inflammation in youth, not all youth exposed to adversity have high levels of inflammatory proteins (e.g., Bernard et al., 2019; Fuligni et al., 2009). Several moderators have been suggested by the literature, including chronicity and timing of adversity, specific genes, and depression. An additional moderator gaining support in the literature is family cohesion. Family cohesion is a broad term that encompasses the affective qualities of family relationships such as affection, support, and helpfulness. It is defined as "the extent to which family members are concerned and committed to the family and the degree to which family members are helpful and supportive to each other" (Moos, Insel, & Humphrey, 1974, p. 4).

Having a concerned and committed family made up of people who are helpful and supportive may lessen the incidence of mental *and* physical health problems by providing youth exposed to adversity with a sense of safety and stability, which in turn reduces the reactivity of the stress response system (Cohen & Willis, 1985; Miller et al., 2011; Nusslock & Miller, 2016). The same plasticity that allows for adversity to negatively impact the immune system in infancy and early childhood, may also permit potential protective factors, like family cohesion, to positively alter immune response patterns (Ben-Shlomo & Kuh, 2002).

Miller and colleagues (2009) measured interpersonal relationship quality with family, friends, and romantic partners in a sample of 103 adolescent girls ages 15 to 19, where lower scores reflected warm, intimate, and supportive relationships and higher scores suggested relationships characterized by conflict, mistrust, and instability. The different relationship domains (i.e., family, friends, romantic partners) were collapsed into one broader index given high correlations between the domains. Among those adolescents who had worse interpersonal relationship quality at baseline, they displayed an increase in IL-6 at 6-month follow-up relative to baseline IL-6. Those youth with warm, intimate, and supportive relationships did not experience an increase in IL-6 from baseline to follow-up.

Miller and Chen (2010) expanded on this study, this time finding that 135 adolescent girls exposed to a non-cohesive family environment in particular showed increasing IL-6 production over 1.5 years. Moreover, participants also became less sensitive to cortisol's anti-inflammatory properties, which in turn increases systemic inflammation. Finally, the investigators explored whether residing in a non-cohesive family environment accentuated participants' inflammatory responses to potentially traumatic life events (e.g., death of a family member) that occurred in the last year and a half. The results revealed a significant interaction between PTLEs and a non-cohesive family environment in predicting IL-6 production. Specifically, adolescents who resided in non-cohesive family environments had greater production of IL-6 at times when they had recently been exposed to a PTLE, relative to times when they had not. In contrast, adolescents who resided in cohesive family environments showed little change in IL-6 production, and even a small decline, after being exposed to a PTLE. These findings suggest the importance of including both adversity and family cohesion in a model predicting systemic inflammation, as family cohesion may serve as a protective factor.

Four other studies lend support for this hypothesis by demonstrating significant interactions. Systemic inflammation, however, is not measured directly in these studies, rather researchers focused on airway inflammation or allostatic load (i.e., a combination of biological markers caused by multiple physiological systems responding to adversity; McEwen, 2002). First, in a sample of children and adolescents ages 9 to 18 with severe asthma perceived as highly stressful, youth who reported having a cohesive family had less airway inflammation and were less resistant to hydrocortisone's anti-inflammatory effects on IL-5 and IFN-ɤ (i.e., two inflammatory proteins associated with airway inflammation) than youth who reported having a less cohesive family, even when controlling for adherence to asthma medications (Miller, Gaudin, Zysk, & Chen, 2009). Second, African American adolescents ages 11-13 residing in a low-income, rural area reported on family cohesion (Brody et al., 2013). Those with highly cohesive families had significantly lower levels of allostatic load (i.e., the sum of six indicators: overnight cortisol, epinephrine, and norepinephrine; resting diastolic and systolic blood pressure; and body mass index) in late adolescence (age 19) compared to those who had reported having less cohesive families. Notably, youths exposed to highly cohesive family environments evinced low allostatic load whether they carried, two, one, or no genes that confer sensitivity for allostatic load. Third, in a sample of adolescents in seventh and eighth grade, youth and parents reported on adversity exposure (i.e., residential density, noise levels, housing quality, family turmoil and child-family separation, and exposure to violence) in the last two to three years (Evans, Kim, Ting, Tesher, & Shannis, 2007). As number of adverse events increased, allostatic load rose, but only for youth whose mothers were low in responsiveness—a construct highly correlated with family cohesion; these results also suggest the importance of dosage of adversity.

Finally, the only study to explicitly measure maltreatment in concert with family cohesion did so in a sample of adults. Carroll et al. (2013) conceptualized maltreatment as the average score of two items: how often an adult pushed, grabbed, shoved, or hit them, and how often an adult swore at them, insulted them, or put them down. Results showed a significant interaction between maltreatment and family cohesion in childhood in predicting allostatic load in adulthood (i.e., sum of 18 biomarkers, including IL-6 and CRP). The results indicated that the strongest positive relation between maltreatment and allostatic load was in those individuals with low family cohesion. In sum, the extant research findings provide support for the role of family cohesion in buffering the effects of adversity on IL-6, airway inflammation, and allostatic load. Although these results suggest that family cohesion is likely an important moderator in the broad adversity-physiology relation, systemic inflammation measured by critical inflammatory proteins must be examined directly, as these proteins are the key players in the disease process and represent the long-term biological sequelae of stress (as opposed to immediate biological effects, such as cortisol) (Miller et al., 2011; Nusslock & Miller, 2016). Moreover, Baumeister and colleagues' (2016) meta-analysis found small to moderate effect sizes for CRP (i.e., Fisher's z = 0.10, 95% CI = 0.05 – 0.14), IL-6 (i.e., Fisher's z = 0.08, 95% CI = 0.03 – 0.14), and TNF-α (i.e., Fisher's z = 0.23, 95% CI = 0.14 – 0.32), suggesting that although the effect of childhood adversity on inflammatory proteins may be somewhat subtle, their impact on health is not.

Conceptualizing Adversity

Beyond possible moderators, it is important to note that past research has indicated that differences in adversity exposure also contribute to the variance in systemic inflammation (e.g., Slopen et al., 2013; Tyrka et al., 2015). Adversity exposure must be carefully conceptualized and

measured when attempting to parse out how it and constructs like family cohesion impact systemic inflammation.

A few studies exploring adversity and systemic inflammation have accounted for the dosage of potentially traumatic life events (Evans et al., 2007; Miller & Cole, 2012; Slopen et al., 2012; Tyrka et al., 2015). Measuring adversity as the total number of PTLEs, however, is usually operationalized as the number of categories, rather than number of total incidents. For example, one child may have experienced one event (e.g., parental job loss) while another child experienced three events (e.g., parental job loss, death of a parent, loss of a close friend). Although it would look like the child with three events experienced more adversity, the child with one *type* of event could have experienced that event more frequently, for example 10 times. Thus, operationalization of adversity by dosage alone may be misleading in identifying the relation between adversity and systemic inflammation, therefore frequency should also be considered (Gabrielli, Jackson, Tunno, & Hambrick, 2017; Jackson, McGuire, Tunno, & Makanui, 2019). The current study focused on the construct of *chronic* adversity, given support in the literature that it is chronicity that confers most risk for high inflammation (Miller et al., 2011); the current study operationalized chronic adversity as both dosage and frequency to better capture the true amount of maltreatment and PTLEs children were exposed to.

Present Study

The present study aimed to replicate and expand upon the growing literature of adversity, family cohesion, and systemic inflammation in several ways. In terms of replicating past research, this project included a sample of young children to investigate the basic association between adversity and systemic inflammation, as so few studies in this area have been conducted with youth, and only two have been conducted with preschool-aged children. The study

expanded on past research by including comprehensive measures of both maltreatment and potentially traumatic life events as well as dosage and frequency of those events, by utilizing a sample with varying levels of adversity exposure, and by measuring the inflammatory proteins critical to immune functioning and associated with disease risk (i.e., CRP, IL-6, IL-1β, IL-8, and TNF-α). Further, the current study also examined the possible moderating role of family cohesion in the adversity-inflammation relation. The following hypotheses were tested:

Hypothesis 1: Adversity (i.e., potentially traumatic life events and maltreatment) is positively associated with systemic inflammation (i.e., CRP, IL-6, IL-1β, IL-8, and TNF-α).

Hypothesis 2: Family cohesion moderates the adversity-inflammation relation such that children exposed to adversity who have high levels of family cohesion show lower levels of systemic inflammation than children exposed to adversity who have low levels of family cohesion.

Method

Participants

Participants included 145 children between ages 3 and 6 and their parents participating in an ongoing prospective longitudinal study, the Preschoolers' Adjustment and Intergenerational Risk (PAIR) project. The PAIR project explores the roles of emotion regulation and cognitive functioning in children's adaptation to adverse events. Participants in the PAIR project complete four study visits over the course of two years (i.e., one visit every six months). Participants completing any time point were eligible for the current study. Parent report of child's lifetime adversity exposure was taken at time one for all participants. At time points two, three, and four parents were asked to report on any new adversity exposures that had occurred in the six months between the previous time point and the current time point. Thus, the child's complete adversity

history up to the day of saliva collection was used for current analyses. Ineligibility criteria for participation in the PAIR project includes 1) child diagnosed with autism spectrum disorder, intellectual disability, or other developmental disability as indicated by parent report; and 2) families whose first language is not English (as all measures are in English and only English-speaking staff served the project). Additional exclusion criteria specific to the current study included: (a) presence of acute illness (e.g., cold, flu), injury (e.g., large, unhealed wound, broken arm), or infection (e.g., sinus infection) within the last seven days (and seven days since last antibiotic/medication use); and (b) presence of oral disease, injury, infection, bleeding in the mouth, or loose tooth/recently lost tooth.

The child portion of the sample ranged in age from 3 to 6 years (M = 4.76, SD = 1.02), and were 47% female; 71% of children were Black or African American, 17% of children were Multiracial, 8% of children were White or Caucasian, and 4% of children were other races. Caregivers ranged in age from 23 to 50 years (M = 30.91, SD = 5.96). Most caregivers were biological mothers (89%), followed by biological fathers (6%), biological grandmothers (3%), and adoptive caregivers (2%). In terms of socioeconomic status, 44% of families reported a total yearly income of $10,000 or less, 18% reported an income between $10,001 and $20,000, 17% between $20,001 and $30,000, 8% between $30,001 and $40,000, 8% between $40,001 and $50,000, 1% between $50,001 and $60,000, and 4% at $60,001 or more.

Children's BMI scores ranged from 12.2 to 31.3 for the whole sample, with a mean of 16.51 (SD = 2.26) and an average BMIz of 0.85 (80th percentile). For girls, BMI scores ranged from 12.2 to 20.2 with a mean of 16.10 (SD = 1.36) and an average BMIz of 0.73 (77^{th} percentile). For boys, BMI scores ranged from 13.2 to 31.3 with a mean of 16.97 (SD = 2.91) and an average BMIz of 0.96 (83^{rd} percentile). According to the Centers for Disease Control and

Prevention, children with a BMI at or above the 95th percentile are considered to be overweight, while children with a BMI between the 85th and 95th percentile are considered at risk of overweight (U.S. Department of Health and Human Services, 2018). Given the positive association between BMI (and BMIz) and inflammation in numerous studies, BMIz was considered as a covariate in the present study (Slopen et al., 2013).

Regarding physical health conditions and medication use, 28% of children were diagnosed with asthma per parent report, 3.5% of children were diagnosed with high blood pressure per parent report, and one child was diagnosed with epilepsy per parent report. No other health conditions were endorsed. Eight percent of children regularly took allergy medication, 2% regularly took asthma medication, and 5.5% took asthma medication (e.g., rescue inhaler) only as needed. The child with epilepsy took regular medication for seizures. Regarding mental health conditions and medication use, 3.5% of children were diagnosed with a psychiatric disorder (i.e., depression, anxiety, or posttraumatic stress disorder [PTSD]) per parent report; 1 child was taking an SSRI. 3.4% of children were diagnosed with attention-deficit/hyperactivity disorder (ADHD), and all were taking stimulants. On the day of saliva collection, 1.4% of the sample had taken an antihistamine for allergies, 5.5% of the sample had taken either control medication or a rescue (i.e., albuterol) inhaler for asthma, and 2.1% had taken a stimulant for ADHD; the child taking the SSRI and the child taking the seizure medication both took these medications on the day of saliva collection. Physical health conditions, mental health conditions, and medication use were examined as potential covariates in the present study.

Recruitment and Procedures

Participants in the PAIR project were recruited from two sources: 1) families who received services from local Head Start centers, and 2) families who received case management

services from Department of Social Services (DSS) and had no history of foster care. Approximately 10% of participants in the current sample were recruited through DSS. Participants were limited to one parent and one child per household. Participants were informed about the study and invited to participate by mail, phone, and in-person recruitment. After expressing interest in participation at the recruitment location, via phone, or via mail, potential participant families were given a follow up call to assess for exclusionary criteria and to schedule a study visit.

Study visits were conducted at community centers located near participants' homes, including churches, schools, and Head Start centers. Research staff explained the study aims, procedures, risks, benefits, and confidentiality protocol to the child's parent, and willing parents completed informed consent. Staff also informed children about the purpose and procedures of the study in language appropriate to their developmental level, and sought the child's assent to proceed with the study. Staff then informed parents about the purpose, risks, benefits, and confidentiality of saliva collection. Parents were informed that they could decline saliva collection and still participate in the larger PAIR project. Four parents during the ten-month saliva collection period declined saliva collection. Parents who agreed to participate in saliva collection were then given a screening measure to determine exclusionary criteria specific to the saliva collection. Upon completion of informed consent and assent, and the exclusionary criteria screener, parents and children began study tasks.

PAIR study visits lasted approximately 2.5 to 4 hours. Measures from the PAIR project that were used in the present study included a demographic measure, a parent-report measure of the child's exposure to adversity, and a parent-report measure of family cohesion. The demographic and adversity measures were administered via paper at the beginning of the PAIR

project ($N = 48$ for the current study). The Audio, Computer-Assisted Self-Interview (ACASI) was then introduced, thus remaining participants completed demographic and adversity measures using the ACASI ($N = 97$ for the current study). All participants completed the measure of family cohesion on paper. The ACASI reads items and answer choices aloud to participants via headphones connected to a laptop computer. This format allows privacy for participants while answering study questions, and accounts for reading difficulties.

At study visits, parents completed demographics and family cohesion measures first, while children completed cognitive, baseline, and frustration tasks as part of the larger PAIR project separately. Height and weight were also obtained from parents and children at the beginning of the visit. Parents then joined children for parent-child interaction tasks. Following tasks, children were taken to a childcare room while parents completed adversity measures. Adversity measures were administered last to minimize the potential for negative emotional reactions that may influence other study procedures. Upon entering the childcare room, children were asked to swish with water to remove any food residue that may contaminate saliva samples. To avoid sample dilution, saliva was collected at least ten minutes after rinsing.

Research staff collected saliva with the SalivaBio Children's Swab (SCS). The SCS method has been validated for children from 6 months to 6 years of age. The swab is made out of durable polymer that withstands chewing. Each swab comes individually wrapped to minimize the possibility of environmental contaminants, and causes no change in sample pH. The swab has been used in previous studies of systemic inflammation in preschool-age children (Tyrka et al., 2015). The swab is placed under the tongue of the child, and remains there for 60 seconds, or until the lower third of the swab is saturated. The swab is then put into a swab storage tube, which is placed in a box specifically designed to hold sample tubes and withstand cold

temperatures. Samples were temporarily stored in a YETI® cooler with dry ice until research staff returned to the PAIR laboratory. Samples were then transferred to a laboratory-grade freezer in the PAIR laboratory where they remained at -20°C until shipped off for assay. All samples were shipped within six months of collection to minimize loss of saliva over time due to evaporation. Samples were shipped overnight on dry ice for next-day delivery to Salimetrics Laboratories (Carlsbad, CA) for assay.

During PAIR study visits, research staff asked parents to rate their level of distress before and after completing the adversity measures, and offered mental health resources to every participant in the project. Parents were compensated $60 at their first study visit, $70 at their second visit, $80 at their third visit, and $90 at their fourth visit. Children were given a small toy in thanks for their participation, as well as a sticker or candy at completion of saliva collection. Parents were contacted via phone within 48 hours of participation in the study visit to assess for study-related distress and offer mental health resources as needed. Shortly after their study visits, parents were mailed packets containing a second copy of local mental health resources to address any possible distress arising from study procedures, a copy of the research consent form, and a letter thanking them for participating in the study. Given that the PAIR project is a longitudinal study, parents also received newsletters, holiday and birthday cards, phone calls, and text messages between study time points. These methods were approved by the University of Kansas Institutional Review Board and the Department of Social Services for the State of Missouri.

Measures

Demographics. Parents completed a demographic information form. Items used for the current study included the child's age, gender, and race, as well as yearly income of the family. Parents were also asked to report on their child's mental health, and indicated "yes" or "no" as to

whether their child had “ever been diagnosed with an emotional or psychological problem.” If they selected yes, they would then write or type what the diagnosis was. Presence of a psychiatric diagnosis was used as a covariate in the current study given prior associations between adversity, depression, and systemic inflammation (e.g., Danese et al., 2011; Miller & Cole, 2012). Parents also reported on their child’s physical health, and indicated “yes” or “no” as to whether their child “had any major health problems (for example: diabetes or chronic pain).” Again, if they selected “yes” they would then write or type out the diagnosis. Finally, parents also reported on medications currently being taken by the child. Physical health problems and medications were tested as covariates in the current study given their potential impact on inflammation.

Childhood adversity. Childhood adversity was measured using the Childhood Experiences Measure (CEM), a 100-item parent-report measure asking whether or not various adverse events occurred in the child’s lifetime. For subsequent time points parents received the same 100 items and were prompted to respond as to whether any of these events had occurred within the last six months. This study-created measure is a combination of previously validated trauma measures (e.g. Child and Adolescent Psychiatric Assessment, Modified Maltreatment Classification System, ACEs Survey). The items include 50 potentially traumatic life events (e.g., parental mental illness, parental substance abuse, community violence, poverty, traumatic loss, accidents, and natural disasters) and 48 childhood maltreatment experiences within 5 overarching categories (i.e., physical and sexual abuse, emotional/ psychological maltreatment, and physical and supervisory neglect). The last two items are 1) an open-ended question asking if there are any additional adverse experiences to report, and 2) a question asking if any of the major events endorsed were connected in any way (e.g. was one event the result of another).

For the 50 PTLEs items, parents were asked to indicate whether or not their child has experienced the event (yes or no). Parents who responded “no” proceeded to the next item. Parents who responded “yes” were asked how many times the event occurred in the child’s lifetime using a Likert scale: 1 time (1), 2 times (2), 3 to 5 times (3), 6 to 10 times (4), and more than 10 times (5). If parents selected that an event happened 1 time, they were then asked when the event happened: within the last week (1), within the last month (2), within the last year (3), two years ago (4), three years ago (5), four or more years ago (6). If parents selected that an event happened more than one time, they would indicate when the first time happened and when the last time happened using the same six answer choices. Parents were also asked an additional question specific to the event. For example, if a parent indicated that someone close to their child had attempted or completed suicide, parents would then be asked “thinking about the last time this happened, who was the person in relation to your child?”

For the 48 maltreatment events items, parents were presented with a list of types of a particular form of maltreatment and asked to select all types that had occurred to their child. For instance, for the physical abuse item, parents would check which of the following types had happened. They may select: hit with something, like a hairbrush, belt, electric cord, or shoe (1), whooped, whipped or popped (2), slapped (3), pushed or thrown against a wall or downstairs (9), burnt or scalded on purpose (10), knocked unconscious (14) or wounded with a gun (15). If parents indicated that one or more of these events had happened, they would then answer the follow up question as to how many times their child had experienced these events. Parents were also prompted to answer when the first event and last event happened. In addition, parents indicated whether their child was injured as a result, and the relation of the person/people who maltreated the child.

For the purposes of this research project, adversity was conceptualized as a latent variable, indicated by dosage and frequency of PTLEs and dosage and frequency of maltreatment (see Figure 1) that had occurred in the child's lifetime. This conceptualization was chosen given the importance of chronicity of adversity (i.e., dosage and frequency) in previous literature on systemic inflammation (Miller et al., 2011). Dosage of PTLEs was operationalized as the sum of all possible events; it was calculated by dichotomizing the first 50 items as 1 = endorsed as having occurred to the child and 0 = not endorsed and summing all events such that scores could range from 0-50. Frequency of PTLEs was operationalized as how frequently each event occurred; it was calculated by summing the frequency items for all events based on the Likert scale. For example, if a child experienced a caregivers' divorce 2 times (2), was forced to leave the home 3 to 5 times (3), and witnessed a terrible event 6 to 10 times (4), their frequency score would be 9.

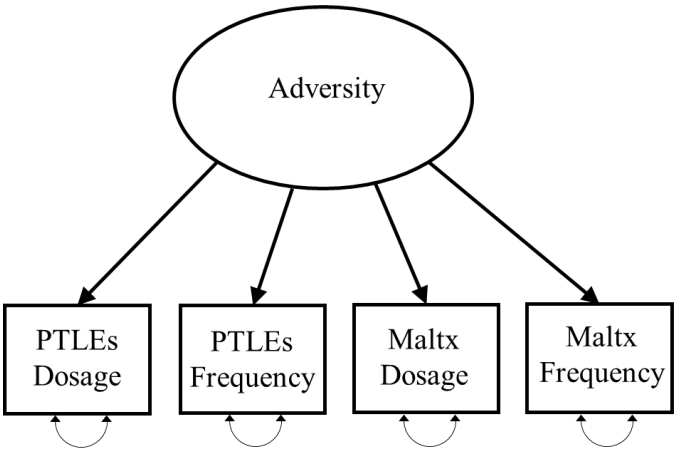

Figure 1. Proposed measurement model of adversity.

Dosage of maltreatment was operationalized as the sum of all possible maltreatment events; it was calculated by dichotomizing the items as 1 = endorsed as having occurred to the child and 0 = not endorsed and summing all events such that scores could range from 0-48. In total, there were 16 possible physical abuse events, 11 possible sexual abuse events, 7 possible physical neglect events, 7 possible supervisory neglect events, and 7 possible psychological/emotional abuse events. Frequency of maltreatment was operationalized in the same way as PTLEs, or by summing the frequency items for all maltreatment events.

Family cohesion. Family cohesion was measured using the Cohesion subscale from the Family Environment Scale (FES; Moos & Moos, 1994). The items that make up the Cohesion subscale of the FES assess the degree to which the family as a whole is concerned and committed to its members. The FES Cohesion scale includes nine items (e.g., there is a feeling of togetherness in our family; family members really help and support one another). The items are in true-false format. A subscale score is generated by summing the scale's items, and ranges from 0 to 9. This subscale score was used as an observed variable for the current project. The alpha for the Cohesion scale of the parent-report FES has ranged from 0.67 to 0.79 (Boyd, Gullone, Needleman, & Burt, 1997; Moos & Moos, 1994). Test-retest reliability for the Cohesion scale was 0.86 (Boyd et al., 1997). In the current study, $\alpha = .67$.

The Cohesion scale of the FES has been widely used in studies on family environment, and has demonstrated convergent validity with other measures, including significant correlations with the FACES III cohesion scale (Edman, Cole, & Howard, 1990; Sanford, Bingham, & Zucker, 1999), the California Q-sort measure of parental warmth (Sanford et al., 1999), the Belsky parent-child synchrony code (Sanford et al., 1999), and parent degree of interpersonal affiliation from the Structural Analysis of Behavior Index Questionnaire (Sanford et al., 1999). Further, parent-reported high family cohesion was associated with significantly fewer child internalizing and attention problems at both ages 6 and 11 (Lucia & Breslau, 2006). In a sample of young children, receiving Early Head Start home-based programming was associated with greater family cohesion (as measured by the FES) at follow-up (Raikes et al., 2014). The FES has also been used to assess family cohesion in other preschool-aged samples, including children in foster care and those receiving cochlear implants (Holt, Beer, Kronenberger, Pisoni, & Lalonde, 2012; Horwitz, Owens, & Simms, 2000).

Body mass index z-score (BMIz). Body mass index z-scores, also called BMI standard deviation scores, are measures of relative weight adjusted for youth age and sex. Height and weight were collected for each participant using the SECA 213 portable stadiometer and SECA 813 scale. Children were weighed without shoes and outer clothing (e.g., jackets, sweaters). Height was measured to the nearest millimeter three times and then averaged, and weight was measured in kilograms three times and then averaged. BMIz was obtained using the Pediatric Z-score Calculator (available at https://zscore.research.chop.edu/) which uses the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (2010) growth charts and formula: weight in kilograms divided by height in meters squared, and converted to z-scores based on children's age and gender. Given that adipose tissue secretes proinflammatory cytokines (Maggio, Guralnik, Longo, & Ferrucci, 2006) and higher BMI is associated with higher IL-6 and CRP levels in some studies of youth (Herder et al., 2007), BMIz is included as a covariate in the present study.

Systemic inflammation. Systemic inflammation was assessed through inflammatory proteins IL-6, IL-1β, IL-8, TNF-α, and CRP. IL-6, IL-1β, IL-8, and TNF-α are the four critical proinflammatory cytokines involved in initiating and maintaining inflammation, and are highly interdependent (Bahramabadi et al., 2017; Holdsworth & Gan, 2015; Irwin & Cole, 2011). Seminal work on the interconnectedness of these four key cytokines, along with the major acute phase protein CRP, is summarized by Gabay and Kushner (1999). This review stipulates that this specific cohort of cytokines operates as both a cascade and as a network, such that one cytokine stimulates the production of another, yet all four cytokines are responsive to changes in the others' levels.

For example, IL-6 stimulates the production of TNF-α, which then stimulates IL-1β; in turn, levels of IL-1β influence levels of IL-6 and TNF-α. Indeed, IL-6, IL-1β, IL-8, and TNF-α have been found to be positively correlated with each other in samples of adults and youth (Elenkov, 2008; Riis, Granger, DiPietro, Bandeen-Roche, & Johnson, 2015). These four cytokines additionally induce acute-phase proteins (i.e., CRP) which comprise the next step of the inflammatory process. The production of CRP requires a combination of cytokines, specifically IL-6, IL-1β, and TNF-α.

Thus, inflammation is not initiated or maintained by any one inflammatory protein, but a combination. Combinations of cytokines and acute-phase proteins have been found to have additive or synergistic effects on overall inflammation in the body, and ultimately on disease (Condon, 2018). Most studies seeking to quantify systemic inflammation measure one or more of these proteins (Baumeister et al., 2016; Coelho et al., 2014; Slopen et al., 2012); however, few studies—especially those interested in associations between adversity, psychosocial factors, and inflammation—include all five proteins together (primarily due to cost) although this is likely the best capture of systemic inflammation (Riis et al., 2015). Therefore, to account for the interconnectedness of these proteins in a statistical model, a latent factor of systemic inflammation with IL-6, IL-1β, IL-8, TNF-α, and CRP as indicators was used in the current study (see Figure 2).

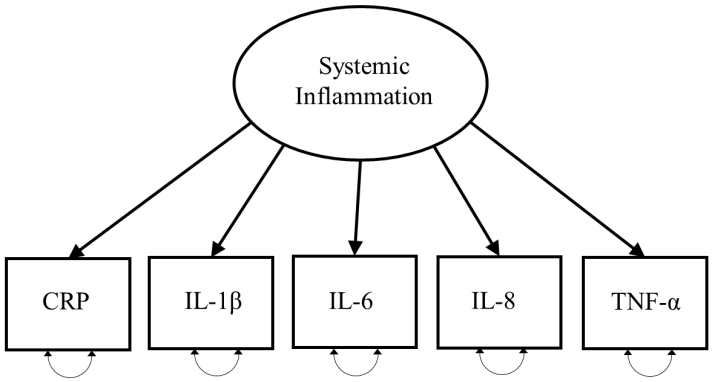

Figure 2. Proposed measurement model of systemic inflammation.

Although these proteins can be measured in either blood or saliva, saliva is frequently used for samples of young children given acceptability and ease with this vulnerable population, and evidence of documented correlations between proinflammatory cytokines in saliva and peripheral blood (Byrne et al., 2013; Megson, Fitzsimmons, Dharmapatni, & Bartold, 2010; Ouellet-Morin, Danese, Williams, & Arseneault, 2011; Riis et al., 2015). Therefore, saliva was used for the current study of 3- to 6-year-old children.

Saliva samples were assayed in duplicate for CRP, IL-1β, IL-6, IL-8, and TNF-α at Salimetrics Laboratories (Carlsbad, CA) using the ELISA (enzyme-linked immunosorbent assay) technique. Lower limits of detection, which represent the lowest measurable analytic level that can be distinguished from zero were as follows: CRP (10 pg/mL), IL-1β (0.07 pg/mL), IL-6 (0.06 pg/mL), IL-8 (0.05 pg/mL) and TNF-α (0.08 pg/mL). The detection rate for CRP was 98% (i.e., only three saliva samples had CRP concentrations below the assays' lower limit of detection). The detection rate for IL-1β, IL-6, IL-8, and TNF-α was 100%. The CRP values that were below the detection limit ($N = 3$) were excluded from the analyses.

The intra-assay coefficient of variation (% CV) represents the degree to which the duplicate results differ within the sample. The % CV is calculated by finding the standard deviation of results 1 and 2, dividing that by the duplicate mean, and multiplying by 100. The average of the individual CVs is reported as the intra-assay CV. The intra-assay % CVs should be less than 10, which suggests that assay results for the current sample are reliable. The intra-assay % CVs were as follows: CRP (7.5%) IL-1β (5.4%), IL-6 (4.8%), IL-8 (2.0%), and TNF-α (5.6%). For the current study, consistent with past research, the mean of duplicate results for each inflammatory protein was used (Tyrka et al., 2015). All inflammatory proteins were measured in pg/mL.

Analytic Plan

Missingness. Regarding missingness in the sample, 0.1% of demographic items were missing for both ACASI and paper measures groups; 0.8% of PTLEs items were missing for the ACASI group, and 1.6% of PTLEs items were missing for the paper measures group. 0.3% of FES cohesion subscale items were missing. Only 0.003% of inflammatory protein data was missing. Thus, excluding maltreatment items, missing data were limited (i.e., less than 3% missing).

Importantly, the maltreatment items were only administered to those participants using the ACASI due to CPS reporting concerns, thus 33% of maltreatment items were missing in the total sample given that they were not administered to those participants using paper measures. For those who were administered the maltreatment items via the ACASI, there were no missing data on these items. Little's (1988) Missing Completely at Random (MCAR) test was not significant [$\chi 2_{(96)} = 125.13$, $p = .15$], indicating that the assumptions of MCAR were satisfied.

Missingness was managed using the full information maximum likelihood (FIML) algorithm available through the lavaan package in R (Rosseel, 2012). FIML results in utilization of parameter estimates that are unbiased and accurate for the model estimation analyses (Enders & Bandalos, 2001). The advantages of FIML over other methods have been demonstrated across different types and levels of missingness, even as rates of missing data exceeded 50% (Newman, 2003). However, it should be noted that when large percentages of data are missing (i.e., typically above 50%), parameter bias and standard error estimates can become unacceptable even under FIML (Newman, 2003). Thus, although missing maltreatment data was still well below 50%, the results as they pertain to maltreatment should be interpreted with caution, and warrant replication.

Data analysis approach. It is well known that sum score approaches, such as ANOVA and regression, suffer from serious limitations in terms of bias and missing data (Lüdtke, Robitzsch, & Trautwein, 2018). Thus, structural equation modeling (SEM) is favored to perform these analyses, but when SEM is applied to a small sample (<100 or <200; Kline, 2016) it likely results in convergence issues, improper solutions, or biased path coefficients and biased standard errors (Gagne & Hancock, 2006; Hoshino & Bentler, 2011; Kelcey, 2018).

Sample size recommendations for SEM in past research have often indicated that at least 10 to 20 cases per parameter are needed to provide a minimal basis for unbiased estimation and inference (e.g., Wolf, Harrington, Clark, & Miller, 2013). The current sample of 145 thus does not allow for sufficient power to test the proposed hypotheses in the latent space. Furthermore, even the minimal requirements put forth by Wolf and colleagues (2013) can be contingent upon the presence of moderate to high magnitudes of factor loadings and latent variable associations.

To circumvent these problems the factor score regression method (FSR; Tucker, 1971) has been used. FSR breaks the SEM method into three steps: 1) estimate the parameters of the measurement model, 2) estimate the factor scores, and 3) conduct the regression or path analysis. Although it substantially reduces parameters in a model and thus is useful for small sample sizes, modeling with estimated factor scores can lead to biased conclusions about the latent structural relations (Hoshino & Bentler, 2011).

Skrondal and Laake (2001) developed an FSR method that avoids this bias by using the regression predictor (Thomson, 1934; Thurstone, 1935) for the independent latent variables and the Bartlett predictor (Bartlett, 1937) for the dependent latent variables. In 2002 Croon developed the bias correcting method which produces a reliable standard error, unlike prior methods. Indeed, a simulation study by Devlieger, Mayer, and Rosseel (2016) found that the bias

correcting method, with the newly developed standard error, was the only suitable alternative for SEM, and had comparable bias, efficiency, mean square error, power, and type I error rate. In the bias correcting method factors scores can be computed using either the regression predictor or the Bartlett predictor; once the factor scores are computed their variances and covariances are calculated. Next, the variances and covariances of the factors scores are used to compute the variances and covariances of the true latent variable scores. These estimates are then used to calculate the regression coefficient. Because the variances and covariances are unbiased, this results in an unbiased regression coefficient estimate. This approach was extended to path analysis, wherein the variance-covariance matrix of the factor scores is calculated, the true variances and covariances for all elements in the variance-covariance matrix are estimated, and then a path analysis is performed using the estimated variances and covariances as the input covariance matrix for the model (Devlieger & Rosseel, 2017).

In addition, there is a FIML-based extension for both the regression predictor and the Bartlett predictor (Estabrook & Neale, 2013; Loncke et al., 2018). Because it makes use of all available data, the regression (or Bartlett) FIML estimator approach makes it possible to estimate factor scores in the presence of missing values. Loncke and colleagues (2018) performed a simulation study comparing the performance of regression FIML, Bartlett FIML, SEM, and ANOVA. Results showed that regression and Bartlett FIML may serve as a valuable alternative for SEM, especially when using bootstrapping to help correctly estimate standard errors.

Analyses were conducted using the statistical software R (R Core Team, 2017), as regression and Bartlett factor scores with FIML are available in the R package lavaan (Rosseel, 2012). Preliminary analyses (i.e., descriptives, group comparisons, correlations) were conducted prior to testing of study hypotheses. Measurement models were then used to perform a factor

analysis for each latent variable separately and to calculate their respective factor scores using the regression predictor for the independent latent variable (i.e., adversity) and the Bartlett predictor for the dependent latent variable (i.e., systemic inflammation). The factor score path analysis method (Devlieger & Rosseel, 2017) was used to test hypothesis one (i.e., adversity is positively associated with systemic inflammation), with gender, BMIz, and presence of a psychiatric disorder as covariates. Hypothesis two was tested by conducting an additional factor score path analysis with adversity x family cohesion (mean-centered) added as an interaction term. A significant interaction term would be probed using procedures outlined by Aiken and West (1991) with simple slopes calculated at low (-1 SD) and high (+1 SD) levels of family cohesion. Although FIML is considered to be fairly robust to departures from multivariate normality (Byrne, 2012), models were estimated using bootstrapped bias-corrected confidence intervals, specified with 1000 bootstraps, given that this type of estimation increases statistical power, produces lower Type-I error rates, and was shown to be effective for the bias correcting factor score path analysis method (Devlieger et al., 2016; Devlieger & Rosseel, 2017; MacKinnon, Lockwood, & Williams, 2004).

Based on Kline's (2016) recommendation, overall model fit for each CFA model and each path model was evaluated with five fit indicators: the chi-square test for model fit, the Root Mean Square Error of Approximation (RMSEA), the Comparative Fit Index (CFI), the Tucker-Lewis Index (TLI), and the Standardized Root Mean Square Residual (SRMR). According to Kline (2016), χ^2/df should be less than or equal to 3.0; for the RMSEA, values less than .05 indicate good fit and values as high as .08 represent acceptable fit; for the CFI and TLI, values greater than .90 and .95 reflect acceptable and good model fit, respectively; and for the SRMR, values less than .08 indicate good model fit.

Regarding path coefficients, regression estimates were inspected for significance (α = .05). The magnitude of regression estimates and the R^2 values were examined to inform the interpretation of each model's results.

Sample size considerations. The minimum detectable effect for a study of this nature is $f^2 = 0.02$, which is classified as a small effect (Cohen, 1988). However, based on prior literature (e.g., Bernard et al., 2018; Baumeister et al., 2016; Tyrka et al., 2015), an effect size of $f^2 = 0.12$ (small to medium) was hypothesized for the current study. A power analysis for hypothesis one was conducted using GPower version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007), wherein the effect size was specified as $f^2 = 0.12$; α = 0.05; and β = 0.95. A total sample size of 111 (1, 105) was required. For hypothesis two, a sample size of 111 (1, 104) was also sufficient. A sensitivity test was also performed post hoc, and showed that with α = 0.05, β = 0.95, and $N = 145$, the effect size must be at least 0.08, which was met.

Results

Descriptive Statistics

The mean score for dosage of PTLEs was 7.39 ($SD = 5.34$) and range was from 0 to 23; 2.1% of the sample endorsed 0 PTLEs, 37.2% endorsed 1-5 PTLEs, 23.5% endorsed 6-10 PTLEs, 28.9% endorsed 11-15 PTLEs, 6.2% endorsed 16-20 PTLEs, 2.1% endorsed 21-23 PTLEs. Normality of data was assessed through visual inspection of histograms (see Appendix A for all histograms) and calculation of skewness and kurtosis. Dosage of PTLEs followed a relatively normal distribution (skew = .81, kurtosis = .02).

Regarding frequency of PTLEs experienced, the mean was 10.34 ($SD = 7.54$), and range was from 0 to 33. Importantly, this number does not reflect a value, such as dosage of PTLEs does, but rather a categorical variable. Specifically, if a parent reported that a PTLE happened

once they would receive a “1”, twice a “2”, 3-5 times a “3”, 6-10 times a “4”, and more than 10 times a “5”. These scores were then summed. Thus, a score of 10 indicates relatively frequent adversity. For instance, a child receiving this score could have had two events happen more than 10 times each, resulting in the score of 10. Alternatively, a child could have had 10 events each happen once, as well as numerous other combinations. Frequency of PTLEs was somewhat positively skewed, though still within normal limits (skew = .98, kurtosis = .76).

Among those who were administered the maltreatment items (N = 97), the mean score for dosage of maltreatment was 1.14 (SD = 1.49), and range was from 0 to 6. In total, 45.3% of this sample had experienced any type of maltreatment. Specifically, 37.0% endorsed a form of physical abuse, none endorsed sexual abuse, 2.1% endorsed a form of physical neglect, 1.0% endorsed a form of supervisory neglect, and 5.2% endorsed a form of emotional or psychological abuse. Normality of the data was examined, and dosage of maltreatment was somewhat positively skewed, but within normal limits (skew = 1.07, kurtosis = .19).

Regarding frequency of maltreatment experienced, the mean was 1.21 (SD = 1.55) and range was from 0 to 6. Again, this number does not reflect a value, such as dosage of maltreatment does, but rather a categorical variable. A score of 1 reflects relatively infrequent maltreatment, particularly that one event happened one time. Normality of the data were examined, and frequency of maltreatment was similarly positively skewed, while remaining in normal limits (skew = .99; kurtosis = -.09).

The mean for family cohesion was 7.21 (SD = 1.79). Scores ranged from 0 to 9, with 9 indicating high cohesion. Family cohesion was negatively skewed and somewhat leptokurtotic (see Appendix A), as many parents endorsed high levels of family cohesion (skewness = -1.48, kurtosis = 2.33).

Upon examination of inflammatory protein data, distributions were very skewed and leptokurtic (skew, kurtosis): CRP: (5.75, 40.98); IL-1β: (3.22, 14.52); IL-6: (5.85, 43.76); IL-8: (3.17, 12.21); TNF-α: (3.55, 16.89). Thus, in line with the literature, all inflammatory proteins were Winsorized and log-transformed prior to data analysis to adjust for skewed distributions and outliers (Cecil, Smith, Walton, Mill, McCrory, & Viding, 2016; Riis et al., 2015).

Winsorization was performed separately for each inflammatory protein; values were brought within three standard deviations of the mean, as is consistent with previous literature (Riis et al., 2015). For CRP, 2.84% of the sample was winsorized; for IL-1β, 2.76% was winsorized; for IL-6, 2.11% was winsorized; for IL-8, 2.76% was winsorized; and for TNF-α, 2.76% was winsorized. Winsorizing less than 4% of each inflammatory protein is considered acceptable (Riis et al., 2015). Winsorization and transformation improved the distribution of inflammatory protein data (skew, kurtosis): CRP: (1.27, 1.46); IL-1β: (.19, -.40); IL-6: (1.25, 1.20); IL-8: (.13, -.61); TNF-α: (.67, .07). Descriptive statistics for raw, Winsorized, and Winsorized/log-transformed inflammatory protein data are available in Table 1. See Appendix A for inflammatory protein histograms.

Table 1

Descriptive Statistics for Inflammatory Protein Data in 145 3- to 6-Year-Old Children

	Mean	*SD*	Minimum-Maximum
CRP			
Raw (pg/mL)	4353.25	9253.52	451.92-83420.66
Winsorized	3753.56	5718.88	451.92-27676.21
Log-Transformed	3.35	0.38	2.66-4.44
IL-1β			
Raw (pg/mL)	47.69	54.43	2.67-404.42
Winsorized	45.61	44.71	2.67-202.90
Log-Transformed	1.51	0.37	0.56-2.31
IL-6			
Raw (pg/mL)	4.41	9.40	0.16-86.33
Winsorized	3.91	6.17	0.16-31.13

Log-Transformed	0.52	0.33	0.06-1.51
IL-8			
Raw (pg/mL)	401.40	490.82	38.96-3100.94
Winsorized	372.96	371.53	38.96-1576.36
Log-Transformed	2.39	0.40	1.60-3.20
TNF-α			
Raw (pg/mL)	2.61	2.83	0.23-21.42
Winsorized	2.46	2.15	0.23-10.18
Log-Transformed	0.48	.22	0.09-1.05

Inflammatory Protein Concentrations

The mean concentration of each inflammatory protein, except TNF-α, was lower than expected for this sample exposed to significant adversity. Albeit very few studies have measured inflammatory proteins in preschool-aged children, thus there is no normal level that has been established by the literature. Further, prior studies of children ages 0-18 have varied significantly in mean levels of inflammatory proteins reported.

CRP. CRP is the most commonly studied inflammatory protein (Kleiner, Marcuzzi, Zanin, Monasta, & Zauli, 2013; Schlenz et al., 2014; Pearson et al., 2003; Ridker, 2003). The Centers for Disease Control and Prevention and the American Heart Association have suggested that concentrations of CRP less than 1 mg/L indicate low risk, concentrations between 1 and 2.9 mg/L suggest moderate risk, and concentrations of 3 mg/L or higher show high risk. In a large (i.e., $N = 7{,}211$), representative sample including children ages 3 to 6 and up to 18 years old, the mean concentration of CRP was 1.22 mg/L and there were no differences based on age (Dowd, Zajacova, & Aiello, 2010). Conversely, all children in the current sample had CRP values substantially less than 1 mg/L (i.e., $M = 0.003$ mg/L), placing them squarely in the "low risk" category for disease.

Among the two prior studies of adversity and CRP in solely preschool-aged children, one showed a similar mean CRP concentration to the present sample, and one quite different. In the

study of 3- to 5-year-olds exposed to maltreatment, PTLEs, and socioeconomic adversity (Tyrka et al., 2015), mean CRP concentration was 0.004 mg/L, which is similar to the mean CRP concentration found in the current study (i.e., 0.003 mg/L). In contrast, Bernard et al.'s (2019) study of 3- to 6-year-olds found that CRP concentration was 0.37 mg/L in the low-risk control group and 0.43 mg/L in the group referred to CPS.

Proinflammatory cytokines. In comparison to CRP, healthy or low-risk levels of proinflammatory cytokines (i.e., IL-1β, IL-6, IL-8, and TNF-α) are much less well-established, even in adult populations. One study, however, provides mean concentration values (Winsorized and log-transformed) for each of those cytokines in a sample of 125 typical, healthy five-year-old children (Riis et al., 2015). In Riis et al. (2015) the mean for IL-1β was 136.77 pg/mL compared to the current sample's 47.69 pg/mL; the mean for IL-6 was 16.70 pg/mL compared to the current sample's 4.41 pg/mL; and the mean for IL-8 was 631.04 pg/mL compared to the current sample's 401.40. IL-1β, IL-6, and IL-8 were lower in the current sample, however, concentrations of TNF-α were similar between the two studies: the mean for TNF-α was 2.47 pg/mL compared to the current sample's 2.61 pg/mL.

Group Differences

Independent *t*-tests were conducted to determine if there were differences between groups on potentially confounding variables, as these differences may need to be accounted for in model tests and in interpretation of results.

Given that children with oral health problems or current illness, injury, or infection may be more likely to experience greater levels of adversity and possibly lack of family cohesion than those without oral health or other health problems (Flaherty et al., 2013; Kvist, Annerbäck, & Dahllöfa, 2018), participants who were eligible to provide saliva samples were compared to

those who were not due to exclusionary criteria (i.e., illness, injury, infection, oral health problem). There were no significant mean score differences between those who provided saliva (N = 145) and those who were ineligible (N = 86) based on mean comparisons of dosage of PTLEs (t = .27; p = .86), frequency of PTLEs (t = .16; p = .47), dosage of maltreatment (t = .70; p = .22), frequency of maltreatment (t = .15; p = .37), and level of family cohesion (t = .95; p = .75). Furthermore, there were no significant differences between the two groups on age, gender, race, income, BMIz, asthma, blood pressure, psychiatric disorder, and ADHD.

Additionally, given previous findings that the time of day samples are collected may impact inflammatory protein levels (Tworoger & Hankinson, 2006), participants that provided saliva in the morning (N = 61) were compared with participants who provided saliva in the afternoon (N = 84). There were no statistically significant differences between the two groups on mean levels of CRP (t = .74; p = .50), IL-1β (t = -.09; p = .82), IL-6 (t = .33; p = .98), IL-8 (t = -.51; p = .20), or TNF-α (t = .60; p = .45).

To determine whether recruitment type impacted study variables, mean score differences between the two groups were examined. Children who were recruited through DSS did not differ significantly from children who were recruited through Head Start Centers based on mean comparisons of dosage of PTLEs (t = .15; p = .20), frequency of PTLEs (t = .33; p = .70), dosage of maltreatment (t = .10; p = .20), frequency of maltreatment (t = .30; p = .29), level of family cohesion (t = -.27; p = .63), CRP (t = .16; p = .91), IL-1β (t = .40; p = .19), IL-6 (t = -.39; p = .10), IL-8 (t = -.12; p = .52), and TNF-α (t = .30; p = .70). The two recruitment groups also did not differ on any covariate.

Given that in the sample, 97 parents completed measures using the ACASI and 48 parents completed measures on paper, mean score differences on adversity variables were

examined to determine whether parents may have responded to items differently based on the type of data collection procedure used. Given that the ACASI provided parents greater privacy, it was possible that parents using the ACASI may have been more open to endorsing adversity events than parents using paper. However, these two groups did not differ significantly on dosage of PTLEs (t = .72; p = .26), frequency of PTLEs (t = .81; p = .24),.90; p = .21), dosage of maltreatment (t = .36; p = .30), or frequency of maltreatment (t = .50; p = .56).

Correlations Among Study Variables

Bivariate correlations were used to examine relations among indicator and measured variables. As shown in Table 2, all proinflammatory cytokines (i.e., IL-1β, IL-6, IL-8, and TNF-α) were positively correlated with each other. IL-8 and TNF-α were particularly highly correlated. CRP, however, was not significantly correlated with any of the proinflammatory cytokines. Dosage of PTLEs and frequency of PTLEs were both positively correlated with IL-8

Table 2

Bivariate Correlations of Study Variables in Sample of 145 3- to 6-Year-Old Children

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Age	1																	
2. Gender	-.10	1																
3. Race	-.03	-.06	1															
4. Income	.03	.08	.00	1														
5. BMI	.20*	-.22*	-.03	.07	1													
6. Asthma	.00	.15	.07	-.05	.05	1												
7. BP	.09	.02	-.03	-.09	-.04	.05	1											
8. Psych	.10	.10	.11	-.04	-.07	.23**	.28**	1										
9. ADHD	.17*	-.06	.08	-.02	.08	.05	-.04	.28**	1									
10. P Dos	.09	-.02	.08	-.07	-.07	.20*	.11	.22*	.07	1								
11. P Freq	.04	-.01	.03	-.02	-.09	.15*	.05	.23*	.06	.55***	1							
12. M Dos	.01	-.07	.05	-.03	-.06	.07	.03	.11*	.03	.42***	.25**	1						
13. M Freq	.09	-.11	.08	-.02	-.04	.15	-.01	.15**	.10	.39**	.47***	.59***	1					
14. Cohes	.10	.07	.05	.19*	.03	-.02	.04	.10	.06	-.15	-.20	-.08	-.11	1				
15. CRP	.01	.09	-.04	.06	-.02	.01	.17	.04	-.01	.14	.13	.10	.08	-.05	1			
16. IL-1β	.07	-.05	.14	.08	-.07	.00	.08	.29**	.10	.10	.12	.11	.14	-.05	.01	1		
17. IL-6	.09	-.26**	.12	.03	.24**	-.08	-.07	.10	.06	.12	.10	.07	.05	-.09	.08	.51**	1	
18. IL-8	-.06	.04	.11	.06	-.04	-.05	.07	.34**	.01	.15**	.21**	.13*	.19*	-.04	.01	.65**	.40**	1
19. TNF-α	.10	.02	.15	.09	-.01	.08	.10	.39**	.08	.18**	.19**	.09	.11*	-.11	.02	.62**	.36**	.82**

Note. BP = High blood pressure. Psych = Presence of a psychiatric disorder. P Dos = PTLEs dosage. P Freq = PTLEs frequency.
M Dos = Maltreatment dosage. M Freq = Maltreatment frequency. Cohes = Family cohesion.
*p<.05, **p<.01, ***p<.001

and TNF-α. Maltreatment dosage was positively correlated with IL-8, while maltreatment frequency was positively correlated with IL-8 and TNF-α. Family cohesion and income were positively correlated with each other, but neither were correlated with any other variable.

To determine whether medication use was associated with inflammatory protein levels, additional bivariate correlations were performed. The seizure medication and SSRI were excluded given that these medications are not known to affect systemic inflammation, and only one child was taking each of those medications. No medications were significantly correlated with any inflammatory protein (see Table 3).

Table 3

Bivariate Correlations of Medications and Inflammatory Proteins

	1	2	3	4	5	6	7	8
1. Asthma-C	1							
2. Asthma-A	.18*	1						
3. Allergy	-.04	.16	1					
4. ADHD	.24**	-.05	.37**	1				
5. CRP	-.07	-.05	-.05	-.01	1			
6. IL-1β	.04	.12	.09	.10	-.01	1		
7. IL-6	-.02	.05	.05	.05	-.08	.51**	1	
8. IL-8	-.09	.08	.07	.01	-.02	.65**	.40**	1
9. TNF-α	-.07	.12	.15	.07	-.01	.62**	.36**	.82**

Note. Asthma-C = control medication for asthma. Asthma-A = as-needed medication (i.e., albuterol inhaler) for asthma. Allergy = antihistamine medication for allergies. ADHD = stimulant medication for ADHD.
*p<.05, **p<.01

Covariate inclusion. Given that child gender, BMIz, and presence of a psychiatric disorder were significantly correlated with one or more inflammatory proteins, these variables were included as covariates in the path models. Child age and race, family income, asthma, high blood pressure, ADHD diagnosis, and any medication were not associated with any inflammatory protein, and thus were considered no further.

Measurement Model Development

Adversity and systemic inflammation factor score estimates were generated in R from confirmatory factor analyses (CFAs). Both CFAs used the fixed factor method of scale setting, in which the latent variance of each construct is fixed to 1.0. The fixed factor method of

identification creates standardized parameter estimates. Both constructs had a fixed variance and at least three indicators. Therefore, both models were overidentified with more observed variance and covariance values available than the number of parameters that were estimated (Kline, 2010).

The CFA model for adversity illustrated in Figure 3 demonstrated excellent fit with the data ($\chi 2_{(2, N = 145)} = .133$, $p = .715$, RMSEA $_{(.000 - .096)} = .000$, TLI = .999, CFI = .999, SRMR = .011). All loadings were moderate to high and significant. Upon inspection of the residual correlation matrix, there were no large residuals, suggesting that this model accounts for the relations among the variables well.

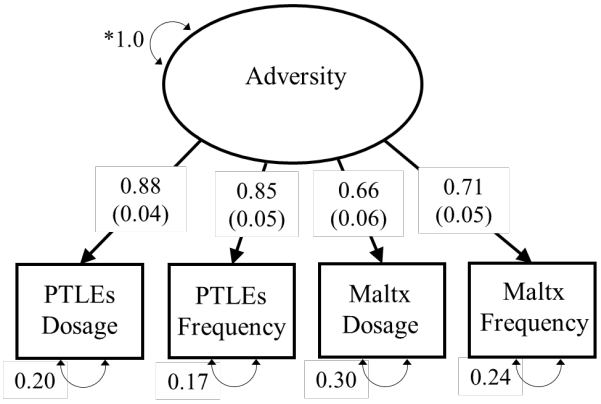

Figure 3. Final measurement model of adversity.

Importantly, all measured variables loaded on the hypothesized factor of adversity consistent with the proposed theoretical framework, providing evidence for construct validity. Construct reliability of the adversity factor was evaluated by calculation of the *H* index (varying from 0 to 1) using the standardized loading estimates (Hancock & Mueller, 2001). The *H* index provides the correlation between a factor and an optimally weighted item composite (Rodriguez, Reise, & Haviland, 2016). If *H* is low, this suggests the latent variable is not well defined by the indicators and thus may change across samples, whereas if *H* is high, the latent variable is well defined by the indicators and should be consistent across samples (Rodriguez et al., 2016). The *H* index for the adversity factor was 0.71, which meets the standard criterion of *H* = 0.70, indicating that this factor had adequate construct reliability and was likely to be replicable across studies using the same indicators (Rodriguez et al., 2016).

Factor score determinacy was also assessed, given that if factor indeterminacies are low any factor score method can be used (i.e., regression, Bartlett, bias avoiding, or bias correcting), and if factor indeterminacies are moderate or high, SEM or the bias correcting method should be used (Devlieger et al., 2016). The factor score determinacy for adversity was moderately high (ρ = 0.812), though did not reach the standard criterion of 0.90, thus the bias correcting method was selected for computing this factor score.

For the latent systemic inflammation factor, the measurement model illustrated in Figure 4 demonstrated poor fit with the data ($\chi 2_{(5, N = 145)} = 13.939$, $p = .001$, RMSEA $_{(.112 - .309)} = .203$, TLI =.889, CFI = .963, SRMR = .035). Upon examination of factor loadings, the CRP indicator did not appear to load on the factor as expected (standardized factor loading = .08). Further, modification indices showed that exclusion of CRP from the model would result in stronger model fit. This is consistent with theoretical rationale that CRP functions differently than proinflammatory cytokines given that CRP is induced by proinflammatory cytokines, and therefore occurs last in the sequence of systemic inflammation (Eklund, 2009). Moreover, it is not produced locally unlike the other four proteins. Thus, a measurement model excluding CRP was tested and demonstrated excellent fit with the data ($\chi 2_{(2, N = 145)} = .800$, $p = .371$, RMSEA $_{(.000 - .091)}$

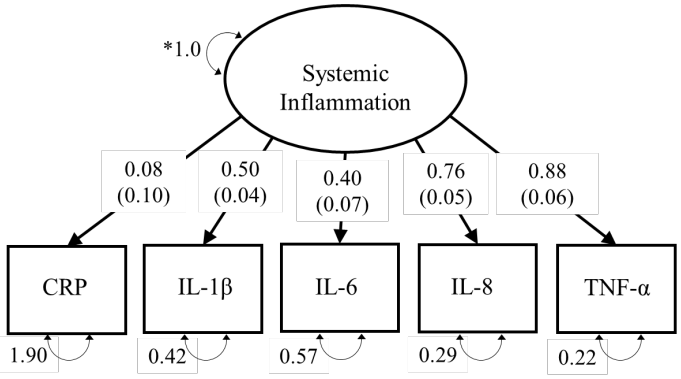

Figure 4. First measurement model of systemic inflammation.

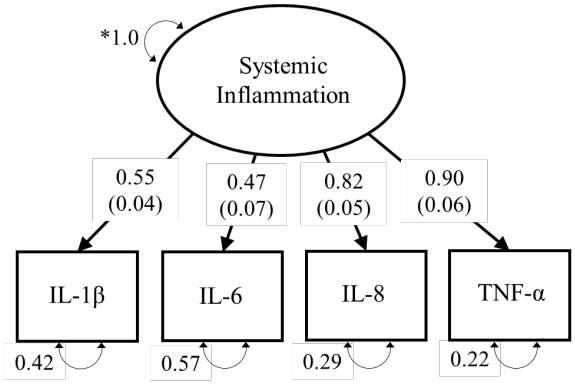

Figure 5. Final measurement model of systemic inflammation.

= .000, TLI =.999, CFI = .999, SRMR = .006). All loadings were moderate to high and significant. Upon inspection of the residual correlation matrix, there were no large residuals, suggesting that this model accounts for the relations among the variables well. This model is pictured in Figure 5.

Importantly, all measured variables loaded on the hypothesized factor of systemic inflammation consistent with a theoretical framework, providing evidence for construct validity. The *H* index, a measure of construct reliability, was 0.84, which exceeds the standard criterion of $H = 0.70$, indicating that this factor had good construct reliability and was likely to be replicable across studies using the same indicators (Rodriguez et al., 2016). The factor score determinacy for systemic inflammation was moderately high ($\rho = 0.935$), thus the bias correcting method is still appropriate for computing this factor score.

The final measurement model including adversity and systemic inflammation as latent variables and CRP as a measured variable demonstrated good fit in the current sample ($\chi 2_{(29, N = 145)} = 80.018$, $p = .160$; RMSEA $_{(0.020 – 0.085)} = .061$; CFI = .964; TLI = .971; SRMR = .043).

Final Path Model: Hypothesis One

The first hypothesis was that adversity would be positively related to systemic inflammation. It was expected that this association would emerge after controlling for relevant covariates (i.e., gender, BMIz, presence of a psychiatric disorder), given the theory that the variable meaningfully linked with systemic inflammation is adversity exposure. The factor score path model using factor scores for the latent variables of adversity and systemic inflammation was tested in R (see Figure 6). This model demonstrated good fit with the data: $\chi 2_{(9, N = 145)} = 23.147$, $p = .245$; RMSEA $_{(.050 - .091)} = .063$; CFI = .953; TLI = .901; SRMR = .030. Results suggest a positive association between increases in adversity and increases in systemic

inflammation (parameter estimates, standard errors, and R^2 values are provided in Table 4). Adversity explained 27% of the variance in systemic inflammation ($R^2 = .271$, $p < .001$). Adversity was not significantly associated with CRP and explained less than 1% of the variance in CRP ($R^2 = .034$, $p = .950$).

Table 4

Parameter Estimates and Standard Errors from the Model Testing Hypothesis One

	B	S.E.	β
Inflammation on Adversity	.815*	.068	.233
Inflammation on Family Cohesion	-.006	.018	-.031
Inflammation on Gender	-.102	.064	-.134
Inflammation on BMIz	.001	.013	.010
Inflammation on Psychiatric Disorder	.008	.018	.093
R^2 (Inflammation) = .271			
CRP on Adversity	.040	.074	.048
CRP on Family Cohesion	-.012	.019	-.055
CRP on Gender	.066	.071	.082
CRP on BMIz	.003	.015	.017
CRP on Psychiatric Disorder	.009	.008	.103
R^2 (CRP) = .034			

*p<.05, **p<.01.

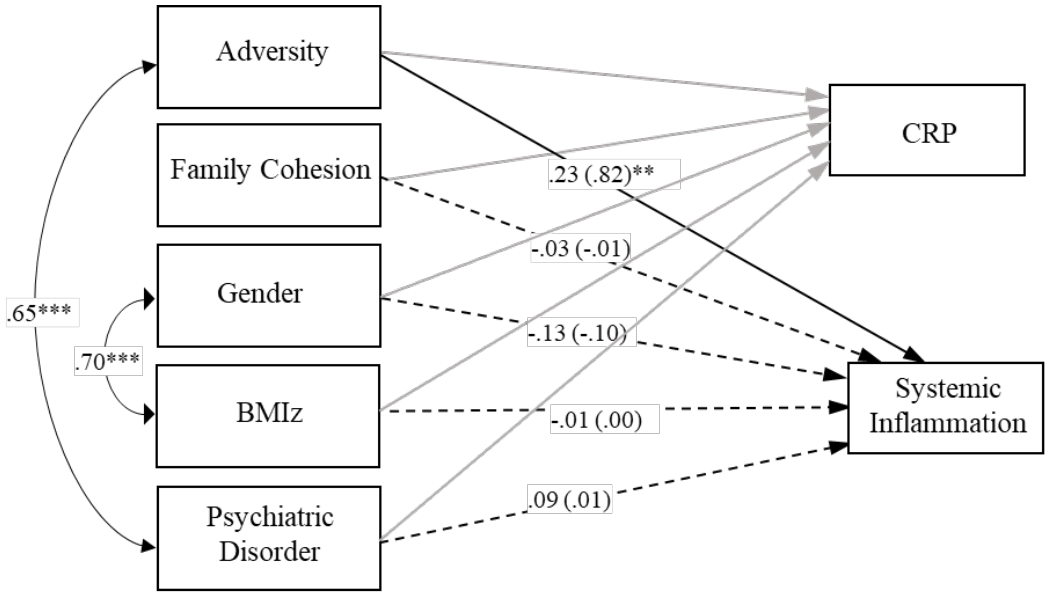

Figure 6. Path model examining associations among adversity, family cohesion, covariates, and systemic inflammation and CRP. The adversity and systemic inflammation variables are factor scores derived from CFAs. Parameter estimates for the regression paths are standardized path coefficients, with unstandardized path coefficients in parentheses. Dashed lines indicate non-significant pathways. All pathways to CRP were non-significant and grayed-out for clarity.
* p < .05. ** p < .01. *** p < .001.

Final Path Model: Hypothesis Two

To identify sources of individual differences in systemic inflammation, the next analytic step was to test family cohesion as a moderator of adversity (see Figure 7). The interaction term was created by multiplying the adversity factor score by the family cohesion total score. The resulting model provided an adequate fit with the data: $\chi 2_{(15, N = 145)} = 50.134$, $p = .431$; RMSEA $_{(.050 - .100)} = .08$; CFI = .921; TLI = .890; SRMR = .075. However, the interaction term was nonsignificant (parameter estimates, standard errors, and R^2 values are provided in Table 5). Next, the pathways from the interaction term to systemic inflammation and CRP were constrained, then the -2loglikehood (-2LL) difference test was conducted to compare the moderation model to the constrained model; the result suggested that the more parsimonious model with the interaction pathways constrained provided adequate information for variance

explained in the systemic inflammation outcome ($\Delta\chi 2_{(2)} = .875$, $p = .406$). This result suggests that family cohesion did not serve as a moderator of the relation between adversity and systemic inflammation. The same pattern was found for CRP.

Table 5

Parameter Estimates and Standard Errors from the Model Testing Hypothesis Two

Moderation Model	B	S.E.	β
Inflammation on Adversity	.138*	.042	.213
Inflammation on Family Cohesion	-.013	.011	-.099
Inflammation on Gender	-.029	.039	-.063
Inflammation on BMIz	.001	.008	.015
Inflammation on Psychiatric Disorder	.004	.005	.077
Inflammation on Adversity X Family Cohesion	.009	.015	.054
R^2 (Inflammation)* = .154*			
CRP on Adversity	.049	.075	.059
CRP on Family Cohesion	-.013	.019	-.057
CRP on Gender	.073	.071	.090
CRP on BMIz	.004	.015	.024
CRP on Psychiatric Disorder	.010	.008	.114
CRP on Adversity X Family Cohesion	.018	.027	.061
***R^2 (CRP)* = .029**			
Constrained Model	B	S.E.	β
Inflammation on Adversity	.824*	.060	.235
Inflammation on Family Cohesion	-.008	.020	-.029
Inflammation on Gender	-.111	.072	-.115
Inflammation on BMIz	.008	.001	.007
Inflammation on Psychiatric Disorder	.005	.020	.100
Inflammation on Adversity X Family Cohesion	---	---	---
R^2 (Inflammation)* = .271*			
CRP on Adversity	.030	.081	.046
CRP on Family Cohesion	-.011	.019	-.061
CRP on Gender	.063	.070	.055
CRP on BMIz	.010	.023	.014
CRP on Psychiatric Disorder	.015	.015	.101
CRP on Adversity X Family Cohesion	---	---	---
***R^2 (CRP)* = .035**			

*p<.05, **p<.01.

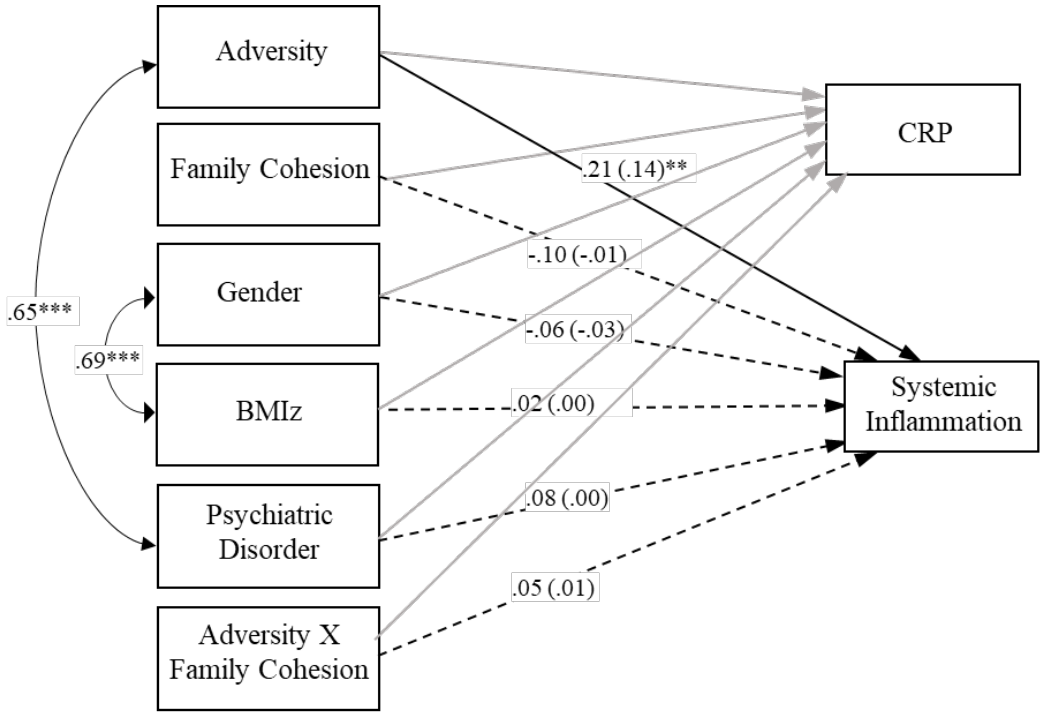

Figure 7. Path model examining the interactive effect of adversity and family cohesion on systemic inflammation and CRP. The adversity and systemic inflammation variables are factor scores derived from CFAs. Parameter estimates for the regression paths are standardized path coefficients, with unstandardized path coefficients in parentheses. Dashed lines indicate non-significant pathways. All pathways to CRP were non-significant and grayed-out for clarity.
* $p < .05$. ** $p < .01$. *** $p < .001$.

Discussion

The purpose of the present study was to examine the association between adversity and systemic inflammation, and to test the possible buffering role of family cohesion, in a sample of preschool-aged children. Findings demonstrated that adversity was associated with most indicators of systemic inflammation, although not CRP. Specifically, dosage and frequency of both maltreatment and potentially traumatic life events were linked to IL-1β, IL-6, IL-8, and TNF-α. This association was present with gender, BMIz, and presence of psychiatric disorder in the model. Unexpectedly, family cohesion was unrelated to inflammation and did not serve as a buffer in the relation between adversity exposure and systemic inflammation.

The Association Between Adversity and Systemic Inflammation: Hypothesis One

A key finding of the study was the positive association between adversity and systemic inflammation. This finding was above and beyond the influence of gender, BMIz, and presence of a psychiatric disorder. Further, this result emerged despite overall "low" levels of inflammatory proteins in the sample. These results lend possible support for the biological embedding of childhood adversity model, which suggests inflammation as an early mechanism by which adversity in childhood deleteriously impacts health over the lifespan (Miller et al., 2011). Results corroborate prior research suggesting adversity is linked to systemic inflammation among children and adolescents (e.g., Cicchetti et al., 2015; Slopen et al., 2013) and adults (e.g., Baumeister et al., 2016). The current results are unique in providing insight on this association in young children, a population in which little work has been conducted on systemic inflammation despite children under age six experiencing the greatest number of exposures to child maltreatment than at any other age (Stahmer et al., 2005). Moreover, understanding the roots of disease clearly requires exploration of risk and protective factors in the very early years of life (Hostinar et al., 2018). Importantly, this is the third study to date to find a positive association between adversity and systemic inflammation in preschool-aged children (Bernard et al., 2019; Tyrka et al., 2015), lending support for a small but compelling body of evidence that biological precursors to disease may be present much earlier than previously thought in children exposed to chronic adversity.

The study results also explain some of the inconsistencies across previous studies of children and adolescents (e.g., Fuligni et al., 2009; Tyrka et al., 2015). Specifically, dosage and frequency of exposure to maltreatment and potentially traumatic life events appear to matter for explaining the variance in systemic inflammation. This study was able to determine this by

assessing a latent structure of adversity indicated by dosage and frequency of both maltreatment and PTLEs in association with systemic inflammation. Thus, if previous studies used presence or absence of adversity, or dosage only, this association may have been more difficult, or impossible, to detect.

As such, comprehensive measurement of adversity is critical to capture the complete nature of a child's experience and subsequent risk, yet is rarely done in practice (Jackson et al., 2019). Several theoretical perspectives have argued that a more complete taxonomy of stressful exposures during early life in humans would greatly aid research in the biological effects of adversity (Humphreys & Zeanah, 2015; McLaughlin & Sheridan, 2016). As has been established in previous literature (Evans et al., 2007; Miller & Cole, 2012; Tyrka et al., 2015), dosage is a critical piece of what constitutes chronic adversity, and indeed explained a significant portion of the variance in systemic inflammation in the current sample. This finding suggests that the buildup of multiple *types* of maltreatment and PTLE experiences are linked to systemic inflammation, which echoes the ACEs studies (e.g., Felitti et al., 1998). Biologically, this plays out such that immune cells become sensitized by dose after dose of adversity, whereby they produce an exaggerated response to stress characterized by release of more proinflammatory cytokines (Glaser & Kiecolt-Glaser, 2005), which may be reflected in the saliva.

What measuring dosage alone fails to explain, however, is the frequency with which each maltreatment or PTLE experience occurs. As mentioned previously, a child with three adverse events appears to have had more exposure than a child with one adverse event, yet the child with one adverse event may have had that incident happen dozens of times over the course of their childhood while the child with three adverse events experienced each incident once. Results showed that frequency of maltreatment and PTLEs was linked to systemic inflammation in

addition to dosage. This finding speaks strongly to the role of chronicity in predicting health-related outcomes, even in children younger than age six, and is aligned with the biological process of immune cell sensitization and increased release of proinflammatory cytokines. In summary, future studies wishing to capture the most variance in systemic inflammation in a youth sample should consider inclusion of both dosage *and* frequency of abuse and neglect events, as well as other potentially traumatic life events, like exposure to community violence or parental arrest (English, Graham, Litrownik, Everson, & Bangdiwala, 2005; Hahm, Lee, Ozonoff, & Van Wert, 2010).

In addition to the nuanced conceptualization of adversity, the construct of systemic inflammation also required careful development to ensure adequate capture of the inflammation throughout the body that arises from chronic adversity exposure. Therefore, the study assessed a latent structure of systemic inflammation indicated by the five critical inflammatory proteins involved in immune functioning. Though most previous studies have modeled inflammatory proteins as separate dependent variables (e.g., Cicchetti et al., 2015; Danese et al., 2011; Miller & Cole, 2012; Slopen et al., 2013), this conceptualization fails to account for the interdependence of these proteins in the body's inflammatory response (Waters et al., 2007), as well as the additive or synergistic effects of cytokines on systemic inflammation (Condon, 2018). Other studies have used a composite of systemic inflammation, wherein all inflammatory protein values are standardized then summed (e.g., Measelle & Ablow, 2018). Although this method may approximate "inflammatory load," it is less clear which inflammatory proteins are accounting for the association with adversity, thus limiting interpretability and clinical implications.

Rather, the current study used the latent construct approach. A few other studies have modeled a latent factor of systemic inflammation indicated by inflammatory proteins (Friedman, Christ, & Mroczek, 2015; Marsland, McCaffery, Muldoon, & Manuck, 2010; McCaffery, Marsland, Strohacker, Muldoon, & Manuck, 2012; Waters et al., 2007), thus supporting the statistical strength of using a latent factor to assess the underlying construct of inflammation. Moreover, the latent factor approach is aligned with prevailing ideas about systemic inflammation as a dysregulated biological process that is linked to health outcomes and should be indicated by several markers (Friedman et al., 2015).

In the current sample, a good-fitting model was obtained with the four proinflammatory cytokines as indicators, excluding CRP—the acute phase protein. Both statistical and theoretical rationale indicated that CRP could be dropped as an indicator of the construct; CRP is not a proinflammatory cytokine like IL-1β, IL-6, IL-8, and TNF-α, but rather is induced by them and as such occurs later in the inflammatory process (Eklund, 2009). Cytokines TNF-α and IL-8 loaded most highly and contributed the most variance to systemic inflammation. IL-1β and IL-6 showed moderate loadings. Given that IL-1β, IL-6, IL-8, and TNF-α are correlated and work together to collectively inform the inflammation process that is predictive of later health outcomes, measuring systemic inflammation as a latent variable may be a useful strategy for future studies interested in measuring the biological process of immune function.

The role of TNF-α. The strong loading of TNF-α, as well as its correlation to each of the adversity indicators, suggest that this proinflammatory cytokine may be particularly driving results. This hypothesis is also supported by results showing that TNF-α concentrations in the current sample were higher than those in a typical, lower-risk sample (Riis et al., 2015), while concentrations of the other inflammatory proteins were not. TNF-α is synthesized by

macrophages, which are immune cells that form the first line of defense against invading pathogens (Chitu, Yeung, Yu, Nandi, & Stanley, 2011). TNF-α is considered a powerful proinflammatory agent as it regulates many aspects of macrophage function; it is rapidly released after perceived or actual trauma, infection, or exposure to bacteria, and has been shown to be one of the most abundant early mediators in inflamed tissue (Feldmann, Brennan, Elliott, Katsikis, & Maini, 1994). Thus, TNF-α plays a pivotal role in orchestrating the production of a proinflammatory cytokine cascade and is thought to be a "master regulator" of proinflammatory cytokine production (Maini et al., 1995; Parameswaran & Patial, 2010). Therefore, it is reasonable that TNF-α, as the leader of the proinflammatory cytokine cascade, might emerge as the protein driving the latent construct of inflammation in a sample of young children, thus contributing to the association between adversity and systemic inflammation.

Lack of association with CRP. In contrast to its association with proinflammatory cytokines, adversity was not linked to CRP. Within the larger systemic inflammation literature, CRP is the most frequently studied inflammatory protein, and shows the most robust association with adversity (Baumeister et al., 2016; Coelho et al., 2014). Thus, the present finding was unexpected. Although adversity was linked to CRP in one study of preschool-aged children (Bernard et al., 2019), another study similarly found no relation (Tyrka et al., 2015), and similar levels of CRP were found in the current sample as were found in that of Tyrka and colleagues' sample. Thus, high levels of CRP may not be consistently evident until later in childhood. In addition, a few studies in older children have found that elevated CRP in association with early adversity may be highest, or may only be evident, in those who also have depression (Danese et al., 2008, 2011; Miller & Cole, 2012). Few children in the current sample had parent-reported psychiatric disorders, and although prescence of a psychiatric disorder also failed to predict CRP,

it may be that the combination of adversity and psychiatric disorders (particularly internalizing disorders) explains elevations in CRP. It is also possible that salivary CRP in particular does not become elevated in response to adversity; indeed, the association of CRP with childhood adversity has been reported primarily in plasma or serum, as it was in Bernard's (2019) preschool-aged sample. Research does suggest however that modest correlations between blood and saliva measures of CRP exist (Byrne et al., 2013; Ouellet-Morin, Danese, Williams, & Arseneault, 2011).

Findings that adversity was associated with TNF-α, IL-8, IL-6, and IL-1β, but not CRP are consistent with the reasoning in the neuroimmune network hypothesis, which asserts that different "layers" of the inflammatory phenotype come "online" sequentially across development (Nusslock & Miller, 2016). The initial layer consists of increased monocyte/macrophage responsivity to microbial threats and decreased sensitivity to anti-inflammatory signals—and this appears in childhood (Hostinar et al., 2018). The second layer consists of increases in proinflammatory cytokines including TNF-α, IL-8, IL-6 and IL-1β. The last layer, which includes circulating CRP, may not be elevated until much later in development. The current study supports this theory, such that adversity was associated with the proinflammatory cytokines (i.e., TNF-α, IL-8, IL-6, IL-1β) that occur in the second layer, but not CRP which occurs in the third layer.

Family Cohesion as a Moderator: Hypothesis Two

In addition to the test of the association between adversity and systemic inflammation, the present study tested the role of family cohesion as a moderator. The findings did not support this hypothesis as family cohesion did not moderate the association between adversity and systemic inflammation, which is contrary to past research that has found family environment

factors, including cohesion, is associated with low levels of inflammation in youth (e.g., Miller et al., 2009; Miller & Chen, 2010). The stress buffering model proposes that a supportive, cohesive family, sensitive parenting, and secure attachment relationships protects children in the face of early adversity by reducing the negative effects of stressors on the physiological processes implicated in physical health problems (Chen, Brody, & Miller, 2017). These family-associated variables may offer important potential targets for intervention in this vulnerable population, but the current study did not support family cohesion as a protective factor as this variable had neither direct nor interactive effects on systemic inflammation.

A few reasons for why family cohesion did not impact systemic inflammation are possible. First, the responses by parents on the measure of family cohesion indicated little variance, as parents tended to report uniformly high levels of family cohesion. It is possible that this is a construct especially susceptible to social desirability bias, wherein parents felt like their parenting was being judged and thus responded most affirmatively to the items. It is also possible that families who agreed to participate in the study were more cohesive than families who did not agree to participate at the recruitment stage, which could also account for the overall high ratings of family cohesion. Second, Cronbach's alpha for the family cohesion subscale demonstrated relatively low internal consistency ($\alpha = .67$), as a reliability coefficient of .70 or higher is considered acceptable in social science research. This suggests that the items within this subscale are not necessarily closely related, and thus may not be a good measure of family cohesion.

Third, family cohesion may not be the construct best able to buffer the negative physiological effects of adversity—rather, parental responsiveness or sensitivity, or attachment may be more important than an overall sense of family support and togetherness. Attachment relationships appeared particularly important in buffering the inflammatory effects of adversity

for infants and preschoolers (Bernard et al., 2019; David et al., 2017; Measelle et al., 2017; Measelle & Ablow, 2018). It may also be the case that family cohesion is more important for older children or adolescents (e.g., Brody et al., 2013; Carroll et al., 2013) while other constructs such as attachment are more salient for preschoolers.

Limitations and Future Directions

Interpretations of the current results are tempered by some notable limitations. First, the current study relied on saliva to assay levels of inflammation rather than using blood samples (Out, Granger, Sephton, & Segerstrom, 2013). This raises the possibility that elevated proinflammatory cytokines in the present sample were due more to oral inflammation than to systemic or circulating inflammation, as has been cautioned (Out et al., 2013). Specifically, the association between adversity and proinflammatory cytokines may be reflecting the role of stress in the development of dental caries (Boyce et al., 2010). For instance, more adversity may be associated with a more chaotic family environment and/or poverty, wherein there is less encouragement of and monitoring around toothbrushing, greater consumption of sugary foods and drinks, and few dental check-ups and cleanings. Thus, the association between adversity and inflammation may be via oral health. Some evidence, however, suggests significant correlations of inflammatory proteins in saliva and blood in youth samples, negating the potential effects of dental caries (Byrne et al., 2013; Riis et al., 2015). Further, the present study's procedures for screening children's oral health, exclusion of those with oral health problems, and, importantly recent evidence of the clinical utility of salivary proteins (Iyengar, Paulus, Gerlanc, & Maron, 2014), increases the author's confidence that these findings are likely not just a reflection of oral inflammation. Nevertheless, future studies should corroborate the current results using blood samples if possible.

Second, these data are cross-sectional, meaning that causal associations are unclear. For instance, it is possible that current levels of systemic inflammation were present prior to adversity exposure, especially given the relatively low levels of proteins seen in this sample. Additionally, the link between adversity and systemic inflammation may be short-lived and could dissipate or reverse prior to adolescence or adulthood. No study to date has measured systemic inflammation at multiple timepoints across developmental periods to elucidate understanding of its trajectory. Like many previous studies in this area, temporal associations among adversity, family cohesion, and systemic inflammation were unable to be ascertained, and therefore prospective, longitudinal data will be critical for illuminating causal pathways over the course of development.

Third, and relatedly, timing of adversity was not considered in the development of the adversity construct, despite some evidence that *when* maltreatment or other potentially traumatic life events occur affects inflammatory outcomes differently (Cicchetti et al., 2015; Slopen et al., 2013). Future research may wish to tease apart how developmental timing of early adversity exposures impacts systemic inflammation, and how these associations change or remain the same as children age, for instance, through latent growth curve modeling. It is possible that proximity to the adverse event(s) – how close in time the child is to the last adversity – impacts the level of systemic inflammation. Relatedly, how a child interprets or perceives an adverse event likely affects the biological response to adversity and will be important to consider in future work. The current study did however include both dosage and frequency of maltreatment and potentially traumatic life events, a method new for the field, and clearly important for explaining variance in systemic inflammation. Moreover, the biological embedding of childhood

adversity model points to chronicity of adversity as the most salient indicator of future health outcomes, possibly more so than developmental timing (Miller et al., 2011).

Fourth, past research has established depression and other psychiatric disorders (e.g., anxiety, PTSD) as important predictors/moderators of systemic inflammation, but few diagnosed psychiatric disorders were present in the current sample—likely given the participants' young age—thus these associations were unable to be tested fully. It is possible that psychopathology must be present for higher levels of systemic inflammation to emerge, as in Danese and colleagues' (2011) study and research by Miller and Cole (2012), which could explain overall lower levels of inflammatory proteins in the current sample. Perhaps adversity exposure increases risk for depression, anxiety, and PTSD (among other psychiatric disorders), which in turn contributes to additional elevation of inflammatory proteins beyond adversity. In the current sample, adversity and presence of a psychiatric disorder were positively correlated, which lends some support for this assertion. Furthermore, other socioemotional variables besides psychopathology should be examined in future research. Specifically, self-regulation, including emotion regulation and physiological reactivity—which dictate a child's conscious or unconscious efforts to control their response to stress—may be a vehicle linking adversity exposure to systemic inflammation. Although research has established links between adversity and self-regulation difficulties in children (Heleniak, Jenness, Vander Stoep, McCauley, & McLaughlin, 2016; McLaughlin, Alves, & Sheridan, 2014) as well as self-regulation difficulties and high levels of inflammation in adults (Appleton, Buka, Loucks, Gilman, Kubzansky, 2013; Gianaros et al., 2014), no study to date has focused on emotion regulation and physiological arousal as central, modifiable mechanisms of the association between adversity and systemic inflammation in children.

Lastly, because the parent-reported measure of family cohesion used in this study showed little variability and low to moderate reliability, future research should consider alternative methods such as behavioral observation and coding of parent-child interactions that may be more sensitive to family interaction patterns. Stress buffering models propose that sensitive and supportive parenting and/or cohesive families may protect children in the face of early adversity by reducing the negative effects of stressors on physiological processes implicated in physical health problems (Chen, et al., 2017), and this may best be captured in a preschool-aged sample through behavioral observation methods.

Despite several limitations, the study's findings offer novel support for an association between adversity and systemic inflammation in young children, which in turn may reflect dysregulated immune processes that could precede or mark increased risk for later health outcomes (Black, 2003). This study addressed a major gap in the literature given that studies of children earlier on in development are necessary in order to more precisely elucidate mechanisms that connect childhood adversity to later disease vulnerability, and to identify potential opportunities to slow the progression of or minimize biological dysregulation.

Conclusions and Implications

The present work does not necessarily lend itself to an applied focus at this time; rather, the greatest merit of the study is that it provides the building blocks for understanding a complex process that may have great significance for those exposed to adversity. This research builds on prior studies, and should the trend of positive, significant associations between adversity exposure and systemic inflammation beginning in early childhood continue, it will be critically important to determine how to translate these findings into applied settings. At this stage, these results reinforce the importance of stakeholders' becoming aware of associations between

adversity and the biological realm; this is particularly significant for those providing clinical and social services to children and families encountering adversity, as well as for policy makers considering the detrimental impact of adversity early on in life.

Although it is not yet feasible (nor advisable) to measure levels of systemic inflammation in an applied setting, these results may signal to those working with adversity-exposed children that although most of these children, especially the little ones, are unable identify and express feeling stressed by maltreatment and other traumatic events, their physiology may show this nevertheless. In fact, the ability to document a positive association between adversity and systemic inflammation early on suggests a need for further investigation of these future health indicators in young children exposed to adversity, which may otherwise have gone unnoticed. Thus, this project sought to increase awareness of the larger scope of adversity, such that maltreatment and trauma are viewed as more than a score on a questionnaire, but as a precipitant for a cascade of biological changes, namely systemic inflammation, that portend future health problems like heart disease.

In conclusion, findings from the current study add to research on the biological embedding of childhood adversity and neuroimmune network hypothesis models, offering preliminary support that dosage and frequency of maltreatment and potentially traumatic life events is associated with systemic inflammation in early childhood. Although there is clearly much more work to be done in this area, the present study provided a strong foundation from which to expand by examining the developmental origins of disease vulnerability, which may ultimately help identify novel opportunities for the prevention of pervasive health conditions later in life.

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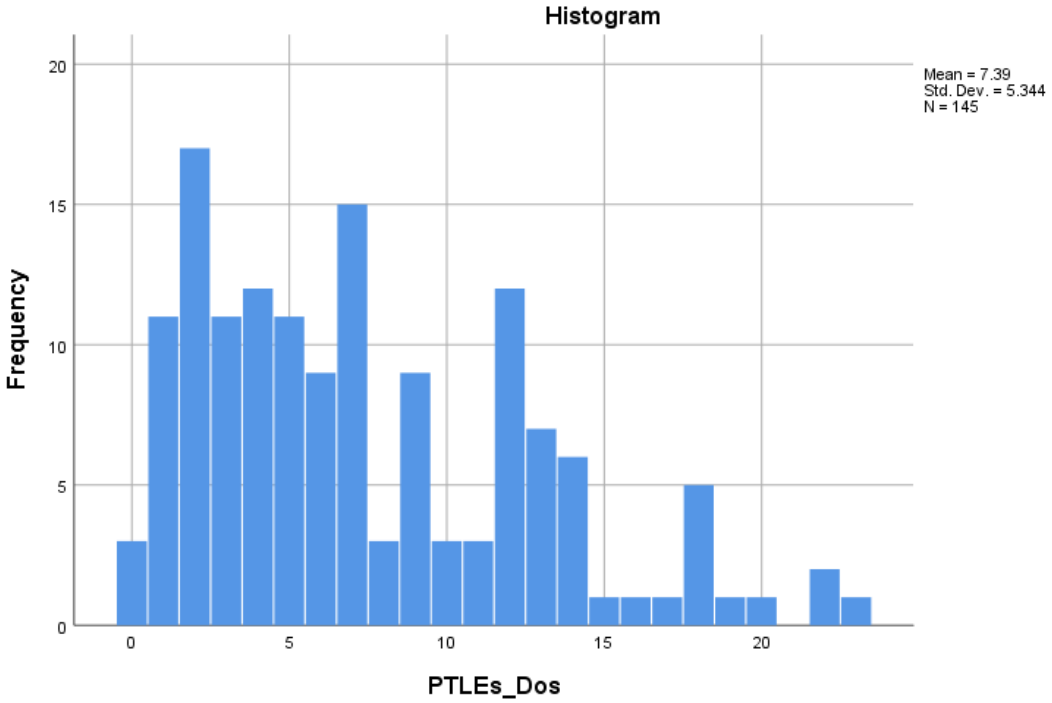
Appendix A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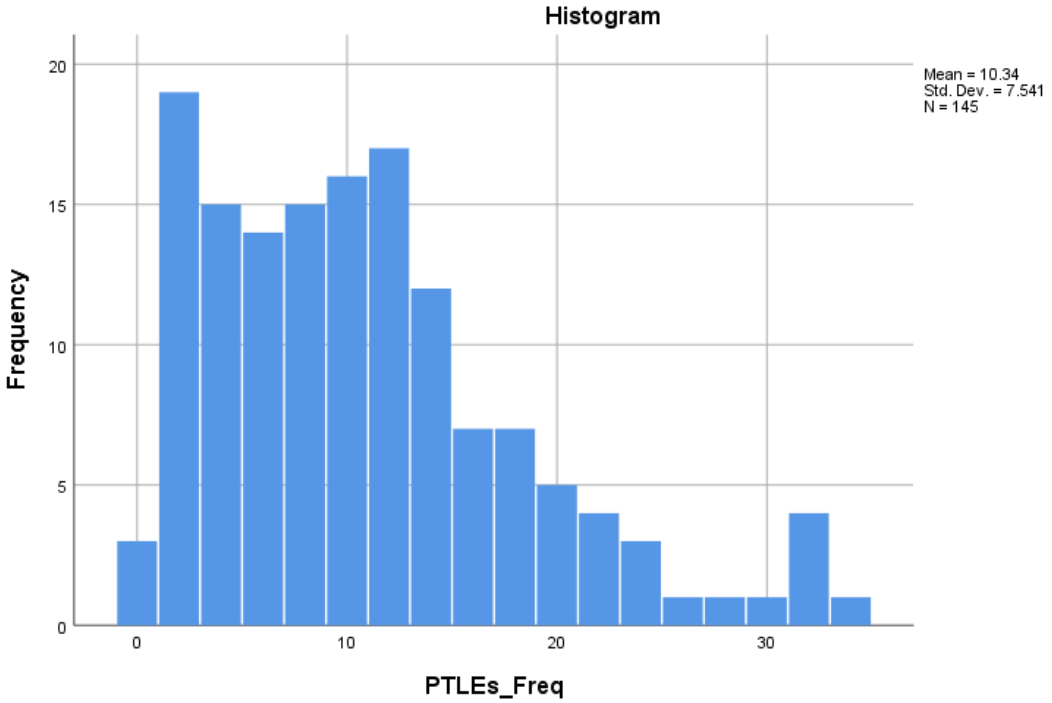
Frequency
PTLEs_Dos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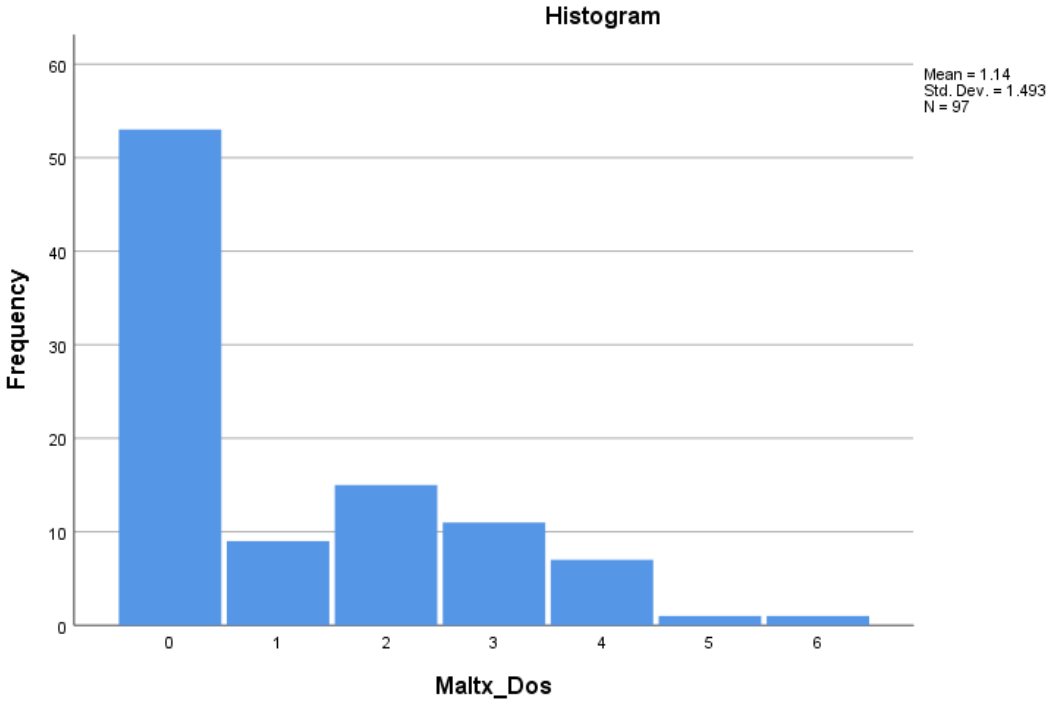
Frequency
PTLEs_Freq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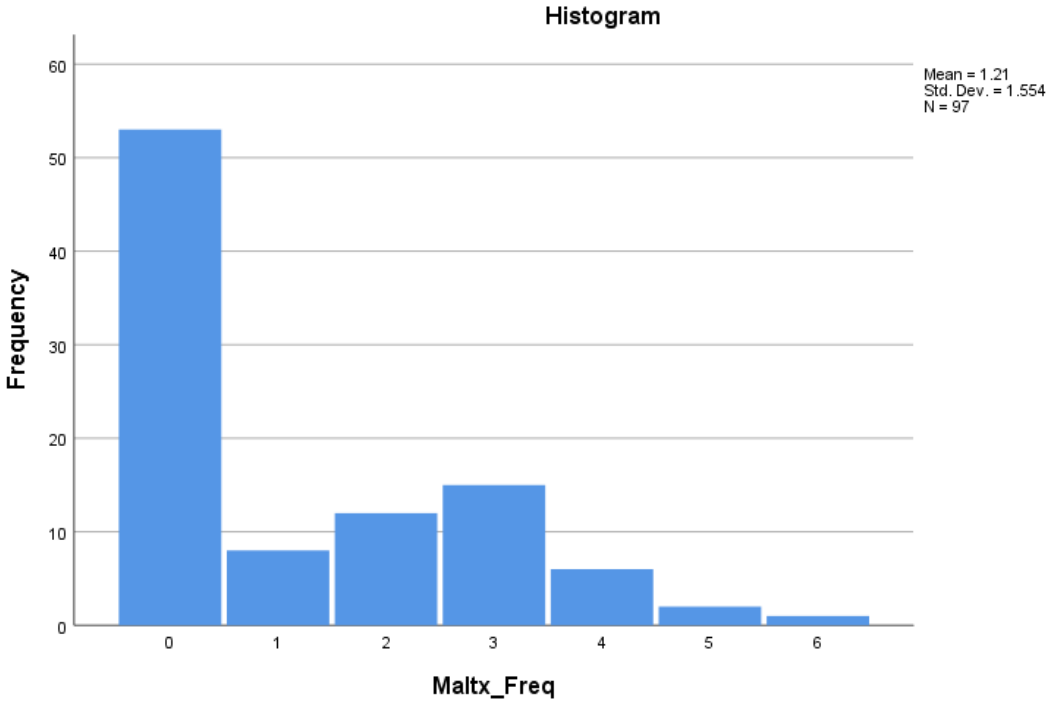
2
3
4
5
6
Maltx_Dos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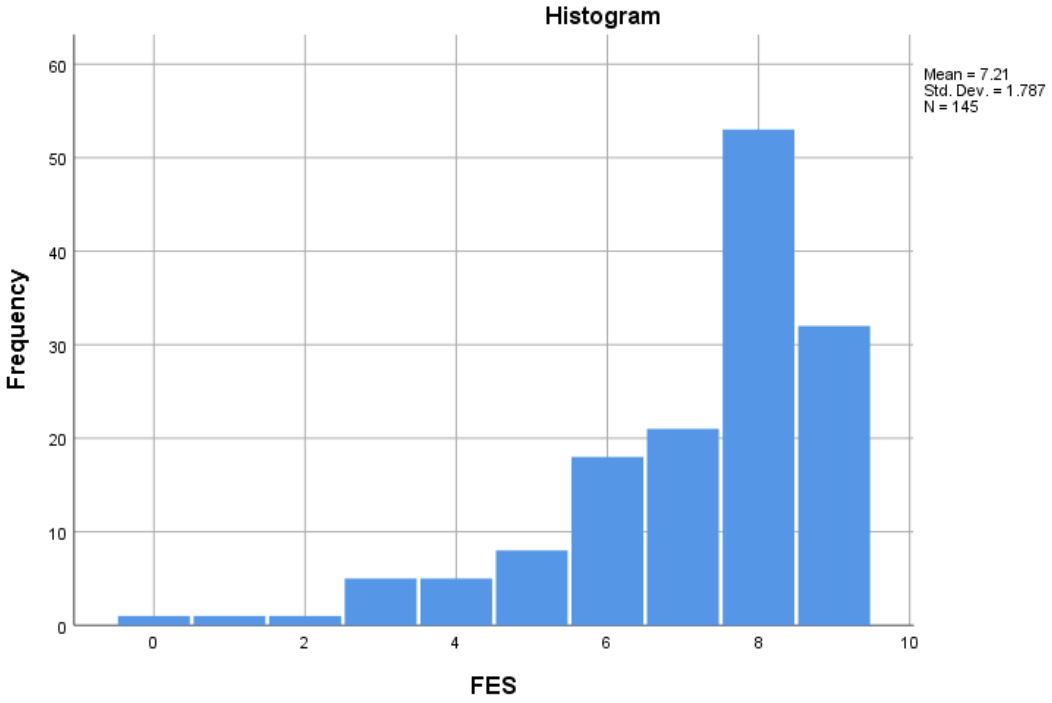
5
6
Maltx_Freq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6
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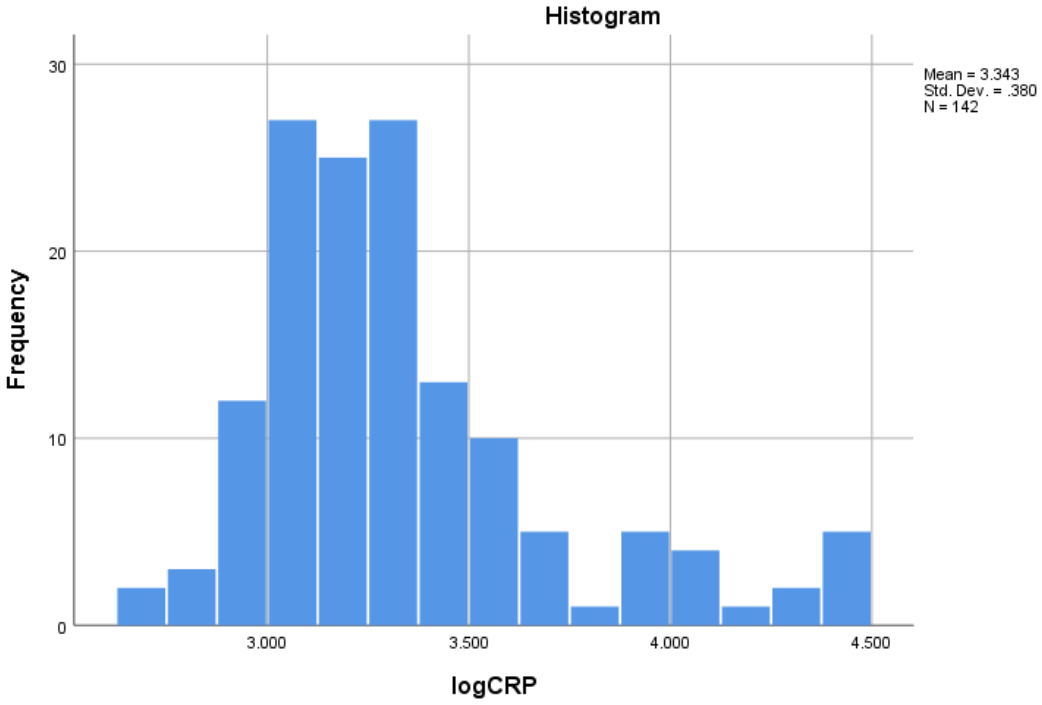
10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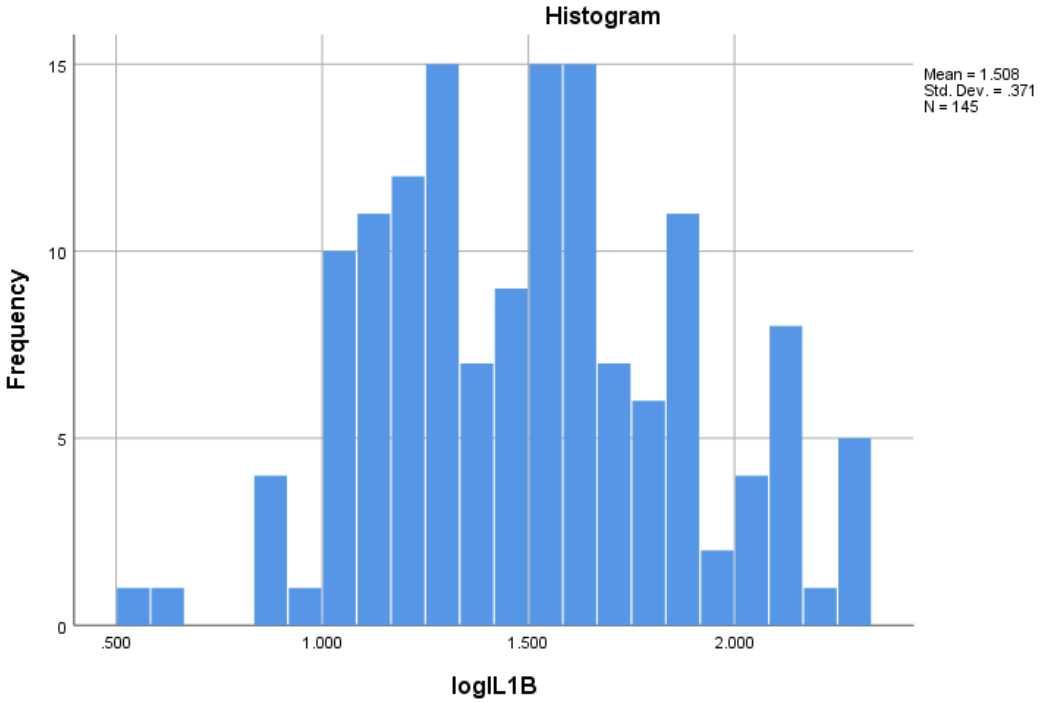
3.500
4.000
4.500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2.000
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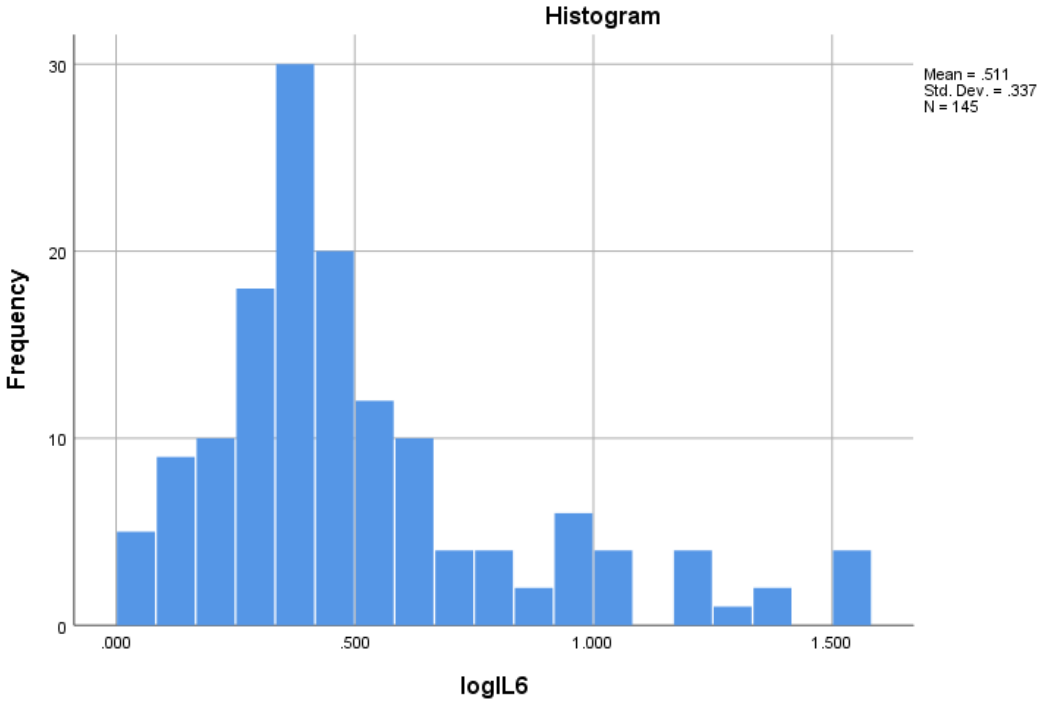
Histogram
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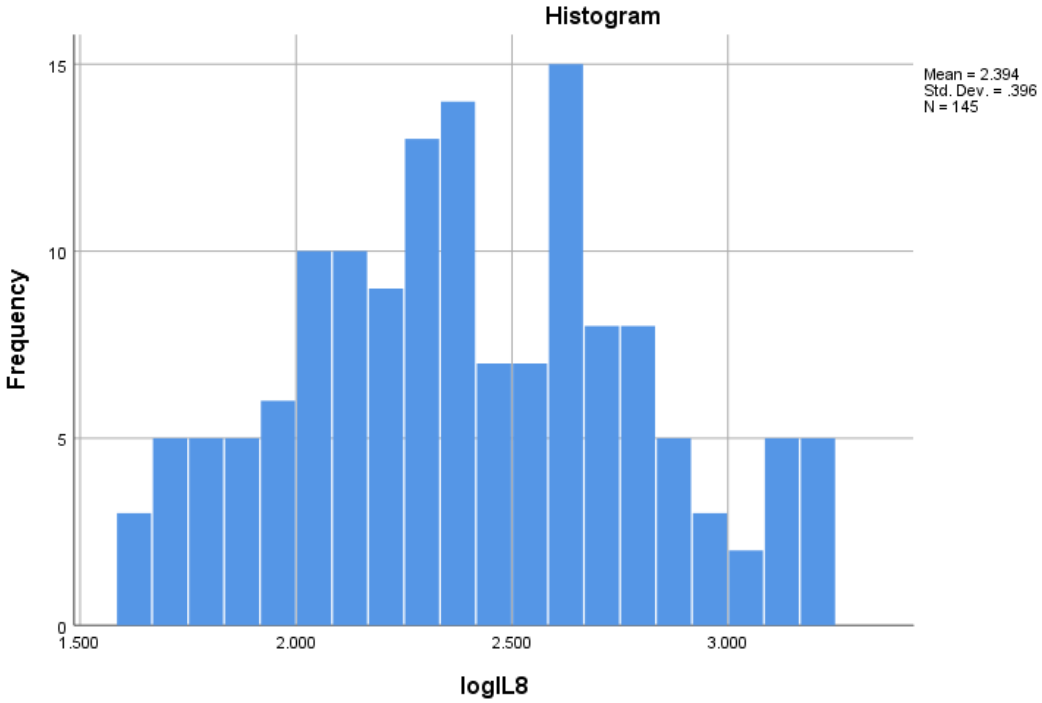
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10
5
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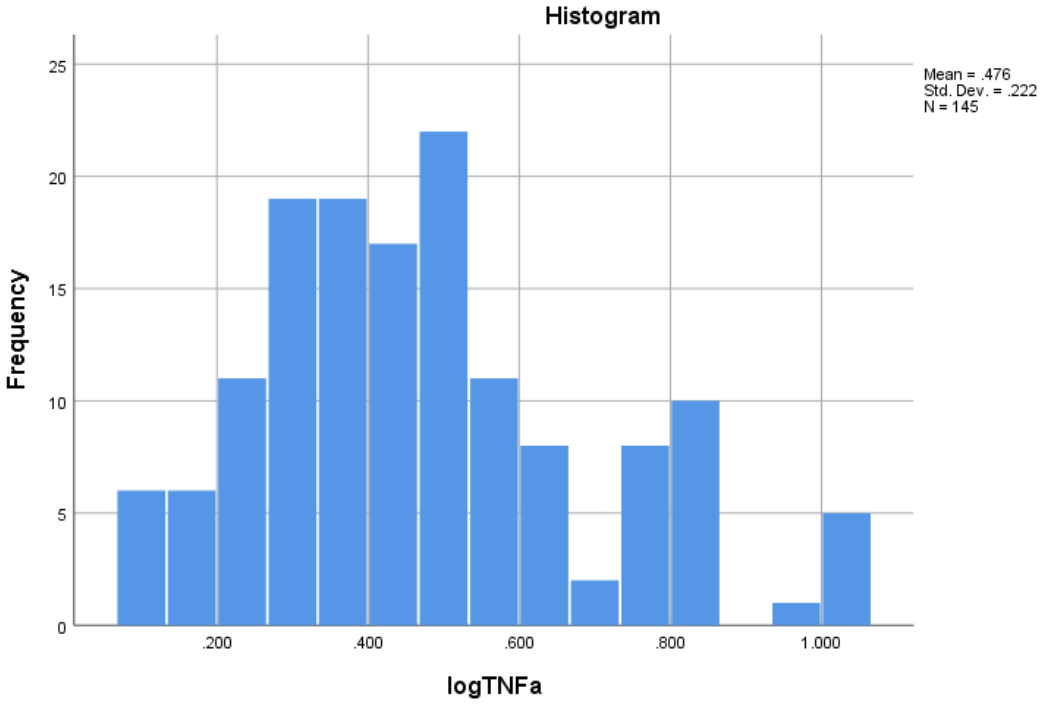
1.500
2.000
2.500
3.000
logIL8


Histogram
Mean = .476
Std. Dev. = .222
N = 145
Frequency
25
20
15
10
5
0
.200
.400
.600
.800
1.000
logTNFa