Development of glucocorticoid resistance and systemic inflammation in mothers whose children have been diagnosed with cancer

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Chronic distress associates with upregulation of innate inflammation and prolonged release of cortisol, known to downregulate levels of inflammation. It is suggested that this paradox is related to distress-related down-regulation of glucocorticoid sensitivity. Caring for a child with cancer is a provocative stressor. Although most mothers cope well, 25-30% show prolonged distress. Here, we assessed distress, interleukin (IL)-6, and glucocorticoid resistance among 120 mothers at 1, 6, and 12 months after their child's diagnosis. A latent factor for distress was indicated by depression, anxiety, and post-traumatic stress. Latent difference score models revealed a significant positive association between change in distress and change in GCR from 0-6 mo. (B = .490) and 6 mo. – 1 yr. (B = .739). The association across the second 6 mo. was retained in analyses that adjusted for peripheral leukocyte counts. IL-6 increased significantly from 0 – 6 mo. (α = 12.94), but this change was not associated with distress or GCR. These findings provide initial longitudinal evidence for an increase in GCR over the first 12 months following onset of a chronic stressor that parallels changes in distress. However, changes in GCR were unrelated to IL-6 over the same period. Future studies should consider additional pathways through which chronic stress relates to increases in IL-6. Given the health consequences of reduced sensitivity to the immunosuppressive effects of glucocorticoids, it is important to investigate the timing and mechanisms through which chronic stress relates to GCR.

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1.0 BACKGROUND AND SIGNIFICANCE

Life event stress associates with increased risk for a range of physical health morbidities that involve inflammatory pathophysiology (S. Cohen, Janicki-Deverts, & Miller, 2007; Tosevski & Milovancevic, 2006), including cardiovascular disease, AIDS, asthma, and rheumatoid arthritis (Cutolo & Straub, 2006; Kozyrskyj et al., 2008; Leserman et al., 2002; Steptoe & Kivimaki, 2013). For example, stressors such as long-term caregiving for a loved one have been associated with a 40-60% increased risk of cardiovascular disease beyond that associated with more conventional risk factors (Steptoe & Kivimaki, 2013). Life stress also associates with the course of other inflammatory-mediated diseases, including decreased cancer survival rates (Chida et al., 2008), and exacerbation of asthma (Sandberg, Jarvenpaa, Penttinen, Paton, & McCann, 2004), rheumatoid arthritis (Cutolo & Straub, 2006), and other immune-mediated conditions (e.g. HIV (Leserman et al., 2002)). Although the pathways that link life stress to adverse health outcomes remain unclear and are likely complex, growing evidence suggests that inflammatory processes may play a role.

Inflammation refers to the body's innate immune response to infection or injury. This response is initiated when immune cells called macrophages are activated by the presence of foreign matter and produce chemical signals, including pro-inflammatory cytokines, that act locally to increase capillary permeability and recruit additional immune components to the site of injury or infection, and systemically via the blood stream to initiate the acute-phase response

(Janeway, Travers, Walport, & Shlomchik, 2005). The acute phase response includes the production and release of proteins by the liver that help to protect against the spread of infection. For example, C-reactive protein (CRP) is a liver-derived acute phase protein that coats bacteria and marks them for destruction (Janeway et al., 2005). The cytokines released by activated macrophages that initiate the acute phase response include tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, and IL-6. Of these cytokines, IL-6 is most readily detected in blood and is primarily responsible for the initiation of the acute phase response (Gruys, Toussaint, Niewold, & Koopmans, 2005; Naugler & Karin, 2008).

In addition to coordinating the acute phase response, circulating pro-inflammatory cytokines, including IL-6, communicate with the central nervous system to bring about a behavioral response known as the "sickness syndrome (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008)." This response includes the experience of lethargy and malaise that often accompanies infectious disease. Pro-inflammatory cytokines, in combination with other signaling molecules, also recruit and activate adaptive components of the immune system, such as B and T lymphocytes, which mount a specific immune response to the invading pathogen. Of note, IL-6 plays a key role in directing the switch from innate to adaptive immunity (S. A. Jones, 2005; Naugler & Karin, 2008).

1.1 INFLAMMATION AND PHYSICAL HEALTH RISK

The size and length of the inflammatory response is critical for health and thus is tightly controlled. If the response is insufficient to contain the pathogen, infection can spread systemically and threaten life. If the magnitude of the response is too large, an individual is at

risk for systemic consequences of prolonged inflammation, including persistence of the sickness response (Dantzer et al., 2008). Prolonged inflammatory responses can also increase risk for inflammatory diseases and exacerbate inflammatory pathophysiology. For example, inflammation may contribute to local plaque development and rupture in cardiovascular disease (Hansson & Hermansson, 2011), increasing risk for myocardial infarction and stroke (Mozaffarian et al., 2015). Inflammation also associates with accelerated progression of cancer, HIV, asthma, and rheumatoid arthritis (Choy, 2012; Deeks et al., 2013; Elinav et al., 2013; Fu et al., 2013; McInnes & Schett, 2011; Naugler & Karin, 2008).

1.2 LOCAL AND SYSTEMIC MECHANISMS THAT CONTROL THE INFLAMMATORY RESPONSE

To protect health, the magnitude of the inflammatory response is controlled by local and systemic mechanisms. Locally, activated cells of the innate immune system (e.g. macrophages) have a short life span (Janeway et al., 2005). This ensures that the local inflammatory response is time limited unless the continued presence of pathogens activates new cells. Additionally, activated macrophages release patterns of cytokines that are fine-tuned to coordinate an immune response that will contain and destroy a specific pathogen as quickly as possible. For example, toll-like receptor 4 (TLR4), present in high density on the surface of macrophages, recognizes lipopolysaccharide (LPS/endotoxin), a component of the membrane of gram negative bacteria, and activates a well-characterized biochemical signaling pathway that results in nuclear factor kB (NFkB)-induced transcription of the pro-inflammatory cytokines TNF-α, IL-1, and IL-6 (Akira & Takeda, 2004; Mosser & Edwards, 2008). This signaling pathway stimulates an

adaptive immune response that is fine-tuned to handle bacterial infection. In addition, activated adaptive immune cells, such as T lymphocytes, produce anti-inflammatory cytokines such as IL-4 and IL-10, which act on macrophages to shut down the pro-inflammatory response (Fiorentino, Zlotnik, Mosmann, Howard, & O'Garra, 1991; Janeway et al., 2005). In sum, at the cellular level, limits on the magnitude and length of the innate inflammatory response include programmed cell death, patterns of signaling and cytokine response, and negative feedback in the form of inhibitory cytokines from the adaptive immune system.

At the systemic level, a number of different pathways play a role in controlling the magnitude of the inflammatory response. First, the acute phase inflammatory response is dependent on ongoing stimulation of hepatocytes by IL-1 and IL-6 (Gruys et al., 2005). As the number of activated macrophages declines, so does the circulating level of these mediators, shutting down the acute phase response. A second systemic pathway involved in the downregulation of the inflammatory response involves the HPA axis. Activation of the HPA axis by the binding of circulating pro-inflammatory cytokines (e.g. IL-1 and IL-6) to receptors in the hypothalamus results in peripheral release of glucocorticoids from the adrenal cortex (Dantzer et al., 2007; Schoneveld & Cidlowski, 2007). Glucocorticoids bind to receptors in peripheral immune cells and down-regulate a number of innate immune processes, including blocking lymphocyte activation, modulating leukocyte trafficking, and down-regulating secretion of pro-inflammatory cytokines (Schoneveld & Cidlowski, 2007). Indeed, glucocorticoid receptors have a high density in peripheral blood mononuclear cells (A. H. Miller et al., 1998), and have been exploited for their role in suppression of inflammation, with widespread use of synthetic corticosteroid therapies for the treatment of inflammatory diseases (Boumpas, Chrousos, Wilder, Cupps, & Balow, 1993). Mechanistically, glucocorticoids bind to intracellular glucocorticoid

receptors (GCR), forming a complex that can inhibit pro-inflammatory transcription factors (e.g. NFkB) in the nucleus of the cell (Medzhitov & Horng, 2009). Thus, systemic activation of the HPA axis and binding of glucocorticoid receptors results in decreased production of IL-1, TNF-α, and IL-6 by macrophages (Boumpas et al., 1993; Raison & Miller, 2003). In addition, the acute phase response includes a decrease in production of corticosterioid binding globulin (CBG), which typically binds glucocorticoids in plasma (Gruys et al., 2005). This results in an increase in biologically available glucocorticoids, further promoting the down-regulation of inflammation. In contrast, at the cellular level, IL-1 can maintain the pro-inflammatory phenotype of the macrophage by inhibiting GCR complex translocation to the nucleus (Pariante et al., 1999). Thus, while IL-1 activates both the HPA axis and the acute phase response systemically, it can act locally to downregulate cellular sensitivity to glucocorticoids. In sum, the magnitude of an inflammatory response is tightly controlled by multiple mechanisms at both systemic and cellular levels; a balance necessary to promote and maintain health.

1.3 NON-IMMUNE SOURCES OF SYSTEMIC PRO-INFLAMMATORY CYTOKINES

Immune cells are not the sole source of pro-inflammatory cytokines that are detectable in peripheral circulation. Other sources include contracting muscle cells (myocytes), adipocytes, and epithelial cells that line blood vessels (Fantuzzi, 2005; Hansson, 2005; Steensberg et al., 2002). For example, low levels of exercise associate with increased circulating IL-6 that is derived from myocytes (Steensberg et al., 2002). In addition, high levels of body fat, particularly visceral adiposity, correlate with circulating levels of TNF-alpha and IL-6 (Fantuzzi, 2005; Khaodiar, Ling, Blackburn, & Bistrian, 2004). The exact conditions under which adipocytes

produce inflammatory mediators is unclear; however, both adipocytes themselves and macrophages within adipose tissue are thought to contribute (Fantuzzi, 2005; van Greevenbroek, Schalkwijk, & Stehouwer, 2013). Finally, subclinical cardiovascular disease is also marked by elevated levels of circulating pro-inflammatory cytokines, likely as a result of inflammatory processes taking place in the walls of blood vessels (Hansson, 2005). Taken together, there are multiple sources of pro-inflammatory cytokines that are detectable in peripheral circulation.

Regardless of source, circulating markers of inflammation such as CRP and IL-6 are associated with physical health morbidity and mortality. Morbidities include persistence of the sickness response, increased risk for disease, and exacerbation of diseases in which inflammation plays a role such as cardiovascular disease, asthma, rheumatoid arthritis, HIV, and cancer (Choy, 2012; Dantzer et al., 2008; Deeks et al., 2013; Fu et al., 2013; Ridker & Silvertown, 2008; Shacter & Weitzman, 2002). Thus, it is important to identify modifiable risk factors that may contribute to elevated levels of systemic inflammation.

1.4 CAREGIVING STRESS AND INFLAMMATION

A large literature examines associations between chronic life stressors and circulating markers of inflammation, and to date, findings have been equivocal (Hansel et al., 2010; Lovell & Wetherell, 2011). One commonly studied model of chronic life stress is caregiving for a significant other who is unable to effectively care for themselves (e.g. a partner, child, or parent with illness or disability) (Lovell & Wetherell, 2011). When compared with age-matched individuals who are not caregiving, a number of studies show elevated circulating markers of inflammation (e.g., IL-6 and CRP) among caregivers (Lovell, Moss, & Wetherell, 2012;

Lutgendorf et al., 1999; G. E. Miller et al., 2008; Rohleder et al., 2009; Segerstrom, Schipper, & Greenberg, 2008; Von Kanel et al., 2006); however, not all findings are consistent (Lovell & Wetherell, 2011). Longitudinal studies investigating the impact of caregiving on systemic inflammation show similarly mixed results. For example, Kiecolt-Glaser and colleagues showed increases in circulating levels of IL-6 over a 6 year period among elderly spousal caregivers of Alzheimer's patients when compared with matched non-caregivers (Kiecolt-Glaser et al., 2003). In contrast, Rohleder et al (2009) found no difference in levels of IL-6 across a 1-year period between younger caregivers and non-caregivers, but did show a steeper increase in CRP in the caregiving group. Reasons for inconsistencies between caregiving and inflammation remain unclear; however, it is possible that psychological responses to the caregiving situation moderates the magnitude of the association, with caregivers experiencing heightened levels of distress at elevated risk for systemic inflammation.

The transactional theory of stress posits that the level of distress that an individual experiences in response to a challenging life event is a function of the degree to which he/she appraises the situation as endangering his/her well-being and exceeding his/her resources to cope (Lazarus & Folkman, 1987). Thus, events appraised as a personal threat beyond a person's ability to cope are perceived as stressful, and result in emotional, behavioral, and physical responses (S. Cohen, Kessler, & Gordon, 1995). In support of this theory, studies investigating psychological responses to negative life events demonstrate widespread inter-individual differences in the magnitude and duration of psychological distress. For example, it is estimated that only 8% of the 50-60% of individuals who are exposed to traumatic events develop post-traumatic stress disorder (PTSD) (Vieweg et al., 2006; Yehuda & LeDoux, 2007). Correspondingly, some studies show that individuals who develop PTSD show elevated levels of

IL-1, TNF-a, IL-6, and CRP when compared to those exposed to trauma who do not develop the disorder, although the literature is small and not all studies are consistent (Pace & Heim, 2011). These studies suggest that individuals who respond to traumatic life events with pathological levels of distress show elevated levels of systemic inflammation compared to those without these clinical disorders; however, it does not inform our understanding of more normative variation in psychological response.

Patterns of distress among parents in the year following their child's cancer diagnosis are also consistent with the transactional theory of stress and typically represent subclinical variation in emotional response. Shortly after diagnosis, when levels of appraised threat are high, most parents endorse emotional distress (Patino-Fernandez et al., 2008). However, patterns of distress vary considerably over the subsequent year. For the majority (65-70%), symptoms of distress fall to pre-diagnosis levels within a year; however, a subgroup of about 25-30% of parents exhibit heightened levels of distress throughout this period, including symptoms of anxiety, depression, and post-traumatic stress (Pai et al., 2007; Poder et al., 2008; Svavarsdottir, 2005; Vrijmoet-Wiersma et al., 2008). Thus, levels of distress vary considerably among parents across the first year after their child is diagnosed. However, to date, no studies have examined the association between the distress of caring for a child with cancer and markers of inflammation.

Psychological distress has been defined as a discomforting emotional state experienced in response to a demand that is perceived to be stressful (Ridner, 2004); however, it has been operationalized in various ways within the caregiving literature. For example, studies have assessed psychological distress using measures of perceived stress (e.g. (G. E. Miller et al., 2008; G. E. Miller et al., 2002; G. E. Miller et al., 2014; Rohleder et al., 2009)), depressive symptoms (e.g. (G. E. Miller et al., 2002; G. E. Miller et al., 2014; Rohleder et al., 2009) and/or symptoms

of anxiety (M. Cohen et al., 2002; Provinciali et al., 2004). In addition, some studies use adjective checklists to derive a measure of anxious, depressed, or negative mood states (e.g. (G. E. Miller et al., 2008; G. E. Miller et al., 2002; Vedhara et al., 2002). Importantly, these measures of psychological distress have been primarily used to differentiate caregivers from non-caregivers, with no studies examining the relationship between individual differences in psychological distress and circulating markers of inflammation within caregivers. In addition, no studies have examined the possibility that the individual symptom measures may be tapping the same higher-order construct (Tanaka & Huba, 1984; Veit & Ware, 1983). Accordingly, a primary aim of the current proposal is to examine the association between the shared variance of individual measures of psychological distress and levels of IL-6, over a 12-month period following the major life stressor of having a child diagnosed with cancer **(Aim 1).** It is predicted that change in the measure of distress will positively associate with change in IL-6 over time.

1.5 PATHWAYS THAT LINK LIFE STRESS AND INFLAMMATION

Individual differences in emotional response to life events are thought to affect levels of inflammation indirectly though health behaviors, and/or directly through alterations in biological pathways. One established pathway through which psychological distress is known to impact inflammation is through engagement in risky health behaviors such as smoking, increased alcohol use, decreased physical activity, and disruptions in sleep pattern (Jackson, Knight, & Rafferty, 2010; Whooley et al., 2008). However, individual differences in health risk behaviors do not fully account for the physical health risk associated with chronic psychological distress

(S. Cohen et al., 2007; McEwen et al., 1997). Thus, it is important to consider biological pathways through which chronic psychological distress may modulate inflammatory processes.

1.5.1 The Autonomic Nervous System (ANS)

The ANS is one of the primary physiological pathways linking the central nervous and immune systems and plays a key role in regulating the inflammatory response. The ANS is comprised of two main effector pathways: the sympathetic and the parasympathetic divisions. Both divisions play a role in the modulation of peripheral inflammation (Elenkov, Wilder, Chrousos, & Vizi, 2000; Janig, 2014). Indeed, the parasympathetic nervous system provides bidirectional communication between brain and peripheral immune processes via the vagal nerve, and is important in the down-regulation of inflammation (Martelli, McKinley, & McAllen, 2014; Pavlov & Tracey, 2015). In contrast, prolonged activation of the sympathetic nervous system can function to elevate levels of peripheral inflammation (Bellinger & Lorton, 2014; Elenkov et al., 2000). The current investigation does not focus on the contribution of the ANS to distress-related modulation of inflammation. However, it is acknowledged that chronic psychological distress can impact activation of both divisions of the ANS, with the sympathetic nervous system interacting with the HPA axis in the regulation of peripheral inflammation.

1.5.2 The HPA Axis

As discussed previously, the HPA axis plays a key role in the regulation of peripheral inflammatory responses, with the release of glucocorticoids acting through the GCR to down-regulate transcription of pro-inflammatory cytokines by immune cells. It is widely suggested that

the HPA axis is activated in response to psychological distress related to major life events, and that it is important for mobilization of adaptive metabolic resources (Castro, Elias, Elias, & Moreira, 2011; Schoneveld & Cidlowski, 2007; Tsigos & Chrousos, 2002). The appraisal of life events as stressful is thought to activate central neural systems that result in the production of corticotropin-releasing hormone (CRH) by the paraventricular nucleus (PVN) of the hypothalamus (Castro et al., 2011; Schoneveld & Cidlowski, 2007). CRH travels to the anterior lobe of the pituitary gland, where it stimulates the secretion of adrenocorticotropic hormone (ACTH) into peripheral circulation. ACTH in turn stimulates receptors in the adrenal cortex, resulting in the production and release of glucocorticoids into the blood stream (Castro et al., 2011).

Both animal and human evidence supports activation of the HPA axis in response to chronic stress. For example, mice who are exposed to the chronic stress of frequent social disruption show more than twice the level of circulating glucocorticoids than undisrupted mice (Stark et al., 2001). However, assessment of the relationship between chronic psychological distress and peripheral levels of cortisol in humans is complicated by heterogeneity of measurement techniques, variability in timing of assessment, and poor reliability of measurement. Nevertheless, a meta-analytic review of over 50 years of research investigating chronic stress and cortisol showed that in response to chronic life events (e.g. caregiving), persistent psychological distress is accompanied by a dysregulated pattern of cortisol hormone secretion (G. E. Miller et al., 2007). This overall pattern is marked by lower than normal morning levels of cortisol, but higher than normal secretion throughout the day, resulting in a flattened diurnal rhythm and an overall higher level of cortisol exposure throughout the body (G. E. Miller et al., 2007). However, the magnitude of the association between psychological distress

and cortisol varies significantly across studies, possibly due to individual differences in the nature and perceived controllability of the stressor, specific emotions elicited by the stressor, and/or individual differences in coping (G. E. Miller et al., 2007).

Given that glucocorticoids like cortisol typically function to down-regulate systemic inflammation, one would expect the increase in HPA activation that accompanies chronic stress to associate with decreased peripheral levels of inflammatory markers. Contrary to this expectation, evidence links persistent psychological distress to elevated markers of systemic inflammation, such as IL-1, IL-6, TNF-a, and CRP (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimaki, 2015; Hansel et al., 2010; Pace & Heim, 2011; Rohleder, 2014). In this regard, it is suggested that chronic exposure to cortisol results in a change in the responsivity of the glucocorticoid receptor such that it no longer downregulates pro-inflammatory gene transcription (A. H. Miller, 2008; Raison & Miller, 2003). Possible receptor alterations that could confer reduced sensitivity to cortisol include changes in the number, binding affinity, or functional signaling capacity of the glucocorticoid receptor (Raison & Miller, 2003).

1.6 LIFE STRESS, GLUCOCORTICOID SENSITIVITY, AND INFLAMMATION

Growing evidence suggests that reduced sensitivity of immune cells to glucocorticoids may accompany chronic life stress. Functionally, reduced glucocorticoid sensitivity can be assessed *in vitro* by isolating cells from peripheral blood and examining pro-inflammatory cytokine production in response to an immune stimulant (LPS) in the presence of increasing concentrations of exogenous cortisol. Individuals who have cells that show a marked reduction in pro-inflammatory cytokine release in the presence of cortisol are thought to be glucocorticoid

sensitive. In contrast, individuals who show little to no reduction in pro-inflammatory cytokines in the presence of cortisol are thought to show reduced glucocorticoid sensitivity (i.e. glucocorticoid resistance). In animals, models of chronic social disruption provide longitudinal evidence that psychosocial stress is associated with elevated levels of glucocorticoids, increased systemic inflammation (e.g. IL-6), and functional glucocorticoid resistance (Quan et al., 2003; Stark, Avitsur, Hunzeker, Padgett, & Sheridan, 2002; Stark et al., 2001). Indeed, isolated immune cells from the stressed mice show functional glucocorticoid resistance (Stark et al., 2001). This effect has been shown to be specific to macrophages, and has been associated with decreased nuclear translocation of the glucocorticoid complex, as well as failure to block pro-inflammatory NFkB-mediated transcription in these cells (Quan et al., 2003; Stark et al., 2001). Concomitantly, these mice show elevated levels of glucocorticoids and pro-inflammatory cytokines in the periphery (Stark et al., 2001, 2002). Because pro-inflammatory cytokines stimulate the HPA axis, it is possible that elevated levels of inflammation could drive the prolonged release of glucocorticoids, rather than chronic activation of the stress pathway. However, mice subjected to chronic stress develop glucocorticoid resistance in the absence of systemic IL-6 (i.e. in IL-6 knock-out mice), providing evidence that the development of glucocorticoid resistance is independent of systemic levels of IL-6 (Stark et al., 2002).

In humans, cross-sectional evidence shows that immune cells from caregivers reporting chronic psychological distress show greater glucocorticoid resistance compared to non-caregivers (Bauer et al., 2000; G. E. Miller et al., 2002). For example, in a cross-sectional study of 49 elderly caregivers of dementia patients, distressed caregivers showed reduced lymphocyte sensitivity to cortisol compared to age-matched non-caregivers at an average of 3.5 years post dementia diagnosis (Bauer et al., 2000). Relevant to the current proposal, Miller et al. (2002)

investigated 25 distressed parental caregivers of cancer patients at the Children's Hospital of Pittsburgh (CHP). In this study, peripheral blood mononuclear cells from parents of cancer patients showed reduced sensitivity to cortisol as assessed by IL-6 production, compared to 25 parents of medically healthy children matched on age, gender, ethnicity, and marital status (G. E. Miller et al., 2002). Importantly, parents in this study were evaluated at an average of 9.6 months post diagnosis, with a standard deviation of 9.5 months and a range of 1-35 months. Thus, this cross-sectional study does not permit examination of the timing in development of glucocorticoid resistance. Interestingly, the researchers found no association between frequency of depressive symptoms and glucocorticoid resistance; however, this could be due to the small sample size, the wide range in timing of assessment post diagnosis, and/or the use of only one measure of psychological distress.

To date, two studies have examined both glucocorticoid resistance and inflammation in distressed caregivers. Rohleder et al. (2009) showed a trend for glucocorticoid resistance in 18 caregivers compared to 19 age-matched non-caregivers over approximately one year, with caregivers showing significant increases in CRP over the same time period. While this study was longitudinal in nature, the initial assessment of caregivers was more than three months after the onset of the stressor, and the group sizes were small (Rohleder et al., 2009). A more recent study found no evidence of functional glucocorticoid resistance in 33 caregivers compared to 47 age-matched non-caregivers, and no differences in CRP; however, the researchers presented evidence of downregulation in glucocorticoid complex signaling in monocytes of caregivers compared to non-caregivers (G. E. Miller et al., 2014). Although this study design was longitudinal, participant attrition prevented longitudinal analyses, and thus these results were based on aggregated group differences collapsed across assessments. Collectively, studies in humans

provide initial evidence for glucocorticoid resistance among chronically distressed caregivers, with some support for the possibility that resistance is accompanied by elevated levels of systemic inflammation. However, available studies are small, largely cross-sectional, and fail to examine associations between psychological distress, glucocorticoid sensitivity, and systemic inflammation. Indeed, to date, no caregiving studies have examined the proposed association between changes in glucocorticoid sensitivity and levels of peripheral inflammation.

Together, evidence from animal and humans studies suggests that changes in glucocorticoid resistance are driven by chronic activation of stress pathways, including the HPA axis, resulting in elevated levels of peripheral inflammation. However, to date, no human studies have provided prospective evidence of the development of glucocorticoid resistance from the onset of a stressor, or examined prospective associations with psychological distress and/or inflammation. Based on the evidence presented here, we propose that prolonged emotional arousal in response to caregiving drives activation of peripheral stress pathways, resulting in peripheral release of cortisol and activation of the immune response. In addition, we suggest that macrophages adapt to chronically heightened cortisol levels by downregulating the anti-inflammatory glucocorticoid receptor response, thereby promoting chronic inflammation. Accordingly, a primary aim of this project was to examine the association between psychological distress and the development of glucocorticoid resistance over time among mothers confronting the stress of having a child newly diagnosed with cancer **(Aim 2)**. It was hypothesized that change in distress will positively associate with change in glucocorticoid resistance over time. In addition, given the role of the GCR in regulation of transcription of pro-inflammatory cytokines by immune cells, an aim of this proposal was to investigate associations between down-regulation of GCR sensitivity and levels of IL-6 over time **(Aim 3).** It was hypothesized that

changes in glucocorticoid resistance would positively associate with change in IL-6 over time. Finally, if indeed psychological distress predicted glucocorticoid resistance **(Aim 2)**, and glucocorticoid resistance contributed to the control of systemic inflammation **(Aim 3)**, it was possible that glucocorticoid resistance was a mediator of the relationship between distress and inflammation **(Aim 4)**. It was hypothesized that change in glucocorticoid resistance would at least partially mediate the relationship between change in psychological distress and IL-6.

1.7 THE CURRENT STUDY

The course of childhood cancer has dramatically changed in recent decades with current estimates showing that 83% of affected children will become long term survivors (American Cancer Society, 2015). As such, the focus of psychosocial care for children with cancer has turned to coping with a chronic disease with an uncertain outcome. Caring for a child with cancer is a particularly provocative stressor that can threaten parental identity, family structure, and quality of life (B. L. Jones, 2012; Svavarsdottir, 2005). While most mothers cope well with this life event, 25-30% of mothers show prolonged symptoms of psychological distress (Pai et al., 2007; Poder et al., 2008; Svavarsdottir, 2005; Vrijmoet-Wiersma et al., 2008). It is these mothers in particular who are thought to be at the greatest risk for immune-mediated physical health outcomes.

Indeed, while there exists mixed evidence regarding the association between caregiving and peripheral inflammation (Lovell & Wetherell, 2011), it is hypothesized that these heterogeneous associations may be a function of differences in psychological distress response. In addition, the association between caregiving distress and inflammation could be mediated by

immune adaptations to the activation of stress systems, such as the HPA axis. Indeed, chronic life events such as caregiving associate with persistent psychological distress, prolonged HPA axis activation, and elevated peripheral levels of cortisol (G. E. Miller et al., 2007). It is this prolonged release of cortisol that is thought to drive the downregulation of glucocorticoid sensitivity in macrophages and concomitant elevations in peripheral inflammation (A. H. Miller, 2008). With regard to mothers whose children have been diagnosed with cancer, preliminary work shows evidence of glucocorticoid resistance in these parents at average of 9 months following their child's diagnosis (G. E. Miller et al., 2002). This project aimed to extend this work by examining prospective relationships between psychological distress, glucocorticoid resistance, and peripheral inflammation among mothers at 3 time points across the 12 months after their children have been diagnosed with cancer.

1.8 EXPLORATORY ANALYSIS

Much of the literature investigating psychological distress and inflammatory outcomes has been cross-sectional in nature, which raises issues regarding interpretation of the directionality of the association. It is widely assumed that chronic psychological distress activates behavioral and biological pathways that modulate inflammation. However, it is also possible that elevated inflammation acts via afferent pathways to influence negative affect. For example, as discussed with regard to inflammation, circulating pro-inflammatory cytokines can induce feelings of malaise and decreased mood associated with the sickness response (Dantzer et al., 2008). When inflammation is chronic, it is proposed to lead to a biologically induced depression (Loftis, Huckans, & Morasco, 2010; Loftis et al., 2013). Thus, while sustained

negative emotional reactions to stress may result in elevations in inflammatory cytokines, these cytokines may also in turn intensify negative affect. Thus, exploratory analyses examined the reciprocal effects between distress and inflammation across time in mothers caring for a child diagnosed with cancer **(Exploratory Aim)**. It is also possible that the association between psychological distress and inflammation may relate to third factors, such as genetic vulnerability, early life adversity, prior psychiatric history, trait characteristics of emotional responding (e.g. neuroticism), and social isolation (Hansel et al., 2010; Rohleder, 2014); however, these factors will not be considered in this project.

2.0 RESEARCH DESIGN AND METHODS

2.1 PARTICIPANTS

One-hundred and thirty one English-speaking biological, adoptive or legal-guardian primary caregivers of a child newly diagnosed with were recruited from the Division of Hematology and Oncology, Children's Hospital of Pittsburgh (CHP) between October, 2010 and February, 2014 to take part in a randomized controlled study designed to examine the efficacy of a supportive stress management intervention (**See Appendix A for Consort Diagram**). Participants were randomly assigned to a usual care control group (N = 67) or an intervention group (N = 64). After mothers provided consent for participation, the baseline assessment was conducted. Research assistants who collected data were blinded to intervention group assignment. Eleven caregivers did not complete the baseline assessment (N = 7 usual care; N = 4 intervention), resulting in a sample of 120 mothers at T1. The usual care group (N = 60) received regular support care that is provided at CHP for families of children diagnosed with cancer, including access to clinical social workers. The intervention group (N = 60) received 6 sessions of psychoeducational and practical intervention in stress management over the course of 3 months.

Eleven mothers did not complete the assessment at the end of the intervention (N = 4 usual care; N = 7 intervention), resulting in a total of 109 mothers assessed at T2. A total of six

mothers were lost at the six-month follow-up time point (N = 4 usual care; N = 2 intervention), resulting in 103 mothers assessed at T3 (**See Appendix A for Consort Diagram**). Participants who did not complete the study were not significantly different than those who completed the study on any of the following variables: age, education, race, intervention group, the child's treatment intensity, BMI, distress measures at T1, levels GCR at T1, or circulating levels of IL-6 at T1 (**Table 1**).

Table 1. Comparison of subjects who completed the study to those who did not

	Completed study (n =103) mean ± SD	**Dropped/Lost (n = 17) mean ± SD**	***p***	**Total Sample (n = 120) mean ± SD**
Age (yrs)	36.0 ± 8.0	35.5 ± 7.7	.791	35.9 ± 8.0
Education (yrs)	14.0 ± 2.0	14.2 ± 2.7	.611	14.0 ± 2.1
Race (%Caucasian, W/AA+other)	86, 89/14	82, 14/3	.657	86, 103/17
Intervention Group (%Intervention, Int/Control)	50, 51/52	53, 9/8	.793	50, 60/60
Treatment Intensity (%most, mild/moderate/very/most)	15, 0/31/57/15	19, 0/3/10/3	.634	15, 0/34/67/18
BDI T1	18.2 ± 11.9	19.9 ± 10.6	.579	18.4 ± 11.7
STAIS-S T1	50.4 ± 13.9	47.1 ± 13.7	.368	50.0 ± 13.9
IES T1	33.2 ± 15.2	32.8 ± 14.1	.368	33.2 ± 15.0
BMI T1 (kg/m^2)	29.4 ± 7.9	26.0 ± 4.8	.919	29.0 ± 7.6
GCR AUC T1 (pg-µmol/mL^2)	$1.7x10^6$ ± $1.6x10^6$	$3.6x10^6$ ± $2.5x10^6$	.128	$1.9x10^6$ ± $1.8x10^6$
GCR AUCwbcs T1 (pg-nmol/cell-L)	3.7 ± 3.2	10.1 ± 6.3	.054	4.4 ± 4.2
cIL6 T1 (pg/mL)	1.44 ± 1.50	0.97 ± 0.87	.221	1.38 ± 1.44

Notes: For continous variables, independent samples t-tests were performed; for categorical variables, $\chi 2$-test was performed; W = White; AA = African American; Int = Intervention; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State version; IES = Impact of Events Scale; BMI = Body Mass Index; GCR = glucocorticoid resistance; AUC = area under the curve; AUCmonos = area under the curve adjusted for concentration of monocytes; cIL-6 = circulating levels of interleukin-6

Circulating inflammatory data was eliminated from the data set and treated as missing for individuals reporting a current diagnosis of an immune related disease and current medication use for that diagnosis (N = 7 usual care; N = 6 intervention). One individual in this group was also lost to follow-up at the six-month post-intervention time point. These individuals were not significantly different from individuals with included immune data on any of the following variables: education, race, BMI, intervention group, the child's treatment intensity, circulating IL-6 values at any of the three time points, or GCR at any of the three time points; however, they were significantly older than individuals with measured values (**Table 2**).

Table 2: Comparison of subjects with and without valid inflammation data

	Those with included IL-6 data (n =101) mean ± SD	**Those with excluded IL-6 data (n = 19) mean ± SD**	***p***	**Total Sample (n = 120) mean ± SD**
Age (yrs)	34.9 ± 7.3	41.4 ± 8.3	.001	35.9 ± 8.0
Education (yrs)	14.0 ± 2.2	13.8 ± 1.9	.652	14.0 ± 2.1
Race (%Caucasian, W/AA+other)[a]	85, 90/16	92, 12/1	.225	86, 103/17
Intervention Group (%Intervention, Int/Control)[a]	50, 58/59	46, 6/7	.612	50, 60/60
Treatment Intensity (%most, mild/moderate/very/most)	14, 31/60/15	23, 3/7/3	.211	15, 34/67/18
BMIavg (kg/m^2)	29.1 ± 7.7	31.1 ± 5.1	.565	29.2 ± 7.6
cIL-6 Time 1	1.93 ± 3.48	1.17 ± 0.58	.625	1.90 ± 3.40
cIL-6 Time 2	1.80 ± 2.64	2.19 ± 1.59	.743	1.82 ± 2.59
cIL-6 Time 3	2.70 ± 3.58	2.56 ± 1.68	.938	2.69 ± 3.50
GCR AUC T1 (pg-µmol/mL^2)	$1.9x10^6 \pm 1.8x10^6$	$1.7x10^6 \pm 1.1x10^6$	.835	$1.9x10^6 \pm 1.8x10^6$
GCR AUC T2 (pg-µmol/mL^2)	$2.1x10^6 \pm 1.7x10^6$	$2.3x10^6 \pm 2.0x10^6$	.818	$2.2x10^6 \pm 1.7x10^6$
GCR AUC T3 (pg-µmol/mL^2)	$2.5x10^6 \pm 1.3x10^6$	$2.4x10^6 \pm 3.1x10^5$	.953	$2.5x10^6 \pm 1.3x10^6$
GCR AUCwbcs T1 (pg-nmol/cell-L)	4.5 ± 4.3	3.5 ± 2.0	.666	4.42 ± 4.17
GCR AUCwbcs T2 (pg-nmol/cell-L)	4.8 ± 3.9	5.8 ± 4.1	.630	4.88 ± 3.92
GCR AUCwbcs T3 (pg-nmol/cell-L)	not enough data	not enough data	-	

Notes: For continuous variables, independent samples t-tests were performed; for categorical variables, $\chi 2$-test was performed W = White; AA = African American; Int = Intervention; BMIavg = average Body Mass Index across all 3 time points; GCR = glucocorticoid resistance; AUC = area under the curve; AUwbcs = area under the curve adjusted for concentration of white blood cells; cIL-6 = circulating levels of interleukin-6

Because structural equation modeling can handle missing data, all cases were included in the analyses. For a summary of sample variables, see **Table 3**. We used all available data to estimate models using full information maximum likelihood (FIML).

Table 3: Means and Standard Deviations for all variables

	Measure	N	Mean	SD
1	Age (yrs)	120	35.94	7.95
2	Education (yrs)	120	13.99	2.12
3	BMIavg (kg/m^2)	106	29.12	7.56
4	Race (%Caucasian, W/AA+other)	120	86, 103/17	
5	Intervention group (%I, I/C)	120	50, 60/60	
6	Treatment Intensity (%most, mild/moderate/very/most)	119	15, 0/34/67/18	
7	Months Since Diagnosis T1	120	1.84	0.82
8	BDI T1	116	18.42	11.69
9	STAI-S T1	117	49.98	13.89
10	IES T1	118	33.17	15.01
11	Glucocorticoid Resistance AUC T1 (pg-μmol/mL^2)	56	$1.9x10^6$	$1.8x10^6$
12	White Blood Cell Count T1 (10^9cells/L)	55	6.86	1.99
13	Glucocorticoid Resistance AUCwbcs T1 (pg-nmol/cell-L)	52	4.42	4.17
14	BMI T1(kg/m^2)	105	28.98	7.61
15	cIL6 T1 (pg/mL)[a]	98	1.39	1.47
16	Months Since Diagnosis T2	102	6.81	1.94
17	BDI T2	96	17.55	12.76
18	STAI-S T2	97	44.69	15.45
19	IES T2	97	27.13	16.82
20	Glucocorticoid Resistance AUC T2 (pg-μmol/mL^2)	62	$2.2x10^6$	$1.7x10^6$
21	White Blood Cell Count T2 (10^9cells/L)	54	7.12	2.05
22	Glucocorticoid Resistance AUCwbcs T2 (pg-nmol/cell-L)	54	4.88	3.92
23	BMI T2 (kg/m^2)	84	30.06	8.08
24	cIL6 T2 (pg/mL)[a]	80	1.43	1.23
25	Months Since Diagnosis T3	97	12.74	2.20
26	BDI T3	85	13.64	12.14
27	STAI-S T3	86	39.67	15.32
28	IES T3	87	23.09	17.98
29	Glucocorticoid Resistance AUC T3 (pg-μmol/mL^2)	47	$2.5x10^6$	$1.3x10^6$
30	White Blood Cell Count T3 (10^9cells/L)	38	7.08	1.60
31	Glucocorticoid Resistance AUCwbcs T3 (pg-nmol/cell-L)	38	5.74	3.22
32	BMI T3 (kg/m^2)	75	29.63	7.56
33	cIL6 T3 (pg/mL)[a]	68	2.05	1.78

Notes: BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State version; IES = Impact of Events Scale; AUC = Area under the curve; AUCmonos = Area under the curve adjusted for concentration of monocytes; AUCwbcs = Area under the curve adjusted for concentration of white blood cells; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

2.2 PROCEDURES

2.2.1 Protocol Overview

Participants were assessed at baseline (0 to 6 weeks post child diagnosis) (T1), at the end of the intervention (approx. 5-6 months post T1) (T2), and 6 months post T2 (T3) (approx.12-15 months post diagnosis). At the time of these three assessment visits, participants were seen by a research nurse who administered a medical history and medication use interview, and drew a blood sample. In addition, participants were given questionnaires to complete and return within 3 weeks via mail, or to the study center/doctor's office to be picked up by the research nurse.

2.2.2 Blood Draw

Participants were instructed to limit physical activity and alcohol intake for 24 hours and caffeine intake for 3 hours before blood draw, participants were screened for these parameters as well as for recent or present symptoms of acute illness as part of the medical history and medication use interview. Blood was not drawn from participants who (1) showed signs or

symptoms of acute illness in the prior 2 weeks (e.g. Cold or flu, including taking prescription antibiotics or over the counter cold medication), (2) showed signs or symptoms of current allergies, or (3) had received a vaccination in the prior 2 weeks. These participants were rescheduled when symptoms resolved or 2 weeks post vaccination. While blood draw was not taken at the same time of day for all participants, to the extent possible, all visits were scheduled at the same time of day within individuals to control for diurnal variations in systemic markers of inflammation. The research nurse drew 3 x 10 mL heparinized tubes of whole blood to be used for analysis of circulating levels of IL-6 and glucocorticoid sensitivity.

2.3 MEASURES

2.3.1 Subject Characteristics

The following variables were self-reported by participants: age, sex, race, and highest educational level completed (an indication of socioeconomic status). Participants' weight and height was taken before each blood draw and Body Mass Index (BMI), a measure of body fat, was calculated (weight in kilograms (kg)/height in meters squared (m^2) (kg/m^2)).

Information regarding the child's disease type and stage, severity of the illness, treatment protocol, compliance with treatment, and response to treatment was assessed by a study physician who completed the Intensity of Treatment Rating Scale for each child (Werba et al., 2007). This scale contains seven questions developed specifically for pediatric cancer diagnoses and categorizes treatment intensity into four groups, from least to most intensive, on the basis of

treatment duration, side effects, and recovery time. This measure has shown high inter-rater reliability (r = 0.87) and content validity (r = 0.95) (Werba et al., 2007).

2.3.2 Psychological Measures

Beck Depression Inventory (BDI-II). The BDI-II is a 21-item measure of depressive symptoms in the past two weeks that reflects DSM-IV diagnostic criteria for depression (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). Coefficient alpha estimates of internal reliability for this sample were .93, .95, and .95 for T1, T2, and T3 respectively. The test-retest reliability over the period of one week has been reported to be .93 (Arbisi & Farmer, 2001). Concurrent validity has also been demonstrated, with BDI showing a correlation with clinical ratings (r = .60) and other measures of depression (e.g., Hamilton Psychiatric Rating Scale for Depression; r = .74) (Beck, Steer, & Carbin, 1988).

State-Trait Anxiety Inventory-State (STAI-S). The STAI-S consists of a 20-item measure of state anxiety at the present moment (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The coefficient alpha estimates for this sample were .94, .96, and .96 for T1, T2, and T3 respectively. It is notable that reliability coefficients (e.g. Chronbach's α) are typically found to be higher for the STAI-S when given under conditions of psychological distress (Spielberger et al., 1983). Construct validity for the STAI-S has been demonstrated in college students; scores were higher under examination conditions, and significantly lower after relaxation training (Spielberger et al., 1983).

Impact of Events Scale (IES). The IES is a 15-item measure that assesses the psychological impact of traumatic experiences including two subscales that assess symptoms of intrusion and avoidance related to the event experienced in the past week. Coefficient alpha

estimates for this sample were .85, .89, and .93 for T1, T2, and T3 respectively. Test-retest reliability for the two subscales has been reported to be .87 and .79, respectively, and correlation between the scales has been reported to be 0.41 (Sundin & Horowitz, 2002). Although the IES is not a measure of PTSD, several studies have shown that the IES can discriminate between people with severe and mild stress reactions (Sundin & Horowitz, 2002).

2.3.3 Biological Measures

Systemic Inflammation. Plasma IL-6 levels were determined from harvested plasma samples frozen at -80°C, and analyzed in batches. IL-6 levels were assessed in duplicate using a high sensitivity quantitative sandwich enzyme immunoassay kit (R&D Systems, Cat # HS600B) according to manufacturer's directions. The coefficient of variability (CV) between duplicates was < 10%, or the samples were re-run. The average intra-assay CV was 7.5% and the inter-assay CV was 8.47%. The range of detection for the assay was 0.156 – 10pg/mL.

Glucocorticoid Resistance (GCR). Whole blood drawn was diluted 10:1 with saline (.9% NaCl) and incubated with increasing concentrations of cortisol in the presence of LPS for 18 hours. One positive (stimulated) control containing LPS, but no cortisol, and one negative (unstimulated) control containing neither cortisol nor LPS were included. Final cortisol concentrations were 276, 27.6, 2.76, 0.276, 0.0276 nmol/L. Final concentration of LPS was 2.5ng/mL. Following incubation, supernatants were harvested and stored at -80^{0}C. Levels of IL-6 in these supernatants were assessed using an enzyme-linked immunosorbant assay (BD Cat # 555220). The coefficient of variability (CV) between duplicates was < 10%, or the samples were re-run. The average intra-assay CV was 3.43%, and the inter-assay CV was 14.5%. The range of detection for the assay was 3.91pg/mL – 300pg/mL. The dose-response curve for each subject

was determined by plotting the cortisol concentration (0-276 nmol/L) on the X-axis and the total IL-6 measured for each sample (pg/mL) on the Y-axis. Area under the curve (AUC) was then calculated according to S. Cohen et al. (2012) by first subtracting the unstimulated control from all values (including the stimulated control), and then using GraphPad Prism version 6.07 (GraphPad Software Inc., La Jolla, Ca) to calculate AUC according to the trapezoidal method, with zero as ground (Pruessner et al., 2003). Note that a larger AUC corresponds to greater levels of IL-6 across cortisol concentrations, and thus increased glucocorticoid resistance.

Because stress has been shown to alter the composition of immune cells in peripheral circulation, specifically increasing the number of leukocytes and modifying the proportion of lymphocytes and monocytes (Dhabar 2014; Segerstrom 2004), and because cytokine gene expression profiles vary across cell populations (Irwin & Cole, 2011), AUC was also calculated for IL-6 values corrected for concentration of monocytes or white blood cells in peripheral circulation. For this, we conducted a complete blood count with differential at each study visit (Laboratory Services, CHP; Pittsburgh, PA). AUC was then re-computed using cortisol concentration (0-276 nmol/L) on the X-axis and IL-6 concentrations divided by the concentration of monocytes or white blood cells (pg/cell) on the Y-axis. Results were similar after controlling for monocytes and white blood cells; thus, we only report results correcting for white blood cells.

2.4 ANALYTIC PLAN

Analyses were performed using SPSS Version 22 (SPSS Inc., Chicago, IL) and Mplus Version 7.4 (Muthen & Muthen, Los Angeles, CA). Outliers were assessed using the leverage statistic (h_{ii}), a measure of the distance for each case from the central distribution of the variable.

Outliers were considered to be influential if h_{ii} was greater than 3(k+1)/n, where k = # of variables (1), and n = # of cases (Belsley, Kuh, and Welsch, 1980). For IL-6 and GCR, there were univariate outliers with influential leverage statistics that were eliminated from the data set at each time point and treated as missing. At T1, there were 3 outliers for IL-6 with a mean value of 19.79 pg/mL (range [17.86-22.86 pg/mL]; mean h_{ii} = 0.28, range [.22-37]). For GCR, one outlier for with influential leverage statistics was eliminated from the data set at T1 ($9.7x10^6$ pg-μmol/mL^2; h_{ii} = 0.26). At T2, there were 2 outliers for IL-6 with a mean value of 16.42 pg/mL (range [14.42-18.42 pg/mL]; mean h_{ii} = 0.40; range [.29-.50]). At T3, there were 3 outliers for IL-6 with a mean value of 17.40 pg/mL (range [15.43-19.81 pg/mL]; mean h_{ii} = 0.26; range [.19-.34]). These outliers together accounted for 6 individuals; 3 of these individuals had high values at 2 time points.

The data were checked for normality at each time point. The data was considered normally distributed if the skewness was +/- 3.0 and kurtosis was within +/- 10.0 (Kline, 2015). Values for circulating IL-6 were found to have excessive skew (T1: 4.57; T2: 4.79; T3: 3.41) and kurtosis (T1: 22.69; T2: 25.87; T3: 12.34), and were natural log transformed for analyses (after transformation, skew [T1: 0.69; T2: 0.60; T3: 0.82]; kurtosis [T1: -0.08; T2: 1.03; T3: 0.42]). Categorical variables were coded as follows: 1) for race, Caucasian = 0 and all others were coded as 1; 2) for children's treatment intensity, the least intensive treatment = 0, moderate = 1, very intensive = 2, most intensive = 3; 3) for intervention group, the control group = 0, and intervention = 1.

Dropout analyses and group comparisons were performed using independent samples t-tests and one-way analysis of variance for continuous variables, and $\chi 2$-tests for categorical and dichotomous variables. All structural equation models used maximum likelihood (ML)

estimation. Data were assumed to be missing at random (MAR) and full information maximum likelihood (FIML) was used in model analyses to accommodate missing data. Preliminary analyses examined correlations between the control variables (age, education, race, treatment intensity, and BMI) and the variables of interest (distress measures, glucocorticoid resistance (GCR), and inflammation (IL-6)). Initial models examined the independent and dependent variables of interest only, while subsequent models examined the relationship between the variables of interest, independent of all control variables.

The possibility that the measures of distress loaded on one factor was explored. First, bivariate correlations among the independent measures (IES, STAI-S, and BDI-II) were examined (**See Appendix B**). Next, confirmatory factor analysis was performed at each time point and the unstandardized indicator loadings were examined for consistency across time (**Table 7**). Finally, measurement invariance was tested across time points (**Table 8**).

2.4.1 Models for Absolute Change in Mean

We used latent difference score models to examine mean change in distress, GCR, and IL-6 over time. For distress, we used an effects coding identification approach: the factor variance was a weighted function of the covariance of the indicators, and the factor mean was equal to a weighted average of the observed indicator means. For GCR and IL-6, we used single indicator latent constructs, with the loading set to one and the error variance fixed at the value for the inter-assay CV (GCR: 0.145; IL-6: 0.085). For all models, the autoregressive paths between time points were set to one and the means and variances at the second two time points were set to zero. At the second two time points, a latent factor representing the difference score was specified with the loading set to one. In this way, the latent difference score represents the

average change between time points and the variance represents the variability in the individual rates of change. Covariances were estimated between the initial time point and the latent variable for the differences scores, and the latent difference scores were allowed to covary with each other. For model specification, see **Figures 1, 2,** and **3**. To determine whether mean differences between time points were significant, unconstrained models were compared to models in which the difference score means were constrained to zero.

Simultaneous latent difference score models were used to examine the association between changes in the following pairs of constructs: change in distress and change inflammation (**Aim 1**), change in distress and change in glucocorticoid resistance (**Aim 2**), and change in glucocorticoid resistance and change in inflammation (**Aim 3**). For this, we used the latent difference score models described previously, with the difference score of the outcome variable regressed on the difference score for the predictor variable between complementary time points. Control variables were regressed on each latent difference score factor. In order to alleviate listwise deletion of data from exogenous covariates with missing data (BMI and treatment intensity), we used single indicator latent constructs, with the loading set to one and the error variance fixed at zero. For model specification, see **Figures 4, 5,** and **6**. To determine whether the path coefficient between difference score factors was significant, the unconstrained model was compared to a model in which these paths were constrained to zero.

2.4.2 Exploratory Analysis

To assess whether change in distress had effects on the level of IL-6 at the following time point, and whether change in IL-6 had effects on the level of distress at the following time point, we added cross-lagged paths between the latent difference score factor(s) and the variable(s) at

the following time point (See **Figure 7**). To investigate whether any of these effects were significantly different than zero, an unconstrained model was compared to a model in which the predictive paths were constrained to zero.

3.0 RESULTS

3.1 SAMPLE CHARACTERISTICS

Subjects were on average aged 36 ± 8 years (range: 19-57 years), primarily Caucasian (86%), and had an average of 14 years education (**Table 3**). The children received a range of cancer treatment intensities, with 15% receiving the most intensive treatment (**Table 3**). Bivariate correlations revealed significant associations between older age and the following variables: higher educational attainment, lower levels of distress at T1 and T2 (BDI, IES), higher BMI, and lower levels of circulating levels of IL-6 (**Appendix B**). Similarly, there were significant bivariate correlations between higher educational attainment and the following variables: lower levels of all three indicators of distress at T2 and T3, lower circulating levels of IL-6, higher levels of GCR at T1, and lower levels of GCR at T2 and T3 (**Appendix B**). One-way analysis of variance revealed a significant difference in age and educational attainment by race, with Caucasians showing an older mean age and more years of education when compared to other races (**Table 4**). Chi-square analyses revealed a significant difference among races by treatment intensity, with Caucasians having a greater proportion of children with the second to highest treatment intensity on our rating scale, and African Americans and other races having a greater proportion of children with the highest intensity treatment on our rating scale (**Table 5**). For treatment intensity, one-way analysis of variance revealed a significant difference in IL-6

across the three time points, with an inverse association between treatment intensity and level of IL-6, such that the lowest concentration of IL-6 corresponded to individuals in the highest treatment level (**Table 6**). Higher BMI was associated with older age, greater children's cancer treatment intensity, higher GCR at T2, lower GCR at T3, and greater circulating levels of IL-6 (**Appendix B**). Given significant associations between the control variables and the independent and dependent variables of interest, all models controlled for age, education, race, and the child's cancer treatment intensity. In addition, BMI was included as a covariate in all models that included IL-6 and/or GCR. Intervention group was explored as a standard covariate after initial models were examined and will be presented after initial model descriptions.

Table 4: One-Way Analysis of Variance by Race

	Caucasian/White		Non-Caucasian/White		
	Mean	sd	Mean	sd	*p*
Age (yrs)	36.63	7.63	31.79	8.79	.019
Education (yrs)	14.17	2.02	12.94	2.41	.027
BMIavg (kg/m^2)	29.34	7.97	28.16	5.19	.581
Months Since Diagnosis T1	1.17	0.85	1.27	0.76	.704
BDI T1	18.24	11.30	19.47	14.10	.691
STAI-S T1	49.68	14.12	51.88	12.57	.560
IES T1	32.78	14.93	35.63	15.77	.484
Glucocorticoid Resistance AUC T1 ($pg\text{-}\mu mol/mL^2$)	$1.95x10^6$	$1.58x10^6$	$1.56x10^6$	$1.27x10^6$	.572
Glucocorticoid Resistance AUCwbcs T1 (pg-nmol/cell-L)	4.43	4.19	4.39	4.38	.980
BMI T1(kg/m^2)	29.17	7.96	27.84	5.03	.533
cIL6 T1 (pg/mL)[a]	1.38	1.50	1.37	1.05	.468
Months Since Diagnosis T2	6.68	1.99	7.75	1.35	.076
BDI T2	17.89	12.60	15.17	14.20	.492
STAI-S T2	45.05	15.19	42.38	17.52	.566
IES T2	27.58	16.59	24.23	18.69	.507
Glucocorticoid Resistance AUC T2 ($pg\text{-}\mu mol/mL^2$)	$2.08x10^6$	$1.58x10^6$	$2.60x10^6$	$2.34x10^6$	.398
Glucocorticoid Resistance AUCwbcs T2 (pg-nmol/cell-L)	4.75	3.96	5.60	3.84	.578
BMI T2 (kg/m^2)	30.34	8.45	28.58	5.65	.474
cIL6 T2 (pg/mL)[a]	1.50	1.34	1.37	0.53	.679
Months Since Diagnosis T3	12.71	2.27	13.07	1.20	.610
BDI T3	14.19	12.25	9.91	11.20	.278
STAI-S T3	39.65	15.63	39.82	13.67	.974
IES T3	23.17	18.15	22.55	17.54	.915
Glucocorticoid Resistance AUC T3 ($pg\text{-}\mu mol/mL^2$)	$2.50x10^6$	$1.37x10^6$	$2.47x10^6$	$1.38x10^6$	.968
Glucocorticoid Resistance AUCwbcs T3 (pg-nmol/cell-L)	5.83	3.24	4.72	3.36	.576
BMI T3 (kg/m^2)	29.51	7.91	30.42	4.93	.726
cIL6 T3 (pg/mL)[a]	2.12	1.89	1.84	0.52	.647

Notes: [a]Race was recoded into a categorical variable (Caucasian/White=0, Non-Caucasian/White=1) due to a small non-white sample; BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State version; IES = Impact of Events Scale; AUC = Area under the curve; AUCmonos = Area under the curve adjusted for concentration of monocytes; AUCwbcs = Area under the curve adjusted for concentration of white blood cells; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

Table 5: Cross-Tabulation of Treatment Intensity by Race

	Race			
Treatment Intensity	Caucasian/White	African American/other	χ^2	*p*
moderately intensive treatment	29	5	6.893	.032
very intensive treatment	61	6		
most intensive treatment	12	6		

Table 6: One-way Analysis of Variance by Treatment Intensity

	Moderately Intensive		Very Intensive		Most Intensive		
	Mean	sd	Mean	sd	Mean	sd	*p*
Age (yrs)	33.36	6.10	37.25	7.61	36.32	11.07	.065
Education (yrs)	13.47	1.81	14.37	2.07	13.56	2.66	.083
BMIavg (kg/m^2)	30.40	8.54	29.26	7.62	26.77	5.24	.326
Months Since Diagnosis T1	1.13	0.75	1.19	0.91	1.27	0.77	.864
BDI T1	18.48	12.66	18.65	11.46	17.41	11.60	.929
STAI-S T1	49.38	15.30	50.62	13.76	48.71	12.43	.846
IES T1	28.97	14.52	34.15	14.56	37.94	16.87	.098
Glucocorticoid Resistance AUC T1 ($pg\text{-}\mu mol/mL^2$)	$2.12x10^6$	$2.03x10^6$	$1.87x10^6$	$1.83x10^6$	$1.50x10^6$	$8.26x10^5$	.743
Glucocorticoid Resistance AUCwbcs T1 (pg-nmol/cell-L)	4.36	3.95	4.62	4.65	3.54	1.74	.848
BMI T1(kg/m^2)	30.05	8.51	29.18	7.62	26.37	4.94	.302
cIL6 T1 (pg/mL)[a]	1.95	2.05	1.09	0.87	1.32	1.41	.039
Months Since Diagnosis T2	6.62	1.82	7.00	2.04	6.53	1.82	.622
BDI T2	17.74	11.17	17.75	13.72	16.17	13.16	.924
STAI-S T2	43.74	14.82	45.30	16.03	44.46	15.56	.905
IES T2	26.71	16.48	27.21	15.90	27.85	22.11	.979
Glucocorticoid Resistance AUC T2 ($pg\text{-}\mu mol/mL^2$)	$2.83x10^6$	$2.00x10^6$	$1.92x10^6$	$1.53x10^6$	$1.81x10^6$	$1.56x10^6$	.155
Glucocorticoid Resistance AUCwbcs T2 (pg-nmol/cell-L)	6.20	4.37	4.50	3.78	4.13	3.59	.336
BMI T2 (kg/m^2)	30.44	8.97	30.71	8.07	27.09	5.79	.355
cIL6 T2 (pg/mL)[a]	1.99	1.86	1.35	0.89	0.96	0.33	.006
Months Since Diagnosis T3	12.59	2.12	12.87	2.28	12.46	1.93	.782
BDI T3	15.50	13.72	13.48	11.63	10.15	10.14	.425
STAI-S T3	40.61	17.17	39.60	14.69	37.92	14.23	.874
IES T3	21.32	18.05	24.02	17.04	23.62	21.98	.820

Glucocorticoid Resistance AUC T3 (pg-μmol/mL^2)	2.76x10^6	1.53x10^6	2.42x10^6	1.34x10^6	2.26x10^6	1.10x10^6	.668
Glucocorticoid Resistance AUCwbcs T3 (pg-nmol/cell-L)	5.39	3.74	5.68	2.90	6.55	3.88	.788
BMI T3 (kg/m^2)	31.19	9.37	29.32	6.98	27.86	5.78	.460
cIL6 T3 (pg/mL)[a]	3.08	2.91	1.79	0.96	1.44	0.74	.040

Notes: BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State version; IES = Impact of Events Scale; AUC = Area under the curve; AUCmonos = Area under the curve adjusted for concentration of monocytes; AUCwbcs = Area under the curve adjusted for concentration of white blood cells; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

3.2 MODEL FOR DISTRESS

Bivariate correlations between distress measures ranged from 0.6 to 0.8 at all three time points (**Appendix B**). Confirmatory factor models were just identified, and indicators of distress loaded similarly on one latent factor across time points (**Table 7**). Examination of measurement invariance for the latent factor of distress revealed weak measurement invariance (**Table 8**). That is, while there was no significant difference in model fit when factor loadings were constrained to be equal across time, there was a significant difference in model fit when intercepts were constrained to be equal. When the intercept for the BDI indicator was freed at T1, the model was not significantly different from a model in which the factor loadings were constrained across time (**Table 8**). All subsequent models using the latent distress factor were thus constrained. This measurement model for distress suggests that the BDI, STAI-S, and the IES contributed similar variance to the distress latent construct at each time point, but the sample reported a lower mean BDI at T1 than at T2 and T3, while reporting similar means at each time point on the STAI-S and IES.

Table 7. Confirmatory Factor Analysis Model(s) for Distress Latent Factor.

	Time 1		**Time 2**		**Time 3**	
	B	**SE**	**B**	**SE**	**B**	**SE**
λ1 BDI	9.953	0.953	11.283	1.084	10.77	1.13
λ2 STAIS	12.617	1.11	14.026	1.296	13.219	1.446
λ3 IES	11.039	1.276	11.966	1.529	11.86	1.789

Note: models just identified, fit statistics not provided

Table 8. Test of Measurement Invariance for Distress Latent Factor.

	Model	χ^2 (df)	RMSEA	CFI/TLI	SRMR	Models Compared	Δχ2 (df)
A	Configural Model	20.044 (15)	0.053	0.993/0.983	0.032	--	--
B	Loading Invariance	20.982 (19)	0.029	0.997/0.995	0.035	B-A	0.938 (4)
C	Intercept Invariance	47.642 (23)	0.094	0.965/0.945	0.062	C-B	26.66* (4)
D	free τ_1 BDI T1	21.601 (22)	0.000	1.000/1.001	0.035	D-B	0.619 (3)

* $p < .05$

A model for latent change in distress over time fit the data well (χ2 (22) = 21.60, p = .484; RMSEA = 0.00, p = .792; CFI = 1.000; SRMR = 0.035) (model not shown). This model was significantly different from a model in which the mean of the first difference score factor, or the mean of the second difference score factor, was constrained to zero (1st: Δχ2 (1) = 22.76, p < .001; 2nd: Δχ2 (1) = 18.57, p < .001). These findings suggest that there was a significant mean change in distress over both time periods. Specifically, the difference score factor means showed a decrease in distress between both T1 and T2 (α (SE) = -5.09 (1.03), $p < .001$), and T2 and T3 (α (SE) = -3.88 (0.85), $p < .001$).

3.3 MODEL FOR INFLAMMATION

The latent change score model for IL-6 values was just identified (model not shown). This model differed significantly from a model in which the mean of the first difference score factor, or the mean of the second difference score factor, was constrained to zero (1st: Δχ2 (1) = 8.38, p = .004; 2nd: Δχ2 (1) = 34.53, p < .001). These results suggest a significant change in mean

circulating levels of IL-6 over both time periods. In particular, the difference score factor means indicated a significant increase in levels of IL-6 between both T1 and T2 (α (SE) = 0.322 (0.112), p = .004), and T2 and T3 (α (SE) = 0.872 (0.154), p < .001).

3.4 MODEL FOR GCR

The latent difference score model for change in mean GCR was just identified (model not shown). This model did not differ significantly from a model in which the mean of the first difference score factor, or the mean of the second difference score factor, was constrained to zero (1^{st}: $\Delta\chi 2$ (1) = 2.76, p = .097; 2^{nd}: $\Delta\chi 2$ (1) = 1.29, p = .255), suggesting no significant change in mean GCR over either time period. Interestingly, the difference score factor means indicated a non-significant increase in GCR over both T1 and T2 (α (SE) = 3.741 (2.207), p = .090), and T2 and T3 (α (SE) = 3.022 (2.647), p = .254).

3.5 EFFECT OF INTERVENTION

Individuals in the intervention and control groups did not differ in age, education, race, the child's treatment intensity, distress measures at T1, BMI, GCR at T1, or circulating levels of IL-6 at T1 (**Tables 9, 10**). We used the latent change score models described above to investigate the impact of intervention on mean change in distress, GCR, and IL-6. For this, we regressed the categorical variable for intervention group on each latent difference factor, while controlling for

age, education, race, and the child's treatment intensity. The model for distress fit the data well ($\chi 2$ (60) = 64.89, p = .310; RMSEA = 0.026, p = .828; CFI = 0.993; SRMR = 0.071; **Figure 1**). A model in which the path between intervention group and mean change in distress from T1 to T2 was constrained to zero was not significantly different from a model in which this path varied freely ($\Delta\chi 2$ (1) = 3.37, p = .066). This suggests that there was no effect of the intervention on distress over the 6 months of active intervention. However, the path coefficient revealed that the effect was in the expected direction (B (SE) = -2.95 (1.61), p = .066; **Figure 1**). A model in which the path between intervention group and change in distress from T2 to T3 was constrained to zero was not significantly different from a model in which this path was freely estimated ($\Delta\chi 2$ (1) = 0.057, p = .811). This finding suggests that there was no significant effect of intervention on change in distress over the 6-month post-intervention period.

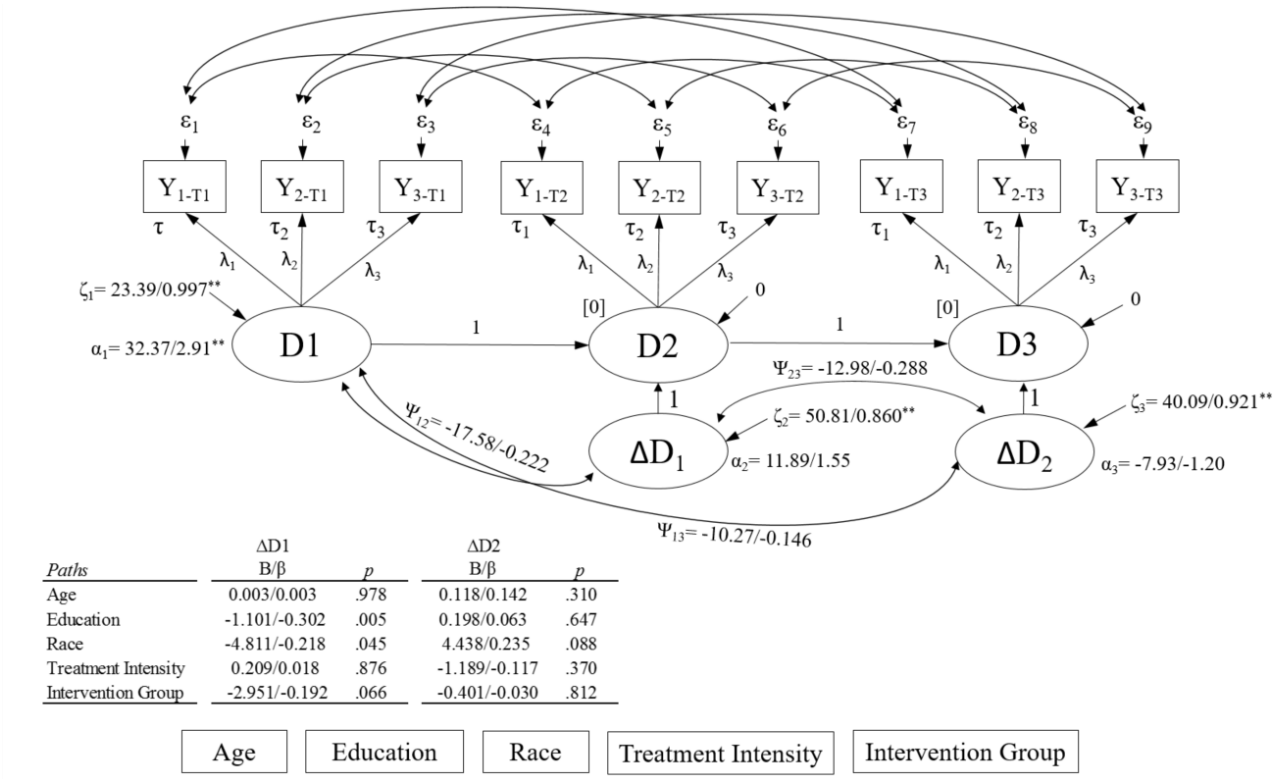
Table 9: Comparison of Intervention and Control groups

	Control (n = 60) mean ± SD	**Intervention (n = 60) mean ± SD**	***p***	**Total Sample (n = 120) mean ± SD**
Age (yrs)	35.3 ± 7.8	36.5 ± 8.1	.415	35.9 ± 8.0
Education (yrs)	14.0 ± 1.8	14.0 ± 2.4	.830	14.0 ± 2.1
Race (%Caucasian, W/AA+other)	83, 50/10	88, 53/7	.432	86, 103/17
Treatment Intensity (%most, mild/moderate/very/most)	12, 0/16/36/7	18, 0/18/31/11	.504	15, 34/67/18
BDI T1	19.6 ± 12.9	17.2 ± 10.3	.266	18.4 ± 11.7
STAIS-S T1	51.5 ± 14.0	48.5 ± 13.7	.249	50.0 ± 13.9
IES T1	35.1 ± 15.9	31.3 ± 13.9	.167	33.2 ± 15.0
BMI T1 (kg/m^2)	30.3 ± 8.8	27.7 ± 6.2	.085	29.0 ± 7.6
GCR AUC T1 (pg-µmol/mL^2)	$1.9x10^6$ ± $1.7x10^6$	$1.9x10^6$ ± $1.9x10^6$	.885	$1.9x10^6$ ± $1.8x10^6$
GCR AUCwbcs T1 (pg-nmol/cell-L)	4.6 ± 4.2	4.3 ± 4.2	.800	4.4 ± 4.2
cIL6 T1 (pg/mL)	1.53 ± 1.41	1.24 ± 1.47	.223	1.38 ± 1.44

Notes: For continous variables, independent samples t-tests were performed; for categorical variables, χ2-test was performed; W = White; AA = African American; Int = Intervention; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State version; IES = Impact of Events Scale; BMI = Body Mass Index; GCR = glucocorticoid resistance; AUC = area under the curve; AUCmonos = area under the curve adjusted for concentration of monocytes; cIL-6 = circulating levels of interleukin-6

Table 10: Cross-Tabulation of Race by Intervention Group

	Race			
Intervention Group	Caucasian/White	African American/other	χ^2	*p*
Control Group	50	10	0.617	.432
Intervention Group	53	7		

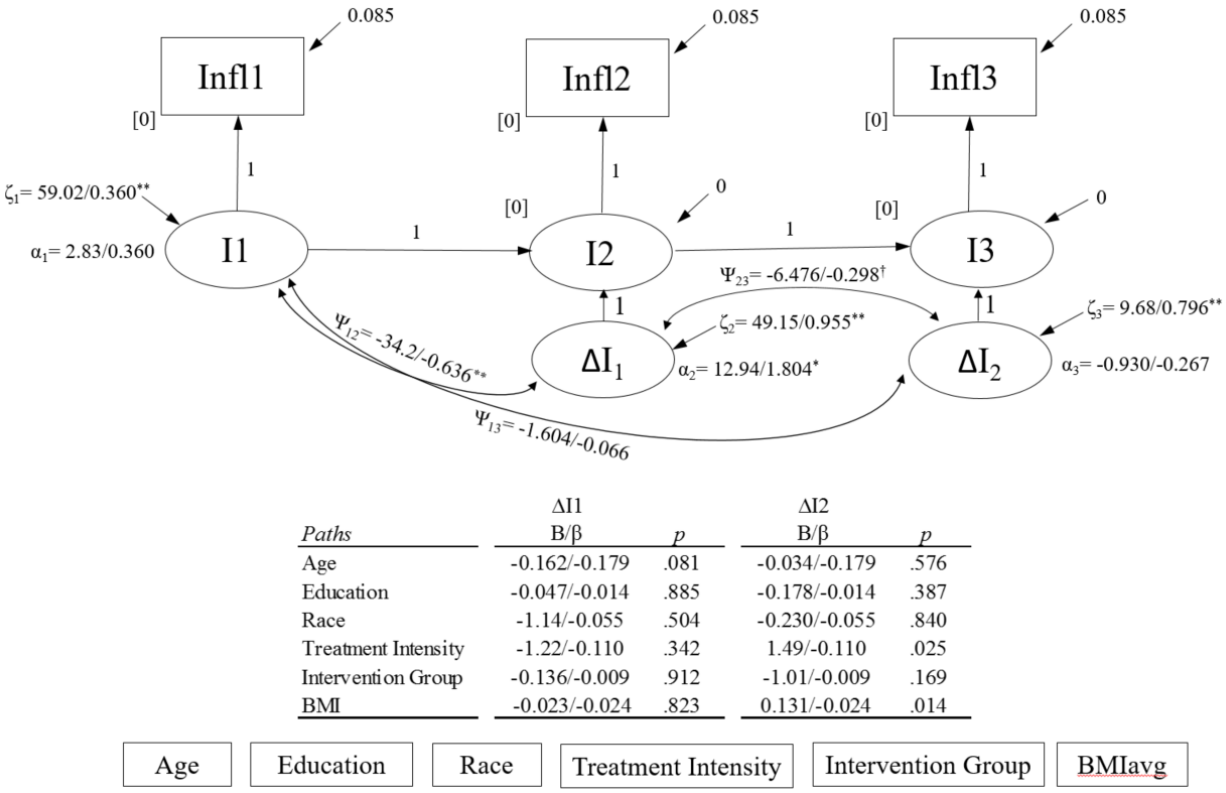


Paths	ΔD1 B/β	*p*	ΔD2 B/β	*p*
Age	0.003/0.003	.978	0.118/0.142	.310
Education	-1.101/-0.302	.005	0.198/0.063	.647
Race	-4.811/-0.218	.045	4.438/0.235	.088
Treatment Intensity	0.209/0.018	.876	-1.189/-0.117	.370
Intervention Group	-2.951/-0.192	.066	-0.401/-0.030	.812

Figure 1. Latent Change Score Model for Distress.

Model fit: $\chi 2$ (60) = 64.89, p = .310; RMSEA = 0.026, p = .828; CFI = 0.993; SRMR = 0.071. ΔD_1 and ΔD_2 were regressed on covariates listed at bottom of figure. Path coefficients for these regressions can be seen in the table. Results are reported as: unstandardized/standardized; $^{*}p <$ .05, $^{**}p < .001$.

There was no evidence of an association between intervention group and mean change in either IL-6 or GCR across either of the 6 month time periods (**Figures 2, 3**). Given these null findings, all subsequent models include intervention group as a standard covariate and report findings independent of any effects due to the intervention.

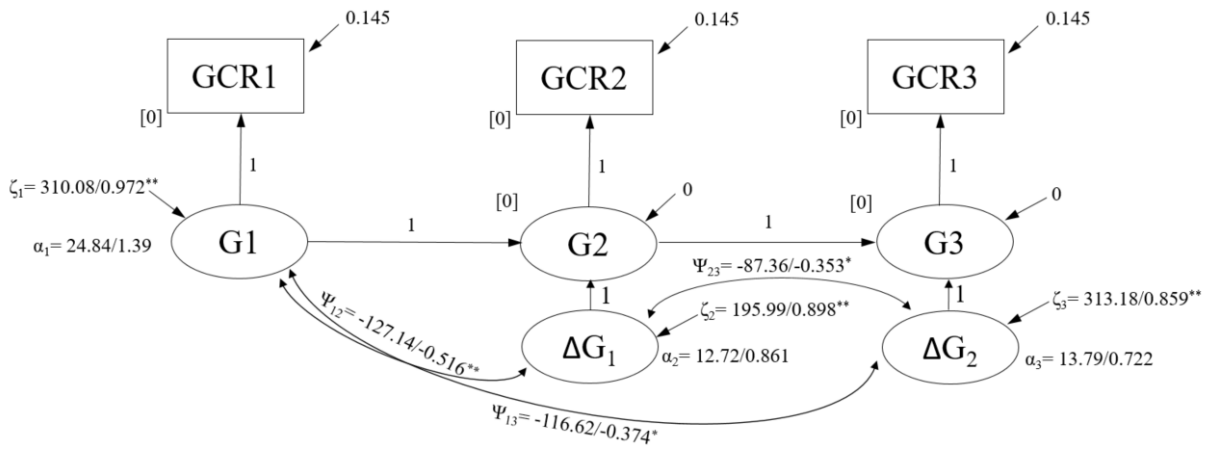


	ΔI1		ΔI2	
Paths	B/β	*p*	B/β	*p*
Age	-0.162/-0.179	.081	-0.034/-0.179	.576
Education	-0.047/-0.014	.885	-0.178/-0.014	.387
Race	-1.14/-0.055	.504	-0.230/-0.055	.840
Treatment Intensity	-1.22/-0.110	.342	1.49/-0.110	.025
Intervention Group	-0.136/-0.009	.912	-1.01/-0.009	.169
BMI	-0.023/-0.024	.823	0.131/-0.024	.014

Age | Education | Race | Treatment Intensity | Intervention Group | BMIavg

Figure 2. Latent Change Score Model for Inflammation (IL-6).

Model fit: $\chi 2$ (13) = 27.18, p = .012; RMSEA = 0.095, p = .070; CFI = .898; SRMR = 0.077. ΔI_1 and ΔI_2 were regressed on covariates listed at bottom of figure. Path coefficients for these regressions can be seen in the table. Results are reported as: unstandardized/standardized; $^{*}p <$ .05, $^{**}p <$ .001.



	ΔG1		ΔG2	
Paths	B/β	*p*	B/β	*p*
Age	0.136/0.073	.560	0.163/0.073	.641
Education	-1.440/-0.205	.112	0.187/-0.205	.882
Race	8.163/0.193	.097	-6.471/0.193	.457
Treatment Intensity	-0.478/-0.021	.887	2.553/-0.021	.532
Intervention Group	2.675/0.091	.433	-4.142/0.091	.391
BMI	0.170/-0.086	.584	-0.802/-0.086	.023

Figure 3. Latent Change Score Model for Glucocorticoid Resistance (GCR).

Model fit: $\chi 2$ (13) = 17.68, p = .170; RMSEA = 0.055, p = .404; CFI = .828; SRMR = 0.053. ΔG_1 and ΔG_2 were regressed on covariates listed at bottom of figure. Path coefficients for these regressions can be seen in the table. Results are reported as: unstandardized/standardized; $^{*}p <$ .05, $^{**}p <$ .001.

3.6 AIM 1: DISTRESS AND INFLAMMATION

We predicted that change in the shared variance of distress measures would positively associate with change in IL-6 over time. First order correlations between distress and circulating levels of IL-6 ranged from .04 to .16 across all three time points, with the highest magnitude correlations occurring at T1 (**Appendix B**). Controlling for age, education, race, BMI, the child's

treatment intensity, and intervention group, the latent change score model associating change in distress with change in inflammation fit the data adequately ($\chi 2$ (104) = 118.11, p = .163; RMSEA = 0.034, p = .825; CFI = 0.983; SRMR = 0.083; **Figure 4**). A model in which the association between change in distress and change in inflammation between T1 and T2 was constrained to zero did not significantly differ from a model in this path was allowed to vary freely ($\Delta\chi 2$ (1) = 0.225, p = .635). Similarly, a model in which change in distress and change in inflammation between T2 and T3 was constrained to zero was not significantly different than a model in which this path was allowed to vary freely ($\Delta\chi 2$ (1) = 0.713, p = .398). These findings suggest that there was no association between change in distress and change in inflammation over either 6-month periods of the study.

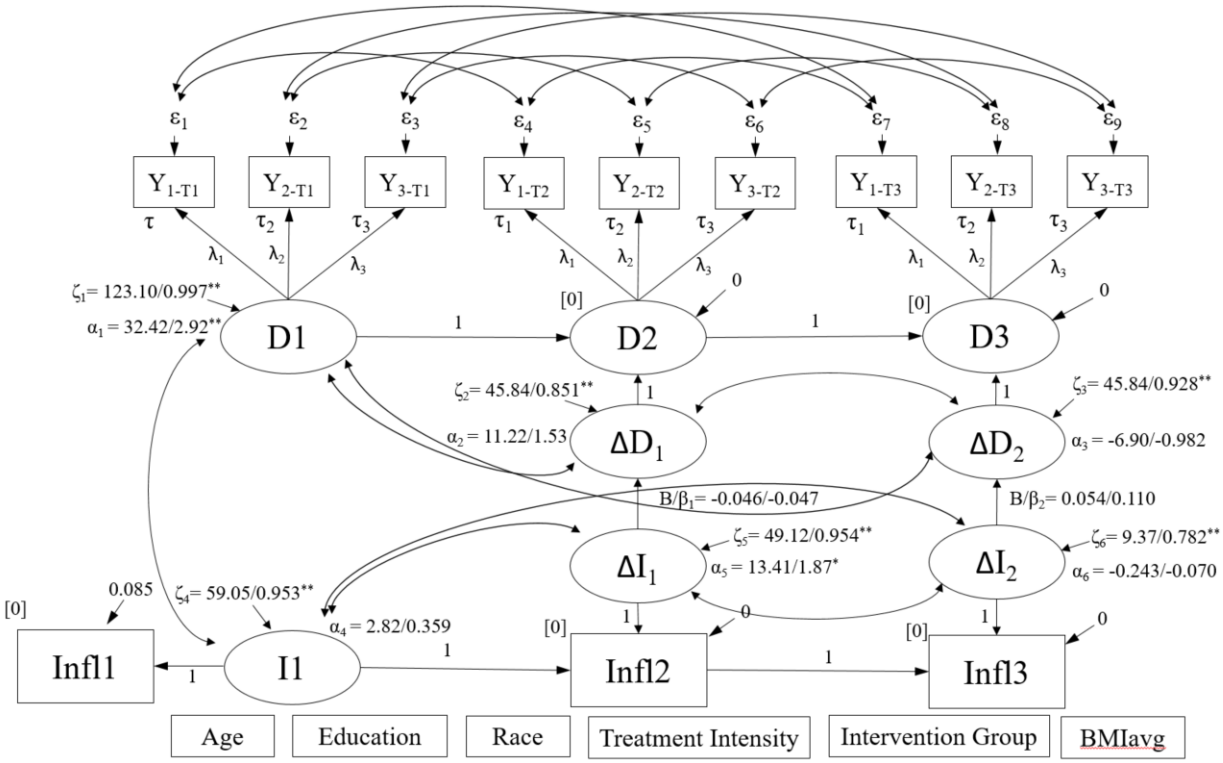


Figure 4. Simultaneous latent difference score model for change in distress predicting change in IL-6.

ΔD_1, ΔD_2, ΔI_1, and ΔI_2 were regressed on covariates listed at bottom of figure. Results are reported as: unstandardized/standardized; $^{*}p < .05$, $^{**}p < .001$. Model Fit: $\chi 2$ (104) = 118.11, p = .163; RMSEA = 0.034, p = .825; CFI = 0.983; SRMR = 0.083.

3.7 AIM 2: DISTRESS AND GCR

We hypothesized that change in distress would positively associate with change in glucocorticoid resistance over time. First order correlations between the indicators for distress and GCR ranged from -.14 (T1) to .28 (T3), with the highest magnitude correlations occurring at T3 (**Appendix B**). Controlling for age, education, race, BMI, the child's treatment intensity, and

intervention group, the latent change score model associating change in distress with change in GCR fit the data adequately ($\chi 2$ (104) = 110.77, p = .307; RMSEA = 0.023, p = .917; CFI = 0.991; SRMR = 0.081; **Figure 5**). A model in which the association between change in distress and change in GCR between T1 and T2 was constrained to zero was significantly different from a model in this path was allowed to vary freely ($\Delta\chi 2$ (1) = 4.24, p = .039). Similarly, a model in which the association between change in distress and change in GCR between T2 and T3 was constrained to zero was significantly different from a model in this path was allowed to vary freely ($\Delta\chi 2$ (1) = 5.07, p = .024). These findings suggest a significant association between change in mean levels of distress and change in GCR over both time periods of the study. Specifically, the path coefficients indicated that increases in distress associated with increases in GCR between both T1 and T2 (B (SE) = 0.490 (0.236), *p* = .038), and T2 and T3 (B (SE) = 0.739 (0.314), *p* = .019) (**Figure 5**). When GCR was adjusted for concentration of white blood cells, only the model in which the path from T2 to T3 was constrained to zero was significantly different from a model in which it was allowed to vary freely (T1/T2: $\Delta\chi 2$ (1) = 0.892, p = .345; T2/T3: $\Delta\chi 2$ (1) = 7.98, p = .005; model not shown). This suggests a more robust association between change in distress and change in GCR independent of cell concentration over the 2nd 6 months of the study. The path coefficients similarly reflected this finding (T1/T2: B (SE) = 0.064 (.068), p = .345; T2/T3: B (SE) = 0.219 (.072), p = .002) (model not shown).

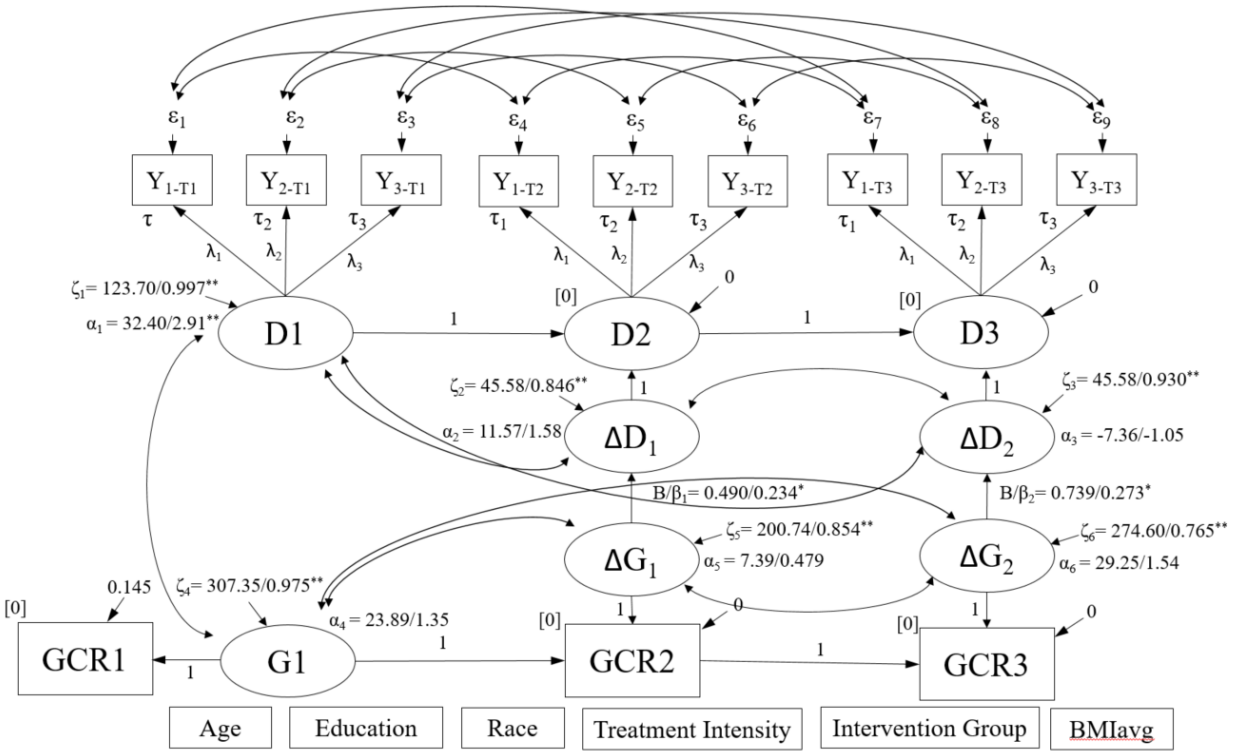


Figure 5. Simultaneous latent difference score model for change in distress predicting change in GCR.

ΔD_1, ΔD_2, ΔG_1, and ΔG_2 were regressed on covariates listed at bottom of figure. Results are reported as: unstandardized/standardized; $^{*}p < .05$, $^{**}p < .001$. Model Fit: $\chi 2$ (104) = 110.77, p = .307; RMSEA = 0.023, p = .917; CFI = 0.991; SRMR = 0.081.

3.8 AIM 3: GCR AND INFLAMMATION

We hypothesized that change in glucocorticoid resistance would positively associate with change in IL-6 over time. Correlations between GCR and IL-6 ranged from to .40 (T2) to -.07 (T3) with the highest magnitude correlations occurring at T2 (**Appendix B**). Controlling for age, education, race, BMI, the child's treatment intensity, and intervention group, the latent change

score model associating change in GCR with change in inflammation fit the data adequately ($\chi 2$ (24) = 30.09, p = .182; RMSEA = 0.046, p = .515; CFI = 0.960; SRMR = 0.072; **Figure 6**). A model in which the association between change in GCR and change in IL-6 between T1 and T2, or between T2 and T3, was constrained to zero did not significantly differ from a model in these paths were allowed to freely vary (T1/T2: $\Delta\chi 2$ (1) = 0.318, p = .573; T2/T3: $\Delta\chi 2$ (1) = 1.05, p = .306). These findings suggest that there was no association between change in GCR and change in inflammation over either 6-month periods of the study. These findings held when adjusting for white blood cell concentration (T1/T2: $\Delta\chi 2$ (2) = 0.170, p = .680; T2/T3: $\Delta\chi 2$ (2) = .102, p = .749; model not shown).

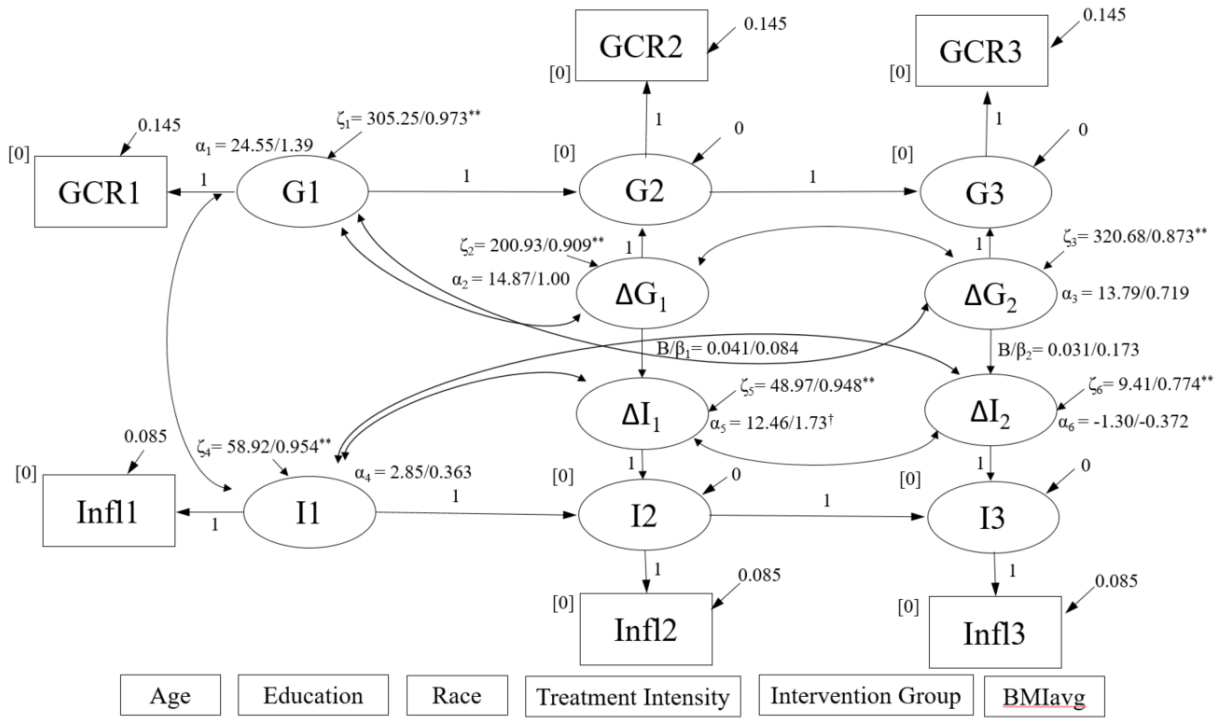


Figure 6. Simultaneous latent difference score model for change in GCR predicting change in IL-6.

ΔG_1, ΔG_2, ΔI_1, and ΔI_2 were regressed on covariates listed at bottom of figure. Results are reported as: unstandardized/standardized; $^{*}p < .05$, $^{**}p < .001$. Model Fit: $\chi 2$ (24) = 30.09, p = .182; RMSEA = 0.046, p = .515; CFI = 0.960; SRMR = 0.072.

3.9 EXPLORATORY ANALYSIS

To investigate reciprocal effects between change in distress and inflammation at the next time point, and change in inflammation and distress at the next time point, we examined a cross-lagged model described earlier (**Figure 7**). Controlling for age, education, race, BMI, the child's treatment intensity, and intervention group, the model fit was adequate ($\chi 2$ (102) = 116.96, p = .105; RMSEA = 0.039, p = .737; CFI = 0.979; SRMR = 0.084; **Figure 7**). A model in which all cross-lagged paths were constrained to zero did not differ significantly from a model in which they were allowed to vary freely ($\Delta\chi 2$ (4) = 1.889, p = .756), suggesting that there were no significant reciprocal associations across time. The path coefficients similarly reflected this finding (**Figure 7**).

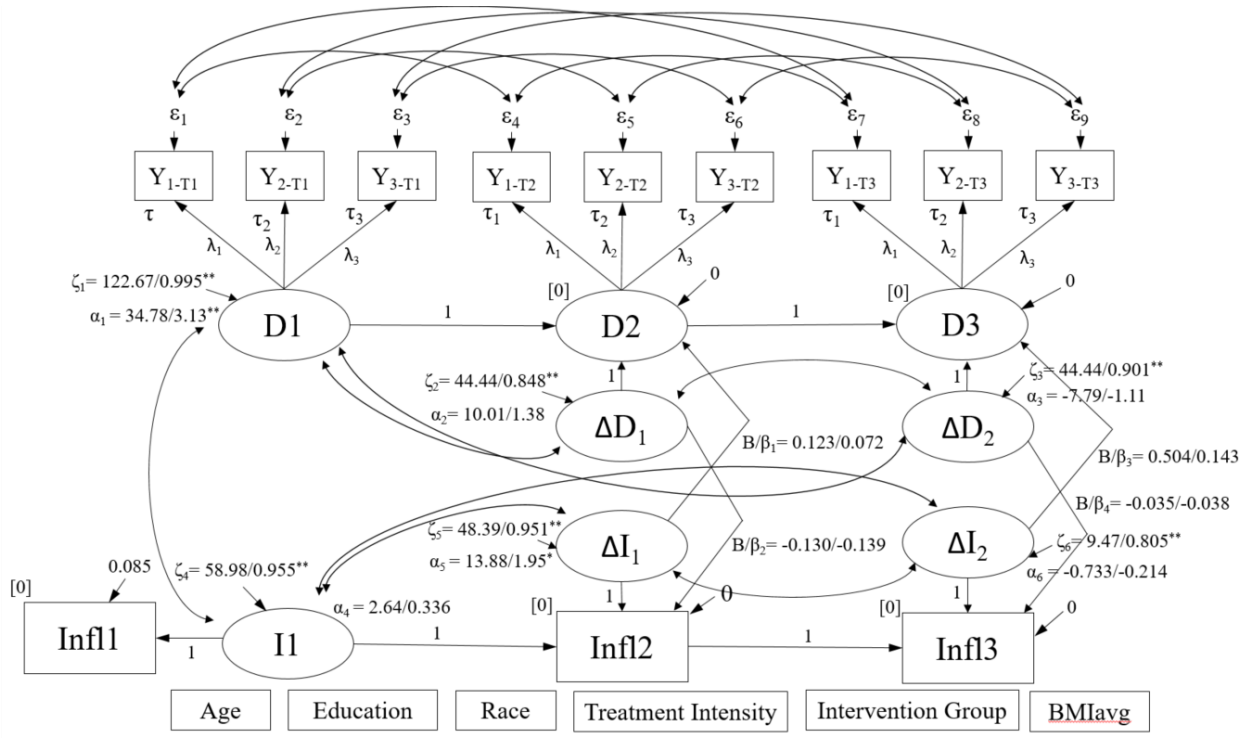


Figure 7. Cross-lagged latent difference score model for change in distress predicting change in IL-6 at the following time point, and change in IL-6 predicting distress at the following time point.

ΔD_1, ΔD_2, ΔI_1, and ΔI_2 were regressed on covariates listed at bottom of figure. Results are reported as: unstandardized/standardized; $^{*}p < .05$, $^{**}p < .001$. Model Fit: $\chi 2$ (102) = 116.96, p = .105; RMSEA = 0.039, p = .737; CFI = 0.979; SRMR = 0.084.

4.0 DISCUSSION

4.1 SUMMARY

Based on existing literature, we proposed a model in which the emotional arousal of caring for a child newly diagnosed with cancer drives activation of the HPA-axis and associated release of peripheral cortisol, as well as activation of the innate inflammatory response. Guided by the theory proposed by Miller, Cohen, and Ritchey (2002), we hypothesized that leukocytes in the periphery would adapt to chronically heightened cortisol levels by down-regulating the sensitivity of glucocorticoid receptors, resulting in increased transcription of peripheral inflammatory cytokines. More specifically, we hypothesized that in our sample of 120 mothers of children newly diagnosed with cancer, changes in psychological distress over the six months after the child's diagnosis would parallel changes in glucocorticoid resistance and peripheral levels of the proinflammatory cytokine, IL-6, as measured at the 6 month follow-up. Similarly, we predicted that there would be an association between changes in distress over the second six months after a child's diagnosis and changes in both glucocorticoid resistance and peripheral levels of IL-6 at one year. Finally, we explored the possibility that glucocorticoid resistance contributed to relationships between distress and IL-6.

4.2 PATTERN OF DISTRESS

As expected, our findings showed heightened levels of distress among mothers in the weeks following their child's diagnosis, followed by a mean decrease in distress over the following 12 months. This pattern was similar for measures of symptoms of anxiety, depression, and post-traumatic stress. The current sample endorsed similar levels of distress to other caregiving samples (Rholeder et al., 2009; Miller et al., 2014; M Cohen et al., 2002; Lovell et al., 2015), as well as to other samples of primary caregivers for children with cancer (Marsland et al., 2002; Kazak et al., 2005; Mullins et al., 2012; Miller et al., 2002). Specifically, 59% of our sample reported mild depression or greater shortly following their child's diagnosis, falling to 52% at 6 months and 42% at one year follow-up (Beck et al., 1961). On the STAI measure of anxiety, 84% of the sample reported symptoms greater than the 50^{th} percentile of population norms shortly following the child's diagnosis, 70.1% at six months, and 58% at one year (Spielberger et al., 1983). For symptoms of post-traumatic stress, 83.9% of the sample reported symptoms above clinically significant cutoffs on the IES at the child's initial diagnosis, 64.9% at six months, and 51.7% at one year (Sundin & Horowitz, 2002). Responses on the BDI, STAI and IES were highly correlated. For this reason, we created a latent variable of distress for use in analyses examining changes in glucocorticoid resistance and IL-6. Our distress variable captured the shared association among measures of depression, anxiety and post-traumatic stress symptoms; however, while the association between the measures was similar at each time point, they showed marked differences in their pattern of change across time. This was reflected in our measurement model, with symptoms of depression, anxiety, and post-traumatic stress showing similar commonality between the measures at each time point, but not showing similarity on their relative means. Specifically, the sample had a lower mean for depressive symptoms at the

time of the child's initial diagnosis compared to six months and one year, while the means were similar across all three time points for anxiety and post-traumatic stress. Given that the sample was measured from the onset of this chronic stressor, and that the pattern of mean depression symptoms differed from the pattern of anxiety and post-traumatic stress symptoms across time, it could be that there was a lag in the onset of depressive symptoms, such that it may take longer for these to appear as individuals move from acute to chronic distress. Thus, it may be that any associations between our measure of distress with biological change over the first six month period after a child's diagnosis with cancer is driven to a greater extent by symptoms of anxiety and post-traumatic stress, while during the second six months after diagnosis, depressive symptoms may play a larger role. Future analyses should test the role of the individual indicators of distress on the outcomes in this study.

4.3 DISTRESS AND GCR

Based on the existing literature, we anticipated that changes in distress in the year following the child's diagnosis would associate with concomitant changes in glucocorticoid resistance. Consistent with findings of others, we observed an increase in glucocorticoid resistance over both the first six months and the second six months after a child's diagnosis, although the change was not significant. Despite the lack of a control group, our findings are consistent with those of other studies demonstrating elevated glucocorticoid resistance in those caring for a significant other with cancer (Bauer et al., 2000; Miller et al. 2002, Rholeder et al., 2009). Given that there was significant variability in individual rates of change in glucocorticoid resistance over time, we went on to examine whether change in distress over time associated

with glucocorticoid resistance. Consistent with our hypothesis and the model proposed by Miller et al., (2002), we observed a positive association between change in our measure of distress over the first six months after the child's diagnosis and glucocorticoid resistance at six months. Similarly, change in distress over the second six months after diagnosis positively associated with change in glucocorticoid resistance at one year. These findings are consistent with cross-sectional findings reported by Miller et al., 2002, showing elevated glucocorticoid resistance in a sample of distressed parental caregivers of pediatric cancer patients. In Miller et al.'s study, parents were evaluated an average of 9 months after their child's initial diagnosis. Although they did not find a significant association between symptoms of depression and glucocorticoid resistance, they postulated that increased distress in response to the child's diagnosis resulted in activation of the HPA-axis and peripheral release of cortisol, resulting in the later downregulation of glucocorticoid signaling. Thus, the authors concluded that their failure to observe a significant association of symptoms of depression with glucocorticoid resistance related to timing issues. Our longitudinal findings lend support to this possibility, providing initial evidence that individual differences in distress across time following a major life stressor moderate sensitivity to glucocorticoids in peripheral immune cells. An examination of the role of stress-related activation of the HPA-axis in the development of glucocorticoid resistance following challenging life circumstances is warranted.

In this study, glucocorticoid resistance was assessed *in vitro* using a whole blood assay. This assay involved incubating whole blood with an immune stimulant (LPS) in the presence of increasing concentrations of exogenous cortisol. Whole blood assays are used because they better approximate the *in vivo* response of the immune system acting in concert to protect against bacterial infection. However, one factor that could influence the magnitude of response and

account for changes over time is the number of leukocytes within the culture. In this regard, there is substantial evidence that naturalistic stress is associated with an increase in the absolute number of leukocytes in peripheral circulation and a change in the relative number of different cell subtypes, favoring increases in cells that play a role in the production of inflammatory mediators, including IL-6 (Segerstrom, 2004). In this regard, we observed a non-significant increase in the mean number of circulating leukocytes between the time of the child's initial diagnosis and six months, and a decrease between six months and one year post diagnosis (**Table 3** for means, change score models not shown). To examine the possibility that distress-related changes in glucocorticoid resistance were accounted for by changes in circulating leukocyte cell subtypes within the whole blood cultures, we assessed the number of leukocytes in peripheral circulation at each time point and calculated glucocorticoid resistance per leukocyte. Using these measures, we found that increases in parental distress over the second six months after a child's initial diagnosis continued to predict an increase in glucocorticoid resistance at one year, but that changes in parental distress during the initial six months after a child's initial diagnosis no longer predicted a significant increase in glucocorticoid resistance at six months. This raises the possibility that during the initial six-month period after a child's initial cancer diagnosis, decreased cellular sensitivity to cortisol *in vitro* may at least partially result from an increase in the circulating number of leukocytes. Future studies should further examine the contribution of stress-related changes in circulating ratios of different cell subtypes to glucocorticoid resistance, particularly in the early stages of the stress response. It is possible that functional changes in glucocorticoid resistance observed close to the onset of chronic stress are attributable to changes in the relative number of different leukocytes subtypes, while functional changes at more distal

time points are attributable to enduring changes in receptor number, binding capacity, or availability.

4.4 THE ROLE OF INFLAMMATION

In addition to examining glucocorticoid resistance, the current study assessed circulating levels of IL-6 among mothers across the 12 months following their child's cancer diagnosis. As expected, we observed a mean increase in IL-6 across this period. More specifically, in analyses adjusted for age, educational attainment, race, the child's treatment intensity, intervention group, and BMI, we observed a significant increase in IL-6 in the first six months after diagnosis, but not the second six months. These findings are consistent with studies showing increased levels of systemic inflammation among individuals exposed to a range of chronic stressors, including the stress of caring for a loved one with cancer (Hansel et al., 2010; Kiecolt-Glaser et al., 2003; Rholeder et al., 2009; GE Miller et al., 2008). However, evidence from these studies is equivocal, especially for associations with levels of peripheral IL-6. More consistent evidence supports positive associations of symptoms of depression and perceived stress with levels of peripheral IL-6 (Howren et al., 2009; Segerstrom & Miller, 2004). Given this evidence, we hypothesized that psychological responses to the multiple challenges that accompany having a child diagnosed with cancer would moderate changes in IL-6 over time, with individuals showing heightened distress to caregiving at elevated risk for systemic inflammation. Interestingly, the pattern of change in our measure of distress paralleled the pattern of change in IL-6 over both the first six months and the second six months after a child's diagnosis (See **Figures 1** and **2**). However, we did not observe a significant association of change in maternal

distress as measured between baseline and 6-months or 6-months and one year with magnitude of change in IL-6 six months later. Interestingly, in separate analyses (data not shown) using a measure of perceived stress collected every month of the 12-month study period, we found that compared to those with decreasing levels of perceived stress over time, individuals with increasing or stable high levels of perceived stress showed increasing levels of IL-6 across the 12 months. Remarkably, this measure of perceived stress did not load onto the latent factor of distress described in the current analysis, suggesting that perceived stress may tap a different psychological construct than that assessed by the combination of symptoms of anxiety, depression, and post-traumatic stress. This raises the possibility that the experience of life events being uncontrollable and unpredictable in the context of a chronic stressor is most closely associated with magnitude of inflammatory response. Further research is warranted to investigate different dimensions of psychological stress and the timing of their impact on changes in peripheral inflammatory markers.

Although increases in peripheral levels of IL-6 in this sample were not explained by our index of psychological distress, changes in distress did predict increases in glucocorticoid resistance. However, we found no evidence that changes in glucocorticoid resistance over either the six-month period after a child's initial diagnosis or the six month follow-up period predicted levels of IL-6 at 6 or 12 months. At least one study has reported a positive association between glucocorticoid resistance and IL-6 (Cohen et al., 2012); however, IL-6 levels were measured in nasal secretions in the context of an experimental rhinovirus challenge. To date, two studies have examined glucocorticoid resistance and inflammation in distressed caregivers (Rholeder et al., 2009; Miller et al., 2014); however, only Rholeder et al. (2009) examined both functional glucocorticoid resistance and levels of peripheral IL-6 in the same study. This study showed an

increase in glucocorticoid resistance, but no change in circulating level of IL-6 across a 10-month follow-up among spousal caregivers of a patient with brain cancer. Taken together with the current findings, preliminary evidence supports an increase in glucocorticoid resistance in response to chronic life stress, but provides little evidence that this change is associated with peripheral levels of inflammation.

These findings raise the possibility that pathways other than immune cell adaptation to prolonged HPA-axis activation may explain the increase in IL-6 that accompanies exposure to chronic stress. One candidate pathway is distress-driven changes in the autonomic nervous system. For example, prolonged activation of the sympathetic nervous system could function to elevate levels of peripheral inflammation through multiple pathways, including, but not limited to, increases in the number and activation state of leukocytes (Bellinger & Lorton, 2014; Elenkov et al., 2000). Indeed, it is possible that a change in the distribution of leukocyte subtypes towards cells that promote the inflammatory response may contribute to the increase in IL-6 that we observed among mothers over the first six months following their child's cancer diagnosis. It is also possible that dysregulation of the parasympathetic nervous system could contribute to stress-related increases in peripheral inflammation. In this regard, the vagal nerve is known to play an important role in the down-regulation of inflammation (Martelli, McKinley, & McAllen, 2014; Pavlov & Tracey, 2015). Another possibility is that increases in inflammation could occur through behavioral pathways, such as dysregulation in sleep, which is known to associate with increased peripheral markers of inflammation (O'Connor et al., 2009). Finally, some studies have found evidence that nutritional status can influence levels of peripheral IL-6 (Ferrucci et al., 2006; Giugliano et el., 2006). In sum, elevations in peripheral levels of IL-6 could have occurred in this sample through a number of unexplored pathways. It is important to note, however, that

we cannot conclude that observed increases in peripheral levels of IL-6 resulted from having a child diagnosed with cancer, as we did not have a control group of mothers whose children were not diagnosed with cancer. Future research should include a comparison group and examine additional pathways through which IL-6 may increase over time in response to chronic stress. These studies should more carefully consider the timing of responses to stress, with more frequent measurements starting closer to the onset of the stressful experience than was possible in the current study (M = 5.07 weeks).

In exploratory analyses, we examined support for the possibility that increased peripheral inflammation may have contributed to the experience of distress in our sample. Growing evidence shows that peripheral pro-inflammatory mediators can access the central nervous system and mediate the experience of sickness behaviors that include fatigue and depressed mood (Dantzer et al., 2008). The current results provide no support for this possibility, with no significant association of change in IL-6 with change in distress in analyses that adjusted for the opposite association over time. In sum, we found no evidence for an association of distress and IL-6 in either direction. These findings are inconsistent with studies that show bidirectional associations between distress and peripheral inflammation in community and clinically depressed samples (Messay et al., 2012; Matthews et al., 2010; Stewart et al., 2009); however, this is the first study to examine such bidirectional predictions contemporaneously, as well as the first to examine these associations in a caregiving sample. Reasons for our null findings are unclear. It is possible that effects in each direction account for the same variance; however, we did not find evidence for significant associations when each path was tested individually (results not shown). Although a majority of our sample reported levels of distress that might warrant clinical attention, we do not have information regarding clinical psychiatric diagnoses. Thus, it could be

that the level of distress in the current sample was too low to see reliable associations. Indeed, meta-analytic evidence shows that community-based samples demonstrate smaller magnitude associations between depressive symptoms and levels of peripheral IL-6 when compared to samples of clinically depressed patients (Howren et al., 2009). Alternatively, it is possible that levels of IL-6 in the current young, healthy sample of adults were too low to result in the elevation of psychological distress. Indeed, studies of both community samples as well as clinically depressed samples report mean circulating IL-6 values well above 2.0 pg/mL (Stewart et al., 2009; Pennix et al., 2003; Uddin et al., 2011; Motivala et al., 2005), whereas the average level of IL-6 in this sample was 1.63pg/mL (Table 1).

4.5 STRENGTHS AND LIMITATIONS

This study had a number of limitations and strengths. First, the parent project was a randomized controlled trial of a psychosocial intervention for mothers of children newly diagnosed with cancer. Although the project provided an opportunity to conduct the first examination of changes in glucocorticoid resistance and IL-6 over time following the onset of an extreme life stressor, it was limited by lack of a control group of mothers of children not diagnosed with cancer and timing of measures were not optimal for studying the onset, growth, and association of our measures of interest. Furthermore, the sample was relatively small for the current analyses.

The study was also limited by the collection of only one peripheral blood sample at each time point. Given the known diurnal variation of cortisol and IL-6 (Nilsonne et al., 2016), as well as the sensitivity of these measures to acute bouts of stress (Marsland et al., 2017), it would be

more reliable to obtain multiple measurements, ideally on successive days. Additionally, we only included one marker of peripheral inflammation in this study. Future studies might examine additional markers such as IL-1β, TNF-α, or CRP as well as the timing of changes in these markers compared to IL-6. Likewise, although glucocorticoid resistance is thought to be relatively stable within individuals (Quax et al., 2013), other studies have shown that bouts of exercise (DeRijk et al., 1997) and/or brief psychosocial stressors (Rholeder et al., 2003; Miller et al., 2005) can affect measures of glucocorticoid resistance acutely. Thus, it will be important in future studies to better characterize the intra-individual stability of glucocorticoid resistance over time. With regard to the glucocorticoid resistance assay used in this study, *in vitro* assays are only a proxy for processes occurring *in vivo*. Although there are some studies to suggest adequate concordance between the *in vitro* assay used in this study and *in vivo* measures of glucocorticoid resistance (Chiguer et al., 2005), it is uncertain the extent to which an *in vitro* assay measuring glucocorticoid sensitivity in peripheral immune cells generalizes to glucocorticoid resistance across different tissue or cell types throughout the body. Further, it is unclear whether *in vitro* measures of glucocorticoid sensitivity are of clinical relevance for health. Improvement in assays that can accurately measure glucocorticoid sensitivity are ongoing (Quax et al., 2013). Finally, as with any study that estimates missing data that is not missing completely at random, results are subject to biased estimates of parameters, bias in standard errors, and weakened generalizability of findings (Dong & Peng, 2013). However, when the assumptions of FIML are met, FIML has been demonstrated to produce unbiased estimates and valid model fit information (Enders, 2001; Enders & Bandalos, 2001).

Despite these limitations, the current study has a number of strengths. To our knowledge, it is the first study to examine the theory that chronic stress results in reduced sensitivity of

immune cells to the immunosuppressive effects of glucocorticoids, which may then contribute to increases in systemic markers of inflammation. Moreover, it is the first study to examine longitudinal associations between changes in psychological distress, glucocorticoid resistance, and peripheral levels of IL-6 from the onset of the chronic stressor through one year. Finally, using structural equation models for latent change allowed us to examine changes in the data at six-month intervals, as well as to examine cross-sectional, longitudinal, and cross-lagged associations across time.

4.6 IMPLICATIONS

Although we did not find our measure of psychological distress to be a modifiable risk factor in the prediction of peripheral levels of IL-6, we did show that the distress of caring for a child newly diagnosed with cancer predicts increased resistance of immune cells to glucocorticoids. Given the substantial health impact of resistance to glucocorticoids, this finding is of potential clinical significance. Glucocorticoids are widely used to treat allergic, inflammatory, and hematologic disorders, as well as for the prevention of allograft rejection (Quax et al., 2013). Increased resistance to such treatments can result in the need for higher doses for effective treatment, which can be associated with serious adverse effects including weight gain, hypertension, type 2 diabetes mellitus, and osteoporosis (Quax et al., 2013). In addition, it is estimated that 1-20% of all genes are regulated by glucocorticoids (Quax et al., 2013). Thus, dysregulation of glucocorticoid signaling may have broad effects on systemic gene regulation, with unknown health consequences. Given the immune compromised state of the children in this study, it is of utmost importance to better understand the increased health risk

among primary caregivers of children with cancer. In addition, it continues to be important to identify and understand processes through which the chronic stress of caregiving may confer increased health risk, including changes in glucocorticoid sensitivity and peripheral inflammatory markers. It is only through careful study of both the psychosocial and biological mechanisms through which these processes occur that we can begin to design better psychosocial and biological interventions to mitigate future health risk.

Appendix A

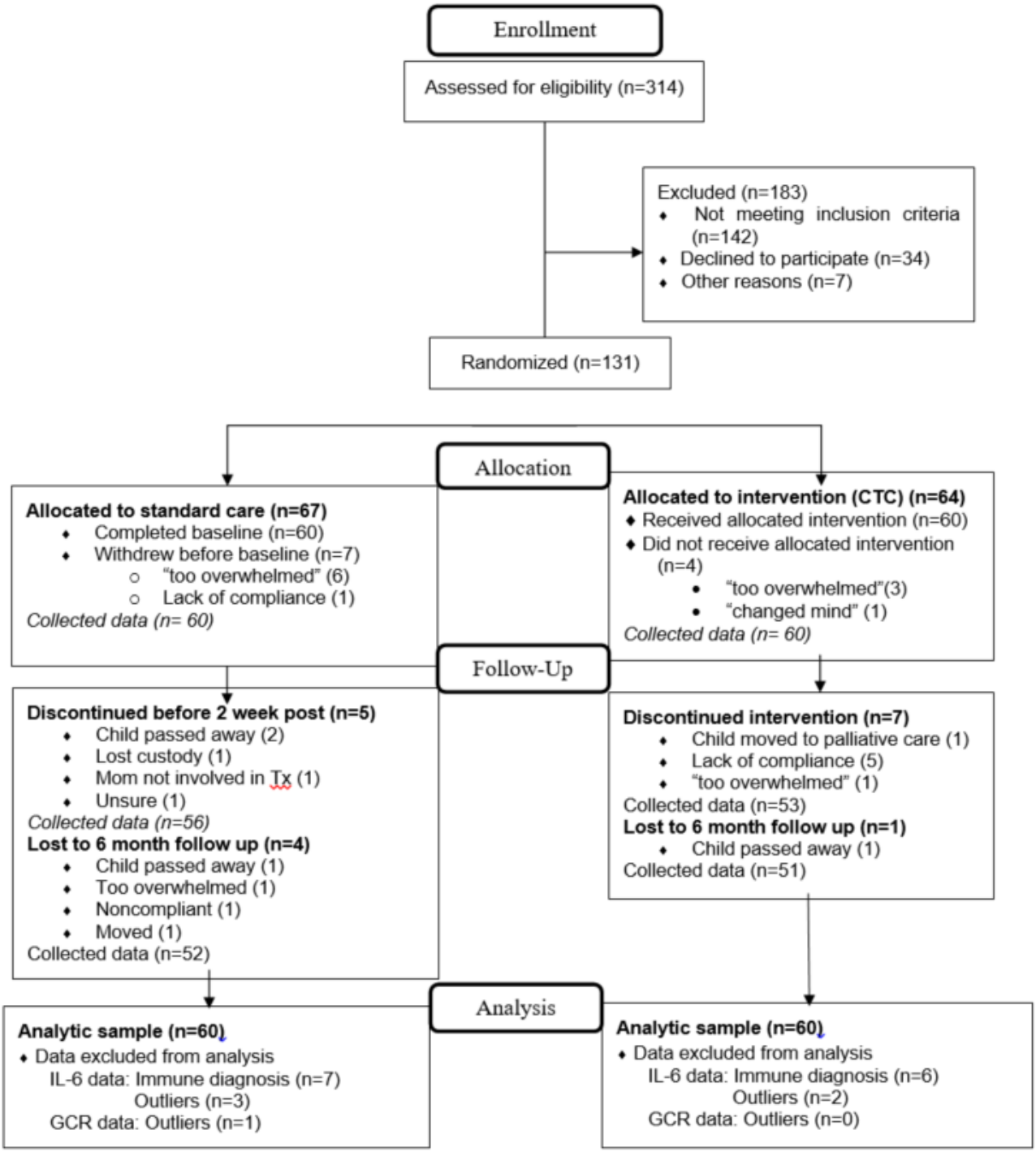


Figure 8. Consort Diagram

Appendix B

CORRELATION TABLE

Table 11: Correlation Matrix for All Variables

part 1

Correlation Matrix for all variables

Measure	1	2	3	4	5	6	7
1 Age (yrs)	1.000						
2 Education (yrs)	0.285**	1.000					
3 BMIavg	0.212*	0.012	1.000				
4 Race	-0.330**	-0.324**	-0.092	1.000			
5 Group	0.094	-0.025	-0.200*	-0.140	1.000		
6 Treatment Intensity	0.175*	0.068	-0.150	0.195*	0.039	1.000	
7 Months Since Diagnosis T1	-0.008	-0.137	0.099	0.078	-0.027	-0.013	1.000
8 BDI T1	-0.222*	-0.040	-0.035	0.053	-0.131	-0.025	0.003
9 STAI-S T1	-0.145	-0.085	-0.019	0.089	-0.135	-0.001	-0.115
10 IES T1	-0.198*	-0.059	0.025	0.101	-0.162*	0.223*	-0.111
11 GCR AUC T1 (pg-µmol/mL^2)	0.077	0.130	0.064	-0.144	0.025	-0.124	-0.137
12 GCR AUCwbcs T1 (pg-nmol/cell-L)	0.114	0.254**	-0.087	-0.006	-0.045	-0.039	-0.004
13 BMI T1(kg/m^2)	0.200*	0.036	0.991**	-0.110	-0.218*	-0.158*	0.094
14 cIL6 T1 (pg/mL)[a]	-0.184*	-0.250**	0.129	0.112	-0.156*	-0.232*	0.133
15 Months Since Diagnosis T2	-0.185*	-0.017	-0.039	0.167*	-0.108	0.062	0.546**
16 BDI T2	-0.196*	-0.171*	-0.015	-0.115	-0.214*	-0.032	0.118
17 STAI-S T2	-0.124	-0.163*	-0.038	-0.091	-0.229*	0.031	0.185*
18 IES T2	-0.151*	-0.162*	0.012	-0.106	-0.111	0.024	0.256**
19 GCR AUC T2 (pg-µmol/mL^2)	-0.070	-0.282**	0.197*	0.145	0.089	-0.244**	-0.068
20 GCRwbcs AUC T2 (pg-nmol/cell-L)	0.007	-0.316**	0.208*	0.115	-0.027	-0.212*	-0.008
21 BMI T2 (kg/m^2)	0.186*	0.031	0.990**	-0.136	-0.241**	-0.119	0.060
22 cIL6 T2 (pg/mL)[a]	-0.259**	-0.157*	0.111	0.075	-0.039	-0.407**	-0.007
23 Months Since Diagnosis T3	-0.076	0.043	0.051	0.157*	-0.026	0.125	0.465**
24 BDI T3	-0.093	-0.145	0.020	-0.207*	-0.283**	-0.154*	0.034
25 STAI-S T3	-0.073	-0.237**	-0.002	0.006	-0.191*	-0.061	0.147
26 IES T3	-0.047	-0.161*	-0.053	-0.019	-0.232*	0.060	0.150
27 GCR AUC T3 (pg-µmol/mL^2)	0.033	-0.243**	-0.204*	-0.012	-0.001	-0.147	-0.053
28 GCR AUCwbcs T3 (pg-nmol/cell-L)	0.217*	-0.038	-0.185*	-0.200*	-0.121	0.123	-0.087
29 BMI T3 (kg/m^2)	0.173*	-0.026	0.988**	0.071	-0.098	-0.169*	0.099
30 cIL6 T3 (pg/mL)[a]	-0.348**	-0.213*	0.360**	0.096	-0.273**	-0.356**	0.188*

Notes: *p < .05 **p < .005; BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State; IES = Impact of Events Scale; GCR = glucocorticoid resistance; AUC = Area under the curve; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

part 2

Correlation Matrix for all variables

Measure	8	9	10	11	12	13	14	15
1 Age (yrs)								
2 Education (yrs)								
3 BMIavg								
4 Race								
5 Group								
6 Treatment Intensity								
7 Months Since Diagnosis T1								
8 BDI T1	1.000							
9 STAI-S T1	0.758**	1.000						
10 IES T1	0.609**	0.651**	1.000					
11 GCR AUC T1 (pg-µmol/mL^2)	0.166*	-0.010	0.179*	1.000				
12 GCR AUCwbcs T1 (pg-nmol/cell-L)	0.076	-0.113	0.161*	0.858**	1.000			
13 BMI T1(kg/m^2)	-0.033	-0.010	0.021	0.058	-0.096	1.000		
14 cIL6 T1 (pg/mL)[a]	0.124	0.074	0.159*	0.079	0.036	0.133	1.000	
15 Months Since Diagnosis T2	0.059	-0.034	-0.052	0.086	0.346**	-0.036	-0.001	1.000
16 BDI T2	0.816**	0.619**	0.544**	0.209*	0.164*	-0.011	0.119	-0.018
17 STAI-S T2	0.625**	0.623**	0.508**	0.228*	0.287**	-0.024	0.085	0.023
18 IES T2	0.505**	0.386**	0.528**	0.309**	0.406**	0.026	0.125	0.046
19 GCR AUC T2 (pg-µmol/mL^2)	0.180*	0.214*	-0.007	0.630**	0.386**	0.193*	0.213*	-0.053
20 GCRwbcs AUC T2 (pg-nmol/cell-L)	0.186*	0.233*	-0.054	0.562**	0.459**	0.207*	0.208*	0.040
21 BMI T2 (kg/m^2)	-0.039	-0.048	0.062	0.079	-0.139	0.975**	0.094	-0.025
22 cIL6 T2 (pg/mL)[a]	0.149	0.162*	0.070	0.138	0.070	0.113	0.532**	0.011
23 Months Since Diagnosis T3	-0.058	-0.100	-0.062	-0.053	0.187*	0.040	-0.011	0.799**
24 BDI T3	0.677**	0.512**	0.391**	0.221*	0.235**	0.024	0.173*	-0.080
25 STAI-S T3	0.550**	0.516**	0.402**	0.236**	0.297**	0.019	0.298**	0.065
26 IES T3	0.502**	0.439**	0.488**	0.144	0.207*	-0.040	0.087	0.114
27 GCR AUC T3 (pg-µmol/mL^2)	-0.065	-0.130	-0.160	0.187*	0.113	-0.139	-0.054	-0.258**
28 GCR AUCwbcs T3 (pg-nmol/cell-L)	-0.102	-0.192*	-0.098	0.062	0.080	-0.102	-0.296**	-0.198*
29 BMI T3 (kg/m^2)	0.019	0.055	0.150	0.153*	0.055	0.961**	0.090	-0.009
30 cIL6 T3 (pg/mL)[a]	0.092	0.075	0.113	0.003	0.094	0.387**	0.681**	0.074

Notes: *$p < .05$ **$p < .005$; BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State; IES = Impact of Events Scale; GCR = glucocorticoid resistance; AUC = Area under the curve; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

part 3

Correlation Matrix for all variables

Measure	16	17	18	19	20	21	22
1 Age (yrs)							
2 Education (yrs)							
3 BMIavg							
4 Race							
5 Group							
6 Treatment Intensity							
7 Months Since Diagnosis T1							
8 BDI T1							
9 STAI-S T1							
10 IES T1							
11 GCR AUC T1 (pg-µmol/mL^2)							
12 GCR AUCwbcs T1 (pg-nmol/cell-L)							
13 BMI T1(kg/m^2)							
14 cIL6 T1 (pg/mL)[a]							
15 Months Since Diagnosis T2							
16 BDI T2	1.000						
17 STAI-S T2	0.812**	1.000					
18 IES T2	0.638**	0.652**	1.000				
19 GCR AUC T2 (pg-µmol/mL^2)	0.210*	0.187*	0.187*	1.000			
20 GCRwbcs AUC T2 (pg-nmol/cell-L)	0.159*	0.190*	0.124	0.905**	1.000		
21 BMI T2 (kg/m^2)	-0.028	-0.066	0.018	0.185*	0.192*	1.000	
22 cIL6 T2 (pg/mL)[a]	0.108	0.082	0.071	0.356**	0.339**	0.184*	1.000
23 Months Since Diagnosis T3	-0.053	-0.022	0.071	-0.094	-0.067	0.046	-0.006
24 BDI T3	0.810**	0.732**	0.545**	0.151*	0.202*	0.025	0.108
25 STAI-S T3	0.645**	0.782**	0.477**	0.242*	0.303**	-0.027	0.048
26 IES T3	0.552**	0.563**	0.664**	0.200*	0.235**	-0.015	0.103
27 GCR AUC T3 (pg-µmol/mL^2)	0.077	0.178*	0.107	0.193*	0.264**	-0.247**	-0.084
28 GCR AUCwbcs T3 (pg-nmol/cell-L)	0.043	0.171*	0.094	0.192*	0.331**	-0.238**	-0.138
29 BMI T3 (kg/m^2)	0.012	-0.004	0.056	0.212*	0.242**	0.981**	0.181*
30 cIL6 T3 (pg/mL)[a]	0.045	0.032	0.092	0.174*	0.219*	0.388**	0.873**

Notes: *p < .05 **p < .005; BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State; IES = Impact of Events Scale; GCR = glucocorticoid resistance; AUC = Area under the curve; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

part 4

Correlation Matrix for all variables

Measure	23	24	25	26	27	28	29	30
1 Age (yrs)								
2 Education (yrs)								
3 BMIavg								
4 Race								
5 Group								
6 Treatment Intensity								
7 Months Since Diagnosis T1								
8 BDI T1								
9 STAI-S T1								
10 IES T1								
11 GCR AUC T1 (pg-µmol/mL^2)								
12 GCR AUCwbcs T1 (pg-nmol/cell-L)								
13 BMI T1(kg/m^2)								
14 cIL6 T1 (pg/mL)[a]								
15 Months Since Diagnosis T2								
16 BDI T2								
17 STAI-S T2								
18 IES T2								
19 GCR AUC T2 (pg-µmol/mL^2)								
20 GCRwbcs AUC T2 (pg-nmol/cell-L)								
21 BMI T2 (kg/m^2)								
22 cIL6 T2 (pg/mL)[a]								
23 Months Since Diagnosis T3	1.000							
24 BDI T3	-0.086	1.000						
25 STAI-S T3	0.018	0.780**	1.000					
26 IES T3	0.033	0.602**	0.591**	1.000				
27 GCR AUC T3 (pg-µmol/mL^2)	-0.377**	0.222*	0.262**	0.212*	1.000			
28 GCR AUCmonos T3 (pg-nmol/cell-L)	-0.302**	0.168*	0.239**	0.274**	0.890**	1.000		
29 BMI T3 (kg/m^2)	0.007	0.031	-0.013	0.037	-0.231*	-0.216*	1.000	
30 cIL6 T3 (pg/mL)[a]	0.018	0.042	0.078	0.132	-0.036	-0.072	0.328**	1.000

Notes: *$p < .05$ **$p < .005$; BMI = Body Mass Index; W = White; AA = African American; I = Intervention; C = Control; BDI = Beck Depression Inventory; STAI-S = State Trait Anxiety Inventory - State; IES = Impact of Events Scale; GCR = glucocorticoid resistance; AUC = Area under the curve; cIL-6 = circulating levels of interleukin-6; [a]valid inflammation data only

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