A Deep Breathing Intervention for Stress Reduction in 5-Year-Old Children

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Abstract

This multi-manuscript dissertation concentrates on child stress, an important area of attention for social workers. Many children we work with are exposed to chronic stressors such as poverty, child maltreatment, and other forms of stressors and/or trauma. These experiences can be damaging to a child's development, especially if they occur early in life, and the effects may be long-lasting. The first manuscript provides an overview of the human stress response and its potential deleterious effects on child brain development. It highlights specific brain regions affected by stress, and possible physical and mental health consequences of stress later in life.

Building on this knowledge, and acknowledging the importance of the biological underpinnings of stress, the second manuscript examines the ways in which physiological stress is being measured in school-based intervention research. 20 studies that used physiological measures of stress, over the last ten years, identified the following physiological stress measures: salivary cortisol (11 studies), serum cortisol (1 study), heart rate (4 studies), ambulatory heart rate (2 studies), heart rate variability (2 studies), resting blood pressure (3 studies), ambulatory blood pressure (3 studies), sodium handling (2 studies), alpha amylase (1 study), and skin conductance (1 study). I discuss each biological measure, its role in the human stress response, sensitivity to intervention effects and feasibility issues of each measure in a school setting.

The third manuscript presents results of a deep breathing intervention for stress reduction in 5-year-old children using a multiple-baseline across individuals design. Five children in pre-kindergarten or kindergarten received a 5-minute deep breathing

intervention daily for four weeks. Salivary cortisol, pulse rate and a perceived stress faces scale were used to measure stress. Although results were inconclusive, I found that deep breathing may be beneficial for stress reduction in some children and five-year-olds are able to participate in a deep breathing intervention. A scripted deep-breathing protocol is presented and limitations for stress measurement with young children and barriers to intervention delivery in a school setting are discussed.

Table of Contents

Introduction 1

References 9

Chapter I: Stress in children: Neurodevelopmental perspective on a cross-cutting social work issue 16

Abstract 17

Introduction 18

Stress in children 21

The human stress response 23

Allostasis, allostatic load, & allostatic overload 25

Brain plasticity & child stress 26

How early life stress influences brain development 27

Limbic system 30

Hippocampus 31

Amygdala 33

Medial prefrontal cortex 33

From stress to illness 34

Conclusions 36

References 37

Chapter II: The use of stress biomarkers in school-based intervention programs 48

Abstract 49

Introduction 50

Methods....52
Results....54
Discussion....54
Cortisol....54
Cortisol measurement....56
Stress reactivity in children....59
Sensitivity, reliability, and validity – cortisol....60
Feasibility considerations – cortisol....65
Blood pressure (BP)....66
BP measurement, reliability & validity....67
Sensitivity – BP....69
Heart rate and heart rate variability (HRV)....69
Heart rate and HRV measurement, reliability, & validity....71
Sensitivity – heart rate/HRV....72
Feasibility considerations – BP & heart rate....73
Sodium handling....75
Sodium handling measurement....76
Feasibility considerations – sodium handling....76
Skin conductance....77
Skin conductance – measurement & feasibility....77
Alpha amylase....78
Alpha amylase measurement, feasibility, & reliability....78

Conclusions and future research 79

References 84

Appendix A 106

Chapter III: A deep breathing intervention for stress reduction in 5-year-old children 111

Abstract 112

Introduction 113

Methods 125

Participants 127

Measurement 128

Selection measures 128

Outcome measures 131

Procedures 135

Treatment fidelity 136

Data analysis 137

Results 138

Colin 140

Christopher 141

Anna 143

Jake 144

Sarah 145

Discussion 146

Conclusions/future research 151

References.....153

Appendix B.....168

Conclusion.....174

Knowledge gaps/future research.....178

References.....183

Vita.....187

List of Tables

Table 1-A. School-Based Intervention Studies Using Stress Biomarkers…….107

Table 1-B. Morning and Afternoon Salivary Cortisol Concentrations for Pre- and Post-Intervention and Normative Cortisol Values for Age and Time of Collection…….169

List of Figures

Figure 1-B. Pulse Rate 170

Figure 2-B. Pulse Rate Difference 171

Figure 3-B. Perceived Stress 172

Figure 4-B. Perceived Stress Difference 173

Introduction

It is well-documented that stress in childhood can lead to deleterious mental and physical health outcomes later in life. Chronic stressors such as poverty, abuse, neglect and exposure to violence are particularly damaging, especially in the absence of a supportive caregiver (Loman & Gunnar, 2010). However, many children experience potentially harmful types of stress, even without these experiences. Bullying is on the rise (U.S. Department of Education, 2016), high-stakes standardized testing is prevalent (Duckworth, Quinn, & Tsukayama, 2012) and less emphasis is being placed on activities that may reduce stress, such as physical education and recess (Slater, Nicholson, Chriqui, Turner, & Chaloupka, 2012).

It is of particular importance to assess how a neurodevelopmental approach can inform early interventions that may reduce the effects of stress on children. It was once thought that once a person reached early adulthood, the brain was fully formed and no longer amenable to changes, although it is now clear that the human brain is malleable throughout the lifespan (Bavelier, Levi, Li, Dan, & Hensch, 2010) and that interventions that change neural processes are best performed during periods of development when brain plasticity is highest. Specifically, there are periods during early childhood and adolescence during which brain plasticity is high and interventions can have the largest preventative impact (Bradshaw, Goldweber, Fishbein & Greenberg, 2012; Zeanah et al., 2003). For example, intervention during the pre-school years, a period that is particularly sensitive to early life events, can be effective in reducing behavioral and/or academic problems in later years (Gunnar & Fisher, 2006; Shonkoff, 2009).

Findings associated with the neurodevelopmental model and its relationship to stress are of particular interest to Social Workers. The mission of the social work profession is to focus our work with individuals who are vulnerable and oppressed (NASW, 2017). Impairment of functioning due to stress is particularly prevalent in vulnerable groups. For example, individuals living in poverty have some of the highest levels of stress (Blair, 2010). Children in poverty are particularly susceptible to adverse outcomes related to stress (Evans et al., 2005; Evans & Schamberg, 2008,), as are children who have been placed in out-of-home care because of neglect or abuse, who have experienced domestic violence, or who have experienced other types of stressors or trauma (Blair, 2010; Bremner & Vermetten, 2001; Fisher, et al., 2006). Adverse outcomes associated with these environmental insults include future mental and physical health problems that can continue throughout the lifespan.

This dissertation has the following aims: 1. To examine child stress, particularly how stress impairs brain structure and function, thus leading to future physical and mental health difficulties, 2. To enhance our understanding of the best ways to measure stress in children in a school-setting, and 3. To test a simple, cost-effective, deep breathing intervention in a school setting in that may reduce stress in five-year-old children.

Chapter 1 offers a conceptual overview and framework for understanding child stress and its detrimental effects, an explanation of the physiological processes involved in the human stress response, and a discussion of how these processes may cause significant negative outcomes. Stressors in early life, especially in the first few years,

impair brain development and the body's physiological response to stress, causing dysregulation of the stress response system (Bremner & Vermetten, 2001). Dysregulation occurs when the stress-response system does not function properly and physiological responses are heightened. This dysregulation can have a long-term impact on social-emotional and cognitive functioning (Bradshaw, Goldweber, Fishbein & Greenberg, 2012). Researchers document that early, preventative interventions for stressed children are significantly more effective than reactive services, and in some situations, may actually reverse damage to the brain caused by stress (Perry, 2009; Thompson, 2014).

In order to translate this knowledge to intervention development, we need to understand how best to measure stress in children, including in out-of-lab settings, such as schools. Use of biological measures has become easier in recent years (i.e. measuring stress hormones through saliva), although feasibility issues still exist. *Chapter 2* offers a literature review examining use of biological measures in school-based intervention research. The research question is as follows: What biological measures are being used to assess stress intervention effectiveness in schools and are these measures sensitive to intervention effects? I also discuss feasibility issues of implementing such measures in a school setting. Since much of social work intervention research takes place outside of traditional research laboratories, examining use of biological measures in these settings is important. Schools are an effective and efficient venue to reach a large number of potentially underserved children. Many children who experience emotional and behavioral issues do not receive treatment (Bratton, Ray, Rhine, & Jones, 2005) so

school-based interventions are a great way to meet the needs of children whose families would not otherwise access services, as well as focus on preventative efforts.

Specifically, mind-body interventions with younger children may fill a gap of lack of strong evidence-based treatment for child stress. Mind-body integrative practices are becoming more assimilated into western culture, with many individuals seeking complementary and alternative approaches to physical and mental health care. For example, a recent *New York Times* article discussed the benefits of mindfulness in children, noting increases in attentiveness and decreases in stress and increased popularity (Holland, 2015). Cognitive Behavioral Therapy (CBT) is sometimes used with younger children, particularly with anxiety and/or depression. Grave and Blissett (2004) question the use of CBT with younger children, however, particularly ages 5-8, and note that limitations in executive functions impede effective problem solving, which may therefore likely diminish these children's capacity to engage in the type of cognitive operations required in traditional CBT. Children who experience stress are more likely to have deficits in executive functions, which may make this type of therapy even more inappropriate. Although a child's unique developmental stage and abilities need to be considered, it may be unlikely for a child under seven to have the analytical ability and abstract thought to engage in CBT or other talk therapies. Mind-body interventions may be a useful alternative.

Mind-body interventions can have a preventative impact on the stress response system and stress-related deficits. Meditation has been shown to increase brain activation in the medial prefrontal cortex in adults, which suggests an improved ability for emotion

regulation (Hölzel et al., 2007; Farb et al., 2007). Deep slow breathing improves autonomic functioning by increasing parasympathetic activity and decreasing sympathetic activity, specifically, improving vagal tone therefore decreasing heart rate and blood pressure (Mohamed, Hanafy, & El-Naby, 2013; Pal, Velkumary & Madanmohan, 2004). Vagal tone is viewed as a marker of various types of psychological adjustment in infants, children and adults and is measured by respiratory sinus arrhythmia (RSA), or the fluctuations in heart rate during respiration (Beauchaine, 2001). Studies employing physiological and psychosocial measures have suggested that these interventions may be effective with children as well (Barnes, Davis, Murzynowski, & Treiber, 2004; Bothe, Grignon, & Olness, 2014) although they have been studied less in younger children.

Coping skills in childhood are linked to future psychological adjustment, particularly related to common daily stressors (Rutter, 1994). Dubow and Tisak (1989) demonstrate that a "toolbox" of coping skills can influence young children's responses to negative events. Daily hassles, defined as the "irritating, frustrating, distressing demands that to some degree characterize everyday transactions with the environment" (Kanner, 1981, p.3) have been identified as perhaps even stronger predictors of well-being than stressful life events (Lazarus & Folkman, 1984) so access to coping skills to alleviate the impact of these events is paramount.

In an attempt to isolate one technique that seems to be an integral part of relaxation and mind-body protocols, *Chapter 3* presents a deep breathing intervention study for 5-year-old children experiencing stress. Pre-school age children were targeted

due to the dearth of literature that exists related to stress intervention for this age group, and teaching an easy, portable coping skill may help children manage stressful situations and possibly prevent development of stress-related problems later in life. Although deep breathing protocols exist, for example as a component of Trauma-Focused Cognitive Behavioral Therapy (Hendricks, Cohen, Mannarino, & Deblinger, (n.d.) the effectiveness of these protocols are not examined separately from the context of other treatment components. In other words, we don't know if a deep breathing intervention alone will reduce stress in preschoolers. A single-subject design was used to test the hypothesis that a deep breathing intervention would decrease physiological and perceived stress in five-year-old children. Stress was measured by salivary cortisol, heart rate, and a perceived stress scale. Deep breathing as a sole treatment component may be effective for a variety of stress-related health issues, although there is not sufficient research to produce definitive evidence, particularly in children.

There are many advantages to deep breathing. It is easily implemented in a school setting. It can be done in a relatively short amount of time, thus minimizing disruption of the school day, on an individual basis or in a group setting. Deep breathing can be implemented with minimal instruction and can be conducted by teachers or other school staff. It does not require advanced cognitive abilities, making it especially promising for younger children.

Breathing exercises may counteract irregular breathing patterns of those who are experiencing stress and/or anxiety (Park, 2013). Breathing techniques are incorporated into, and adjuncted with, many treatment modalities including Cognitive Behavioral

Therapy (CBT), Trauma-Focused CBT, Mindfulness-Based Stress Reduction (MBSR), yoga, guided imagery, progressive muscle relaxation, music therapy, meditation, biofeedback, hypnosis and others.

In adults, studies have measured the effect of deep breathing on both physiological and psychosocial stress (Chicayban & Malagris, 2014, Dissanayake, Liyange, & Senanayake, 2014; Nida et al., n.d.; Seppala et al., 2014), while many focused solely on psychosocial stress with a variety of stress-related outcome measures. Studies found positive effects in overall anxiety (Baker, 2012, Nida et al., n.d.; Seppala et al., 2014), test anxiety (Paul, Elam, & Verhulst, 2007), general perceived and psychosocial stress (Arasumani & Kuppusamy, 2013; Baker, 2012; Dissanayake, Liyange, & Senenayake, 2014; Hayama & Inoue, 2012; Miller & Spence, 2013; Parsotam, 2009; Thorat, 2014; Williams, 2014) psychological distress (Busch et al., 2012; physical fatigue (Hayama & Inoue, 2012) and PTSD scores (Seppala et al., 2014).

In children, less is known. Although deep breathing has been used as part of manualized-based treatment protocols (i.e. MBSR; TF-CBT) or with other types of mind-body interventions (such as guided imagery and meditation), deep breathing research as a sole intervention with children is limited. No known deep breathing studies exist for children under age eight that examined variables other than pain. Few exist even with older children. There is some preliminary evidence that deep breathing may be an effective treatment modality, although it is inconclusive. In high school students, deep breathing was found to reduce overall anxiety, depression and test anxiety (Akinsola & Nwajei, 2013), improve impulse control (Gains & Barry, 2008) and reduce music

performance anxiety in 3rd-6th graders (Su et al., 2010).

This gap in knowledge provides an opportunity to explore the use of deep breathing with younger children in a school-based setting. This work aligns with the first of American Academy of Social Work and Social Welfare's (2016) Grand Challenges for Social Work, to "ensure healthy development for all youth" and promotes "social progress powered by science" (American Academy of Social Work and Social Welfare, 2016).

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Chapter I
Stress in Children: Neurodevelopmental Perspective on a Cross-Cutting Social Work Issue

Abstract

Child stress should be an important focus for social workers. Many children we work with are exposed to chronic stressors such as poverty, child maltreatment, and other forms of stressors and/or trauma. These experiences can be damaging to a child's development, especially if they occur early in life. This chapter provides an overview of the human stress response and its deleterious effects on child brain development. I highlight specific brain regions affected by stress, as well as outline possible physical and mental health consequences later in life. This chapter highlights the need for social workers to understand stress as a physiological construct. Moreover, social workers should recognize how this research is key in informing social work interventions, particularly as it relates to early intervention.

It is difficult to conceive of an area of social work practice that is not related in some way to stress. Child abuse and its many damaging effects, mental illness, disease and physical illness, and certainly poverty and discrimination all impose high levels of stress on the human body, costing billions of dollars and wasting untold human potential. New understanding of the effects of stress on physiology provides a mandate for social work to understand and attend to stress, especially when it occurs in early life.

A neurodevelopmental perspective has been limited in social work until recently. Matto and Strolin-Goltzman (2010) discuss the importance of integrating neuroscientific principals into the development of psychosocial interventions and explain how social workers can play a key role in interdisciplinary neuroscience research, thus helping to facilitate more brain-based interventions for clients. Anthony, Williams, and LeCroy (2014) support the idea that new neuroscience knowledge should inform practice interventions, and highlight how these findings enhance our understanding of adolescent development, as well as implications for practice.

Egan, Combs-Orme, and Neely-Barnes (2011) offer a case-based model for integrating neuroscience content into social work education, citing that students armed with this knowledge are better prepared for practice. Lefmann and Combs-Orme (2013) further support this integration with a discussion of how to incorporate knowledge of brain development, a critical component of the bio-psychosocial model, into a classic model of development (Piaget) that is typically taught to social workers. Similarly, Sayre and Walker (2014) discuss the relevance of neuroscience to evolutionary theory and discuss its compatibility with social work frameworks and values. In addition, two recent

books highlighting neuroscience content applicable to social work practice and the human services have been authored (Farmer, 2009; Johnson, 2014).

Montgomery (2013) supports the assertion that neuroscience findings are critical to the development of social work practice, but cites some challenges. Most social workers and social work educators do not have strong backgrounds in biology or neuroscience and much of the research, with its technical hard-science language, can be daunting. Keeping current with ever-changing neuroscience research is also a challenge. Moreover, the relevance and importance of neuroscience to social work practitioners is not obvious to all. The slow but existing influx of neuroscience-related literature in social work journals geared towards practitioners and educators should enhance social workers' understanding of this important link.

Stress is of particular interest to social workers because of the constituency we serve. For example, Blair (2010) suggested that individuals living in poverty have some of the highest levels of stress, and children in poverty are particularly susceptible to adverse outcomes related to stress (Evans, Gonella, Marcynyszyn, Gentile, & Salpekar, 2005; Evans & Schamberg, 2008), as are children in out-of-home care because of neglect or abuse, who have experienced domestic violence, or who have experienced other types of stressors or trauma (Blair, 2010; Bremner & Vermetten, 2001; Fisher, Gunnar, Dozier, Bruce, & Pears, 2006). More than six million American children under the age of six experience the stress of poverty every year (National Center for Children in Poverty, 2015); over half a million spend time in foster care (U.S. Department of Health and

Human Services, 2015). World-wide, millions of children experience the stress of war, famine and hunger, and natural disasters.

Stress is a construct that is defined differently across the literature; the terms *stress*, *crisis* and *trauma* often overlap in meaning (Yeager & Roberts, 2003). Chronic stress, crisis and trauma may have similar physiological responses; however, chronic stress is conceptualized to occur over time, while a crisis or traumatic event is more acute. In some cases, an acute event may be followed by a lengthy period of chronic stress, such as occurred with Hurricane Katrina. Severe chronic or toxic stress can lead to Post-Traumatic Stress Disorder or more commonly, post-traumatic stress symptoms (PTSS; Perry, 1999). Although chronic or toxic stress is not identified as a "disorder" in and of itself, its neurobiological effects can present serious mental and physical health effects across the lifespan. A clear example of this is the Adverse Childhood Experiences Study (ACE Study; Felitti et al., 1998), a large-scale collaboration between Kaiser Permanente and the Center for Disease Control (CDC), discussed later in this manuscript.

Of course, additional individual factors are prevalent in the impact of the stressor or traumatic event, both psychologically and physiologically. These include previous trauma history, pre-existing neuropsychiatric disorders, cognitive abilities, coping skills, and social support, among others (Perry, 1999).

While the term *stress* is often used loosely to indicate *distress* or *distressing events*, in this manuscript we discuss the physiological state of stress, "a state in which an organism's internal regulatory balance (or homeostasis) is disturbed by real or perceived challenges in its external environment" (Ali & Pruessner, 2012, p. 65). Dysregulation can

occur after a single traumatic episode or due to ongoing stressors over time. Although *stress* and *anxiety* are sometimes used as the same construct, they are different: excess stress may (or may not) lead an individual to experience anxiety (Smith, Hancock, Blake-Mortimer, & Eckert, 2007). Nevertheless, chronic or toxic stress on its own can have a detrimental impact on development, especially in children. For example, in a study of adolescent rats, exposure to chronic, unpredictable stress increased anxiety symptoms about six months later (Chaby, Cavigelli, Hirrlinger, Carusi, & Braithwaite, 2015). In a study with pregnant rats, noise stress (or sound pollution) increased anxiety symptoms and levels of corticosterone (equivalent to cortisol in humans) in the maternal rat and her offspring (Barzegar, Sajjadi, Talaei, Hamidid, & Salami, 2015). This is important, because noise pollution is one of the many stressors many poor children and families deal with on a daily basis.

This paper provides an overview of stress in childhood, when it is most dangerous, briefly discussing a framework for understanding children's stress, explaining the physiological processes, and discussing how these processes may cause significant negative outcomes. In this way, I respond to the American Academy of Social Work and Social Welfare's (2016) Grand Challenges for Social Work, promoting "social progress powered by science."

Stress in Children

Although there is a common belief that childhood is a carefree time, children do experience stress and its harmful consequences. A conceptual framework proposed by the National Scientific Council on the Developing Child lays out three specific types of

childhood stress: positive, tolerable, and toxic (Shonkoff et al., 2012). Positive stress, an important part of healthy development, includes normal daily frustrations and challenges, which stimulate physiological fluctuations in heart rate, blood pressure and cortisol levels. Positive stress might include school exams or conflict with friends, and it can contribute to growth, particularly when it occurs in the context of caregiver support. Tolerable stress includes more potentially disruptive events such as natural disasters, chronic violence, or death of a friend or family member. This type of stress has the potential to alter brain architecture through cortisol- (commonly known as the "stress hormone") induced damage, but can be buffered by social support.

Toxic stress, according to the National Scientific Council on the Developing Child (Shonkoff et al., 2012, p. e236) is "strong, frequent, or prolonged activation of the body's stress response systems in the absence of the buffering protection of a supportive, adult relationship." Toxic stress produces the most negative impacts on early brain development and dysregulation of the physiological stress response (Shonkoff et al., 2012). Impairment of brain structures has been noted in various studies related to child abuse and neglect, poverty, and exposure to domestic and community violence (Blair, 2010; Blair & Raver, 2012; Bremner & Vermetten, 2001).

Although there are ways to objectively measure anxiety (DSM diagnostic criteria, anxiety scales, etc.) in both children and adults, measuring stress is challenging, especially in young children. Self-report measures are virtually non-existent for younger children. It is unclear if young children can self-report stress and if so, what is the best way to measure this? Parent reports are often used, which measure stressful (or more

properly *distressing*) life events, such as the Coddington Life Events Scale (Coddington, 1972) or daily stressors/hassles, such as the Children's Hassles Scale (Kanner, Feldman, Weinberger, & Ford, 1987) which may be an even stronger predictor of well-being than stressful life events (Lazarus & Folkman, 1984). Physiological measures of stress, such as salivary cortisol and heart rate variability, can be relatively easy to use with young children and should be incorporated into future research studies of child stress.

The Human Stress Response

The human stress response is a complex set of physiological processes designed to enhance survival in the face of threat. It enhances physiological processes that promote survival, down-regulating other processes until the threat is over. When an individual perceives a threat (and this may not be a conscious, explicit cognition), the stress response system is activated via two neural systems originating in and signaled by the hypothalamus, an area of the brain that governs hormone expression: the sympathetic adrenomedullary system (SAM) and the Hypothalamic-Pituitary-Adrenal (HPA axis). The SAM then produces epinephrine (or "adrenaline") and norepinephrine, orchestrating what is commonly known as the *fight-or-flight* response (Gunnar & Quevedo, 2007). SAM's role is to support arousal and vigilance (Gunnar & Quevedo, 2007). The heart beats faster, blood pressure rises, and energy rushes to the muscles; other processes not critical to immediate survival (such as digestion, reproduction, and higher-order thought) are suppressed to conserve energy.

Activation of the other system, the HPA axis, begins when neurons in the hypothalamus produce a hormone that triggers the adrenal gland to release cortisol, a

glucocorticoid often called the *stress hormone*. Cortisol distributes to various parts of the brain, including the hypothalamus, hippocampus, pre-frontal cortex and amygdala, completing a feedback loop and starting the process of return to homeostasis (physiological stability). However, dysregulation of the HPA axis due to chronic stress may cause the body to become insensitive to cortisol and remain in a constant stress state, even when threat is past. This dysregulation can damage brain structure as well as function (discussed below) (Davidson & McEwen, 2012).

In healthy people, there is usually a strong physiological reaction to threat, then a quick return to baseline levels. A person whose stress response is impaired may either not respond adequately to the threat (blunted response) or require a longer recovery period. That is, dysregulation of the stress response can cause either over- or under-production of cortisol, both of which are problematic to physical and mental health. Cortisol typically follows a diurnal pattern, with peak levels in early morning to facilitate waking and a decline throughout the day until time to sleep. In some cases, individuals have lower than normal daytime levels, levels that do not follow this diurnal pattern, and/or blunted response to stressors. Gunnar and Vasquez (2001) reported that in several neuroendocrine studies of institutionalized children, lower cortisol levels as well as decreased cortisol reactivity to stressors were noted, contrary to expectations.

Age and gender have varying influences on cortisol secretion, as do other factors, including previous trauma history, pre-existing neuropsychiatric disorders, cognitive abilities, coping skills, and social support, among others (Perry, 2002). Children in low socio-economic status (SES) environments are particularly at risk for impaired brain

development due to stress (Hackman, Farah, & Meaney, 2010). Growing up in a low SES environment is a major, early life stressor in and of itself, due to substandard housing, higher community crime rates, and food insecurity.

Allostasis, Allostatic Load, and Allostatic Overload

Allostasis is the body's process of returning to homeostasis or physiological stability through adaptive actions (McEwen, 2008), such as sweating when body temperature is too high or shivering when it is too low. Although the stress response is necessary for humans to survive in the face of threat, chronically stressful environments increase *allostatic load*, the physiological costs incurred from adaption. For example, the increased energy shunted to the muscles in the face of threat uses calories; higher blood pressure challenges the arteries; and down-regulation of the digestive system may cause digestive problems. Although allostasis is normal and necessary, *allostatic overload* occurs when stress exceeds the body's coping resources, and poor health and chronic disease may result (McEwen, 2008).

Researchers hypothesize that allostatic overload is correlated with chronic environmental stressors in children, particularly children in poverty (Blair, 2010). Early life stress compromises functioning of the HPA axis and maintenance of a stable equilibrium, so that the risk of allostatic overload is higher. Children are at particular risk because they are still developing; their systems may adapt to stress by organizing to meet this constant need. The impact on the HPA axis has been shown to affect individuals throughout the lifespan, and to increase risk for various diseases, such as heart disease, type II diabetes, and alcoholism (Francis, Caldji, Champagne, Plotsky & Meaney, 1999).

Stress vulnerability can also emerge as a product of generational transfer via changes in genetic expression (Champagne & Mashoodh, 2009). This issue is discussed in more detail below.

Brain Plasticity and Childhood Stress

The first potential impact of stress occurs in utero. If a pregnant woman is exposed to either a single or repeated episode of stress, this increases her glucocorticoid secretion, which can be transferred to the fetus through the placenta (Lupien, McEwen, Gunnar & Heim, 2009). This transfer may increase HPA axis activity in the fetus, modifying brain development (Lupien et al., 2009). The good news is there is evidence that quality of postnatal care can moderate the effects of prenatal stress (Lupien et al., 2009).

Specific periods during early childhood and adolescence when the brain is developing most rapidly are called *sensitive periods,* "periods during which an experience (or its absence) has a more marked impact on the neural organization underlying a particular skill or competence" (Gunnar & Quevedo, 2007, p. 153). Because of the rapidity of brain development *in utero* in the first few years of life, chronic stress is especially damaging during this time (Gunnar & Fisher, 2006). The brain develops in a sequential fashion, from bottom to top and inside out; each more complex structure is built on the integrity of earlier ones. For example, preclinical (animal) research indicates that high levels of stress may influence neurogenesis, neuron migration, myelination, and other basic processes that are necessary for further brain development (see review by Dackis et al., 2012).

Brain regions related to executive function (cognitive flexibility, working memory, goal-directed behavior, and self-control) and stress regulation, which in turn affect cognitive development and susceptibility to learning disorders and psychopathology, are predominantly at risk due to high levels of childhood stress (Blair, 2010; Bradshaw, Goldweber, Fishbein, & Greenberg, 2012; Perry, 2009). Perry et al. (1995) emphasized that whereas the adult brain responds to trauma by encoding a fear state into memory, a developing brain organizes around the fear state, leading to traits.

Sensitive periods for different neural circuits and brain areas occur at different ages (National Scientific Council on the Developing Child, 2012; Perry, 2002). For example, young children who experienced institutionalized care in Romanian orphanages were subject to social deprivation due to low levels of care, causing lower levels of cognitive function and other psychological and behavioral issues. Those removed from institutional care at younger ages showed less severe cognitive deficits (Davidson & McEwen, 2012). Overall, maturity of the brain dictates how stimuli are received and will damage brain development.

How Early Life Stress Influences Brain Development

Much of what we know about stress derives from pre-clinical (animal) studies, particularly the impact of early life experiences on the physiological stress response. There are many barriers to experimental studies with children and the physiological effects of stress in terms of invasiveness, ethics, and research design barriers (Cicchetti & Gunnar, 2008; Gunnar & Fisher, 2006). Despite widespread belief that animal models are not relevant for humans, similarities between mice and humans are remarkable. On

average, we share about 85% of coding DNA with mice, and animal research has been able to identify genes with similar functions in both species, called "homologues" (National Human Genome Research Institute, 2015).

One commonality between mice and humans is that exposure to long-term, chronic stress is linked to negative impacts on specific neural systems, particularly the HPA axis (Bremner & Vermetten, 2001; Fisher et al., 2006). Animal studies show long-term negative effects of extended maternal separation (maternal absence or lower levels of care) on the HPA axis, suggesting the importance of sensitive and responsive caregiving (Fisher et al., 2006). Research also has demonstrated that early stress and poor maternal care have effects on the brain through altered gene expression, particularly genes related to the stress response system.

Meaney and colleagues' pioneering animal research is fundamental to understanding the effects of stress on children (Meaney, 2001; Weaver et al., 2004). Early in the 1990s these researchers observed mice mothers and their offspring and confirmed that the quality of parenting (defined as posture during breastfeeding and amount of licking and grooming offspring) followed a fairly normal curve. Just as is true with humans, they observed neglectful and abusive mothers at one end of the distribution (stepping on their offspring, making nursing difficult, providing low levels of physical contact), and "soccer moms" at the other end: those who provided the most nurturing care. They also found that the nature of parenting was positively correlated with stress responses in offspring. Well-nurtured mice reacted to stress less extremely and recovered

more quickly. Moreover, well nurtured female mice were more nurturing as mothers themselves.

Meaney and others demonstrated that high levels of cortisol alter expression of the gene governing glucocorticoid receptors in the hippocampus, resulting in fewer receptors and thus higher levels of circulating glucocorticoids that may dysregulate the stress response system. Good nurturing activates genes that promote formation of corticosteroid receptors in the hippocampus; more receptors are able to take up more stress hormones for a healthier response and quicker recovery. Meaney (2001) showed that the stress induced by poor maternal nurturing was responsible for these effects by observing similar effects when synthetic stress hormones were administered, and by cross-fostering offspring of poorly- and well-nurturing mothers.

Although many aspects of Meaney and colleagues' research may not be directly replicated in humans, researchers are finding new ways to examine parallels in humans. The relatively recent development of functional magnetic resonance imaging (fMRI), which involves lower radiation exposure and fewer health risks than earlier scanning methods, has improved researchers' capability to image human brains (Carrion & Wong, 2012). That brain tissue of deceased abused suicide completers shows similar effects to those observed in stressed mice provides further evidence of the similarities of the effects of early stress on mice and humans (McGowan et al., 2009). Moreover, technological advances in genetic analysis now enable testing of changes in human genes with similar functions to genes that have been demonstrated to change in animals (homologues; Romens, McDonald, Svaren, & Pollak, 2015). For example, Romens et al. (2015)

demonstrated differences in abused children's glucocorticoid receptor gene expression that mirror findings with mice. These advances should help researchers continue to develop safe protocols for neuroscience research with children and adolescents, with less reliance on animal studies.

The following sections briefly discuss some of the specific effects of early stress to several inter-related neural structures.

Limbic System

In humans, the limbic system in the lower brain is critical to emotional memory and expression. It begins development in gestation and is dominant over the more rational and regulatory prefrontal cortex (PFC) until the PFC is fully mature in young adulthood. After that the two systems work together to use emotional memory as critical information for decision-making. The limbic system is particularly susceptible to high stress because it has a high density of glucocorticoid receptors, and glucocorticoids are believed to have strong organizing effects on the brain (Dackis et al., 2012).

Teicher (2000) examined "limbic irritability" (a collection of symptoms such as somatic and perceptual distortions, brief hallucinations, and dissociation), thought to indicate hyper-sensitivity to threat. He reported the symptoms to be 113 percent greater in those who experienced both childhood physical and sexual abuse. Teicher, Samson, Polcari, and McGreenery (2006) also reported that limbic irritability was significantly linked to emotional abuse, in addition to physical and sexual abuse, and that those who were exposed to two or three types of abuse were even more affected. Limbic irritability has been linked to increased hypervigilance and dysregulated stress response, as well as

aggressive behavior and dysphoria (Teicher, 2000). Damage to the limbic system during sensitive periods is believed to create vulnerability to psychiatric disorders (Dackis et al., 2012), in part by increasing children's vigilance, and thus responses, to threat (Shackman, Shackman & Pollak, 2007).

Hippocampus

The hippocampus is located in the limbic system (the medial temporal lobe), and its main function is information processing, learning, and memory (Gould & Tanapat, 1999; Mizomuri, Smith & Puryear, 2007). This critical part of the brain is especially sensitive to stress because environmental stressors elevate cortisol and stimulate release of glutamate, inhibiting neurogenesis in the part of the hippocampus that formulates new memories (the dentate gyrus) and resulting in production of fewer receptors to take up cortisol in the hippocampus (Gould & Tanapat, 1999). High levels of stress are associated with decreased volume in the hippocampus (Davidson & McEwen, 2012). Memory and learning functions of animals exposed to chronic stress due to absent maternal care are degraded (Gould & Tanapat, 1999; McEwen, 1999). More attentive maternal care in the first ten days of life also results in increased glucocorticoid receptors in the hippocampus and decreased corticotropin-releasing hormone (CRH), programming the HPA axis to respond more favorably to later stress (Liu et al., 1997).

Luby et al. (2012) extended these findings to humans, examining brain images of 92 children, ages 7-13. Controlling for variables that influence hippocampal volume (sex, depression severity, maternal depression, and stressful life events), they found early life maternal support (positive parent interaction at ages 3-5) to be correlated with larger

hippocampal volumes in non-depressed children. They hypothesized that the negative impact of depression impeded positive effects of maternal support, since effects were not found in depressed children. They had no retrospective data on parenting quality from birth to three, a period critical to brain development, so the quality of maternal care during this time may have influenced these findings. However, parenting tends to be fairly stable over time (Dallaire & Weinraub, 2005).

Some adults diagnosed with Post Traumatic Stress Disorder (PTSD) have smaller hippocampi, although several MRI studies with children and adolescents have demonstrated different results (see review by McCrory, De Brito, & Viding, 2010). A meta-analysis by Woon and Hedges (2008) examined eight cross-sectional studies of hippocampal volumes, including 105 children (ages 11-13) with maltreatment-related Post-Traumatic Stress Disorder (PTSD) and 160 healthy controls, and 65 adults with child maltreatment-related PTSD and 66 healthy controls. They found deficits in left and right hippocampal volumes in adults, but none in children, suggesting hippocampal damage from maltreatment-related PTSD may not become apparent until later in life. An alternate theory with support is that a smaller hippocampal volume is a risk-factor for the development of PTSD. For example, Gilbertson and colleagues (2002) examined monozygotic twin pairs, one who experienced military combat and subsequent PTSD and one who did not, and found similarly small hippocampal volumes in the twin that did not experience combat compared to those who did experience combat but did not develop PTSD, which supports a pre-existing vulnerability.

Carrion, Weems, and Reiss (2007) however, did find evidence of hippocampal reduction in a clinical sample of 15 maltreated children (ages 8-14 years) in a longitudinal study. MRI scans and salivary cortisol levels revealed that hippocampal reduction was predicted by severity of PTSD symptoms and baseline cortisol levels, 12-18 months after baseline measures.

Amygdala

The amygdala is located in the limbic system next to the hippocampus. It attaches emotion to sensory information and relays this information to the stress response system, even before the higher "thinking" parts of the brain register the threat. Larger amygdala volumes have been found in both children and adults who have experienced significant stress in childhood. Larger amygdala volume is associated with anxiety in children, adolescents, and adults (DeBellis et al., 2000; MacMillan et al., 2003; Barros-Loscertales et al., 2006) and depression in adults (Frodl et al., 2002).

Tottenheim et al. (2010) examined children in institutional care and found amygdala size to be associated with age adopted out of the system. Lupien et al. (2011) reported that amygdala volumes were higher in 10-year-olds who had been exposed to maternal caregivers with depression (which is associated with poor maternal nurturing) since birth, compared to children who had not.

Medial Prefrontal Cortex

The Prefrontal Cortex (PFC) is a large brain region located in the front of the frontal lobe, also connected to the hippocampus, which regulates higher-order thinking, mood and emotion, and manages executive functioning (working memory, reasoning,

flexibility and problem solving). Because the PFC is the last region of the brain to develop, it depends upon the integrity of earlier brain development.

The PFC is the brain region most sensitive to stress (both mild acute and more chronic long-term stress) (Arnsten, 2009), and high levels of stress are associated with decreased volume in the PFC (Davidson & McEwen, 2012). Moreover, architectural changes in the PFC occur more rapidly than in other regions, perhaps even after a single occurrence of stress exposure (Izquierdo, Wellman, & Holmes, 2006). Children who experience damage to the PFC due to chronic stress may have damage to the lower structures of the brain discussed above.

The PFC is a primary region associated with early adverse experience and SES; children in low-income families may be at higher risk for difficulties with executive functions due to early stress and resulting damage to the PFC (Lipina & Posner, 2012). Van Harmelen et al. (2010) reported that children who experienced emotional maltreatment (emotional neglect and/or psychological abuse, without physical or sexual abuse) showed a 7.2% reduced volume in the medial prefrontal cortex.

From Stress to Illness

The relationship of childhood stress due to child abuse and neglect to mental illness has been known for some time. Dackis et al. (2012) reviewed research demonstrating increased risk of numerous psychiatric diagnoses in adults who were abused as children, as well as earlier onset and greater disability. Keyes et al. (2012) examined data from a large nationally representative study to correlate child maltreatment and 12 DSM diagnoses, reporting that relationships between child

maltreatment and all diagnoses were completely mediated by vulnerability to internalizing and externalizing symptoms. Moreover, abused children are more likely to develop chronic disease, as stress disrupts the body's immune system (Danese et al., 2008).

As stated above, however, child abuse is not the only form of extreme childhood stress. In 1980, Kaiser Permanente obesity clinic physicians noticed high drop-out rates in their obesity programs, though the drop-outs tended to be losing weight successfully (Felitti, 2002). Further research revealed startling rates of childhood sex abuse among these drop-outs. The researchers hypothesized that survivors of childhood sex abuse were leaving the program that had been successful for them because they feared that weight loss made them more vulnerable to abuse.

Anda, Felitti, and colleagues designed a large epidemiological study of Kaiser members (ACE study) to investigate whether the same was true with other negative childhood experiences, retrospectively measuring adverse childhood experiences and documenting members' health in adulthood, roughly a half-century later. The survey measured recurrent physical abuse; recurrent severe emotional abuse, contact sexual abuse; growing up in a household where: someone was in prison, the mother was treated violently, an alcoholic or a drug user lived, someone was chronically depressed, mentally ill, or suicidal; and where at least one biological parent was lost to the patient during childhood.

Felitti and colleagues' work showed that the accumulation of adverse experiences in childhood as measured by the total Adverse Childhood Experiences (ACE) Score was

proportionately associated with the ten most common causes of death in America, as well as with mental illness and morbidity (see summary in Felitti & Anda, 2010). This study, which is consistent with the findings of research examining outcomes of numerous stressful events during childhood, suggests that future research should look not just at child maltreatment as the independent variable and psychiatric diagnoses as the dependent variables.

Conclusions

With millions of children experiencing high levels of stress caused by living in poverty in the United States, it is particularly important to target them for prevention and intervention, due to increased brain plasticity during childhood. Increasing knowledge about the physiological effects of early stress can inform new interventions for children, particularly those whose cognitive and language skills have not developed adequately for effective psychotherapy.

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Chapter II
The Use of Stress Biomarkers in School-Based Intervention Programs

Abstract

The objective of this review is to examine the ways in which physiological stress is being measured in school-based intervention research. 20 studies that used physiological measures of stress, over the last ten years, were identified. Results are as follows: salivary cortisol (11 studies), serum cortisol (1 study), heart rate (4 studies), ambulatory heart rate (2 studies), heart rate variability (2 studies), resting blood pressure (3 studies), ambulatory blood pressure (3 studies), sodium handling (2 studies), alpha amylase (1 study), and skin conductance (1 study) were used to assess physiological stress. Further, I discuss each biological measure, its role in the human stress response, sensitivity to intervention effects and feasibility issues of each measure in a school setting.

Neuroscience research provides important insight as to how we may intervene with children who are at risk for psychosocial/behavioral problems. A neuroscience framework calls for the use of biological measures, or biomarkers, to strengthen the assessment of outcome variables in intervention research. The importance of using such a framework in schools has been documented and school-based intervention research provides a unique opportunity to intervene with large numbers of children at various levels of development, and therefore potentially high levels of brain plasticity (Bradshaw, Goldweber, Fishbein, & Greenberg, 2012).

Using biomarkers potentially offers an opportunity to assess intervention effectiveness more comprehensively than questionnaires or self-report. This may be especially important for younger children who cannot articulate subjective feelings, such as distress or anxiety, or children who have communication deficits due to disability. Research has consistently shown poor agreement across informants (i.e. parent, teacher, etc.) with regard to a child's behavioral or emotional functioning (Frick, Siverhorn, & Evans, 1994; DeLos Reyes & Kazdin, 2005), though if a child is unable to self-report, this is often what is used. There is also question as to how accurate a child's self-report may be due to issues such as social desirability bias (Miller et al., 2014).

Social workers perform a large majority of mental health interventions (NASW, 2017), therefore should be at the forefront of developing effective interventions. However, social work has been slow to incorporate biological principles into their work (Montgomery, 2013). Because most social work intervention research takes place in out-of-lab settings, such as schools, it is important to examine the utility of biological

measures in these settings. There are approximately 40,000 practicing school social workers in the US (Bureau of Labor Statistics, 2016) and school-based intervention programs have the potential to reach many school-age children.

Examining constructs such as stress and anxiety is particularly timely, given the relatively recent focus on standardized testing (Duckworth, Quinn, & Tsukayama, 2012), which has been linked with increased student anxiety (Segool, Carlson, Goforth, Von Der Embse, & Barterian, 2013) and less emphasis on recreational school activities that may reduce stress, such as physical education and recess (Slater, Nicholson, Chriqui, Turner, & Chaloupka, 2012). And although bullying has decreased in recent years, over 20 percent of students ages 12-18 report having been bullied during the school year (U.S. Department of Education, 2016).

There is a clearly established link between childhood stress and many physical and mental health challenges. The Adverse Child Experiences (ACEs) study has shown how such early adversity can damage physical and mental health (Felitti et al., 1998). For example, ACEs have been associated with greater risk of lung cancer (Brown et al., 2010), liver disease (Dong et al., 2004), alcoholism (Dube, Anda, Felitti, Edwards & Croft, 2003) and depression (Chapman et al, 2004), among other things. Armed with this knowledge, as well as an increased understanding of the neurophysiology of stress (Combs-Orme, 2013, 2017), it is critical to find ways to decrease physiological indexes of stress in schools. Schools are a practical venue for delivery of youth interventions due to access to large numbers of children and children whose families may not otherwise seek social service programs. Many children who experience emotional and behavioral

issues do not receive treatment (Bratton, Ray, Rhine, & Jones, 2005), and schools are an effective and efficient venue to reach a large number of potentially underserved children.

Here, I review intervention studies that aim to improve psychosocial functioning related to stress or anxiety and use at least one biomarker as an outcome measure to assess intervention effectiveness in a school setting. My goal is to show to what extent biological measures are being used in school-based stress-intervention research, provide an overview of the biological measures being used, describe which measures may be most sensitive to intervention effects, and discuss the feasibility of using biomarkers in school-based intervention research. Results suggest that certain biological measures can be successfully integrated into intervention protocols in schools and may provide valuable information. Cortisol, the most commonly used physiological indicator of stress (Michels et al., 2013) is not surprisingly the most often used biomarker in the school-based intervention research reviewed here. Others include heart rate, heart rate variability, blood pressure, sodium handling, alpha amylase, skin conductance, and serum cortisol, and although more research is needed to provide definitive conclusions, this review offers a current state of the literature on the use of stress biomarkers in school-based intervention research.

Methods

I conducted a comprehensive search for school-based interventions over approximately the last ten years (due to the fact that technology changes rapidly, which includes the way we assess biomarkers) that used at least one biological measure related to stress.

I selected articles published in English peer-reviewed journals and whose participants were children under the age of 18. Included were those that examined school-based psycho-social interventions as the sole independent variable and anxiety and/or stress, as measured by at least one biological measure, as the dependent variable. Not included were studies of non-psychosocial interventions such as aromatherapy or exercise. I defined "school-based" as any services provided at a public or private child-care center, pre-school, elementary school, middle-school or high school. The search was restricted to articles published between January 1, 2006, and April 30, 2017.

I searched the following databases: CINAHL, ERIC, PsychINFO, Web of Science and Social Work Abstracts and used the following search terms: (stress OR anxiety) AND (intervention OR curriculum OR training OR prevention) AND (child* OR teen* OR adoles* OR youth) AND (school* OR "learning center" OR "early intervention") AND (biomarker* OR "bio* measure*" OR "physiological measure*" OR "pulse rate" OR "heart rate" OR "heart rate variability" OR "skin conductance" OR cortisol OR "alpha amylase"). In addition, I hand-searched the reference pages of the studies found in the database search.

My research question is as follows: What biological measures are being used to assess stress intervention effectiveness in schools and are these measures sensitive to intervention effects? I also discuss the feasibility of each measure for use in school-settings based on feasibility issues identified in each manuscript. Notably, it is not the purpose of this systematic literature review to examine the interventions employed in these studies, but rather to focus on the measures employed to evaluate outcomes.

Results

Of the total 1,024 studies discovered by the search, 57 met initial criteria after title and/or abstract screening, and 33 studies remained after duplicates were removed. Of these 33, 20 met criteria for inclusion in this review.

Table A-1 (all tables and graphs are located in the appendix) provides a brief description of each of the 20 studies found, which vary in intervention type, sample size, age and measurement.

Discussion

In the 20 studies identified, the following biological measures were used: salivary cortisol (11 studies), serum cortisol (1 study), heart rate (4 studies), ambulatory heart rate (2 studies), heart rate variability (2 studies), resting blood pressure (3 studies), ambulatory blood pressure (3 studies), sodium handling (2 studies), alpha amylase (1 study), and skin conductance (1 study). I included all stress-related biomarkers that were used in school-based interventions related to stress and/or anxiety, although others do exist (for example, hair cortisol). I will briefly discuss each biological measure used in the studies in this review and discuss their feasibility in a school setting.

Cortisol

The HPA axis works in conjunction with the sympathetic adrenomedullary system (SAM) to activate the physiological stress response when stressors occur (Gunnar & Quevedo, 2007). SAM produces epinephrine, commonly known as adrenaline, and norepinephrine, which produces a “fight or flight” response (Gunnar & Quevedo). This fight or flight response orchestrates physiological responses such as increased heart rate

and/or blood pressure, among other things. The end product of the HPA axis is cortisol, which is an indicator to return to homeostasis. Cortisol can be toxic at high levels, impairing brain development via dysregulation of the HPA axis due to the body's being in a constant stress state (Davidson & McEwen, 2012). This can have major negative implications on the brain, both structurally and functionally. Specifically, dysregulation has been linked to decreased volume in a portion of the hippocampus (an area important for learning and memory; Woon & Hedges, 2008) and prefrontal cortex (PFC, the most advanced part of the brain, which is responsible for higher-order thinking and executive function; Davidson & McEwen, 2012) and both increased (De Bellis et al., 2000) and decreased (Rogers et al., 2009) size in segments of the amygdala, which regulates fear and fear memories.

We know, largely based on animal research, that exposure to long-term, chronic stress is linked to damaging impacts on neural systems, particularly the hypothalamus-pituitary-adrenal (HPA) axis (Bremner & Vermetten, 2001; Fisher, Gunnar, Dozier, Bruce & Pears, 2006). Dysregulation has been associated with many psychosocial difficulties in children and adolescents, including depression (Halligan, Herbert, Goodyear, & Murray, 2007), anger (Lupien et al., 2013), and anxiety (Howell, Rice, Carmon & Hauber, 2007). The impact on the HPA axis and physiological response to stress has been shown to affect individuals throughout the lifespan, and increase risk for various diseases, such as heart disease, type II diabetes, and alcoholism (Francis, Caldji, Champagne, Plotsky & Meaney, 1999). There is also evidence that patterns of cortisol secretion may be altered with appropriate interventions (Slopen, McLaughlin, &

Shonkoff, 2014; Fisher, Gunnar, Dozier, Bruce & Pears, 2006; Gunnar & Vazquez, 2001), due to brain plasticity, especially in childhood.

Cortisol measurement. Cortisol, the most commonly used stress biomarker (Michels et al., 2013) can be measured from blood, urine, hair, and saliva (Noppe et al., 2014), the latter being commonly used in intervention research (Fisher et al., 2016). Blood and saliva are effective in examining cortisol levels at a particular point in time, whereas urine is typically measured over a period of 24 hours (Noppe et al., 2014). Hair cortisol, a more recently developed collection method, allows measurement of chronic stress over time (Staufenbiel et al., 2012). Blood and saliva have shown to correlate with one another (Hellhammer, Wüst, & Kudielka, 2008), although the timing of the response elicited after a stressful event is slightly different and tends to occur about two minutes later for salivary cortisol (vs. plasma), which is important if measuring cortisol in response to a stressor (Gunnar & White, 2001). Salivary cortisol is viewed as a better measure of adrenocortical functioning than plasma, since saliva offers free, unbound cortisol which produces glucocorticoid effects (Jessop & Turner-Cobb, 2008). Venipuncture has shown to elicit a stress response in some participants (Weckesser et al., 2014) and salivary collection methods are considered non-stressful and easy to collect (van Andel, Jansen, Grietens, Knorth, & van der Gaag, 2014), making it a desirable option, especially for children. Commonly used is a passive drool method, where a child drools or spits directly into a test tube, or saliva swab collection. Both methods can be used with children or adults, however for children under six, saliva swabs are recommended (Salimetrics, 2017).

Neuroendocrine factors may be measured during a baseline (i.e. non-stress) period, or in response to a stressor. At either time point, diurnal fluctuation in cortisol secretion should be accounted for. A dysregulated stress system can produce either higher or lower than normal levels of cortisol, placing a child at greater risk for mental and physical health challenges. The complex nature of cortisol can make interpretation challenging. There is some evidence that type of adverse exposure may predict cortisol profile (Suor, Sturge-Apple, Davies, Cicchetti, & Manning, 2015). For example, varied family problems (Suor et al., 2015), or different types of abuse (Gunnar & Fisher, 2006) may dictate different patterns (high or low) of neuroendicrine dysregulation. Various types of psychopathology are associated with varied patterns of cortisol secretion, though this literature is inconsistent. Higher levels of cortisol have been associated with depression (Ulrike, Reinhold, & Dirk, 2013). However, a meta-analysis by Knorr, Vinberg, Kessing, and Wetterslev (2010) did not find a difference in cortisol levels when comparing depressed adults to controls. Lower cortisol has been associated with PTSD (Yehuda, 2002), however a meta-analysis found this relationship only in females or those who have experienced sexual or physical abuse (Meewisee, Reitsma, De Vries, Gersons, & Olff, 2007). More research is needed on the relationship between cortisol and different psychopathologies, including the usefulness of cortisol as a predictor of illness.

Dysregulation may appear as blunted or flat cortisol levels with lower daytime levels, or a flatter decline throughout the day (Gunnar, 2007), which is why examination of diurnal patterns is important. Cortisol follows a diurnal pattern with highest levels seen within 20-30 minutes of awakening; (this is referred to as the cortisol awakening

response or CAR) and a decline throughout the day. Low patterns of cortisol, either at awakening or throughout the day, have been associated with child maltreatment and have been highlighted in studies with children living in orphanages and thus receiving lower levels of care (Gunnar & Vasquez, 2001). This has been found in the animal literature as well, specifically with abused infant rhesus monkeys who showed suppressed cortisol levels in infancy (Sanchez, McCormick & Howell, 2015). In humans, these effects have been reversed with increased levels of care in some situations (Dozier, Peloso, Lewis, Laurenceau, & Levine, 2008).

Chronic stress, reflected in the chronic activation of the HPA axis, has been associated with higher CAR (Fries, Dettenborn, & Kirschbaum, 2009) and various health problems, although it has been suggested that CAR may not be a robust measure of stress in children (Michels et al., 2011). Findings related to CAR in children are inconsistent, and this remains and area of exploration. One reason may be due to compliance issues in collection, since a full diurnal pattern, which would include CAR, requires at-home collection and adherence has been shown to be an issue (Valentino, De Alba, Hibel, Fondren, & McDonnell, 2017). Area under the curve (AUC) calculates the average of cortisol output throughout the day, although this method is used less often in research (Khoury et al., 2015) and requires many cortisol collections throughout the day, including the CAR.

Cortisol patterns in response to a stressor provide another indicator of cortisol regulation. In a person whose stress-response system is compromised, a perceived

stressor may not elicit a typical cortisol response, in that cortisol may be blunted or may not return to normal levels in a timely fashion.

Stress-reactivity in children. Examination of stress-reactivity and cortisol may be challenging in younger children. In a review of stressor paradigms used in stress research with children, Gunnar, Talge, and Herrera (2009) found that in children ages two to five, it was difficult to elicit a stress response that increased cortisol, where out of 11 studies, only nine percent of children showed an increase in cortisol after a stressor. This may be due to a stress hypo-responsive period (SHRP), a hypothesized period in which children do not respond with cortisol reactions to a stressor (Gunnar et al., 2006), perhaps due to care elicited from supportive adults. There is evidence to suggest that stress may be buffered by supportive, nurturing and stable relationships. Healthy, supportive relationships are key to resilience and coping with stress and trauma, thus social connectedness is considered a protective factor for many forms of child maltreatment (Perry, 2009).

Meaney's work with rat pups provided a framework to understand these mechanisms (Meaney, 2001; Weaver et al., 2004). They found that rodents who provided quality maternal care (high levels of licking and grooming) had rat pups who recovered more quickly to stress, whereas rodents who received lower-level maternal care (low levels of licking and grooming and difficult nursing) didn't fare as well, showing higher levels of stress dysregulation and presenting as more aggressive or skittish. Although this research has been replicated in humans (Hostinar, Sullivan & Gunnar, 2013) the exact timing of the SHRP in humans is unknown, but it is hypothesized to end around the

transition to adolescence (Tottenheim, 2014). Longitudinal research is needed to determine how children's SHRP changes as they mature.

Factors such as age and gender may also have varying influences on cortisol secretion (Alink et al., 2008; Daughters, Gorka, Matusiewicz, & Anderson, 2013). Gunnar and Vasquez (2001) suggest that cortisol levels may vary due to developmental stage, with higher basal levels in infancy and toddlerhood leading over time to a downregulation of the HPA axis, therefore resulting in lower basal levels in older children. A meta-analysis by Alink et al. (2008) examined both basal levels of cortisol and cortisol reactivity to a stressor in children. They found no correlation between externalizing behaviors and cortisol reactivity to a stressor, but there was a small relationship between basal (baseline) cortisol levels and externalizing behavior, which was moderated by age. In preschoolers, basal cortisol levels were higher, but in elementary school students, levels were lower and no significant relationship was found for adolescents. Reactivity to stress in terms of cortisol production may be similar for males and females (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004), however some studies show males as having slightly greater reactivity (Daughters et al., 2013). Of course, additional individual factors are important in the impact of the stressor or traumatic event, both psychologically and physiologically, including prior trauma, cognitive abilities, coping skills, and social support, among others (Perry & Azad, 1999).

Sensitivity, reliability, and validity – cortisol. There is evidence to suggest that cortisol may be useful to evaluate stress-related treatment effects in a school setting. Eight out of the eleven studies reviewed here that examined salivary cortisol found some

effects. Butzer et al. (2015) examined a 10-week yoga intervention with second and third graders and found a significant decrease in salivary cortisol in the second graders but not the third graders at post-intervention. No changes were found after a single yoga session.

Stueck et al. (2016) also found significant decreases from pre-to post-intervention cortisol with four- to six-year-olds, when examining a 10-week dance and movement intervention. Specifically, significant effects were found in "high" and "medium" cortisol subgroups, yet no significant changes in the "low" cortisol group. They also found short-term effects, with significant reductions in cortisol in three out of five pre-post session collections. It is important to note that neither of these studies had control or comparison groups.

Lupien et al. (2013) examined the Destress for Success program (five sessions over approximately one month), which focuses on coping skills, and did not find a significant pre-post change in cortisol between a treatment and control group after a stress-coping intervention, but did find significant effects for a subgroup of children who reported higher levels of anger and presented with higher cortisol levels. Students with higher levels of anger who did not received the intervention did not show reduced cortisol, suggesting that this subgroup may have benefitted the most from the intervention. These findings are important and future research should include examination of differential treatment effects to assist in the development of more comprehensive and effective prevention efforts.

Yoo et al. (2016) found significant pre-post salivary cortisol changes after an 8-week meditation intervention, when compared to a comparison group. Schonert-Reichl et

al. (2015) also found post-intervention changes after a 12-week mindfulness program in morning cortisol levels (not mid-morning or afternoon levels) when compared to a control group. Neither of these studies examined short-term intervention effects.

Lozada, Carro, D'Adamo, & Barclay (2014) did not find pre-post intervention effects for cortisol after an eight-week mind-body integration intervention, but did find a significant change at a five-month follow-up, compared to a control group. The authors stated that 85% of the children reported using some parts of the intervention during stressful situations at the five-month follow up, so these results may indicate that a longer duration of the intervention facilitated the cortisol change, although it is difficult to know the specificity of the frequency and dose of these intervention techniques when practicing on their own.

A few studies found changes that were more challenging to interpret. Hatfield and Williford (2016) examined Banking Time, an intervention focused on development of sensitive and responsive child/teacher interactions over seven weeks (2-3 times per week), and found changes in morning cortisol decline as compared to a control group, but no difference existed between a comparison intervention group that focused on equivalent child/teacher time. More needs to be understood about what aspects of the intervention and/or teacher-student relationship may have accounted for these changes. Lindblad, Hogmark, & Theorell (2006) examined a music intervention that included concentration and relaxation and found lower levels of afternoon cortisol post-intervention but not compared to a comparison group, so benefits may have existed but were inconclusive.

Three studies found no significant changes in cortisol (Roden, Zepf, Kreutz, Grube, & Bongard, 2016; Sibinga et al., 2013; Sieverdez et al., 2014). Two examined area under the curve (Sibinga et al., 2013; Sieverdez et al., 2014) and one examined stress reactivity (Roden et al., 2016). All three had control groups and examined a music program, mindfulness, and yoga respectfully, although the sample sizes were relatively small so it is possible that treatment effects weren't detected.

It's important to note that salivary cortisol responses vary across individuals and situations (Kudielka, Hellhammer, & Wust, 2009). For example, when measuring cortisol reactivity to a stressor, it is essential to understand what type of stressors will elicit a stress response in your target population (Kudielka et al., 2009). Some types of cortisol measurement (basal, reactivity, diurnal, CAR, etc.) may be more sensitive and/or appropriate based on the population or outcome measure. For example, assessing basal cortisol at one time point may not provide a full picture of HPA axis activity when examining links with mental health disorders and/or intervention effectiveness (Bruce, Gunnar, Pears, & Fisher, 2013).

When assessing the reliability and validity of salivary cortisol, specificity, accuracy and precision of the assay need to be considered, as well as collection methods, storage considerations and sampling conditions. Although it is unclear as to whether commercial immunoassay kits vary in terms of results (Groschl, 2008) it is important to select an assay that is highly sensitive and specifically validated for measurement of salivary cortisol, as well as to follow manufacturer instructions for collection and handling of saliva samples (Salimetrics, 2017).

Collection methods are critical and can influence the accuracy of the cortisol concentrations. Saliva can be collected in children using a passive drool method (drooling directly into a test tube) or a saliva swab, keeping in mind that different size saliva swabs are used with different age groups. For example, Salimetrics (2017) offers three types of saliva swabs – infant (under six months), child (six months to six years) and adult (six years or older). They do not recommend the passive drool method for children under six. Gallagher, Leach, Massey, McAllister-Williams, and Young (2006) found that both a passive drool method and salivette (a type of swab) collection correlated with plasma cortisol and with each other in a group of healthy adult males, although some studies have found that swab-like devices lowered cortisol concentrations (Putnam et al., 2012).

Sampling conditions as they relate to timing of collection is also paramount in enhancing the reliability of cortisol values. Due to the diurnal pattern of cortisol, basal cortisol collections should occur at the same time each day (when comparing or averaging). When assessing cortisol reactivity to a stressor, a typical cortisol response occurs approximately 10-30 minutes after the stressor occurs (Lupien, 2013), with recovery from a stressor typically occurring between 20 to 40 minutes post-stressor, and pre-stressor levels typically being reached between 40 and 60 minutes, but this may vary based on the type of stressor induced (Dickerson & Kemeny, 2004). Even seasonal variations of cortisol can occur, which should be considered in longitudinal research (Hansen, Garde, & Persson, 2008). Outside influences such as eating times, exercise, and the use of certain drugs may influence cortisol results and need to be considered (Hansen et al., 2008).

While salivary cortisol is fairly durable, improper handling and/or storage may impact the reliability of the samples. Differences exist in terms of storage protocols, but it is recommended that samples be refrigerated within 30 minutes of collection, and frozen at or below -20 degrees Celsius within four hours (Salimetrics, 2017). Research suggests that samples can be thawed and refrozen without impacting the concentration values (Kirschbaum & Hellhammer, 2007). Although saliva samples can be stored in room temperature for at least two weeks without degradation, samples may get moldy and produce a foul odor if not refrigerated (Kirschbaum & Hellhammer, 2007).

Feasibility considerations – cortisol. While there is evidence that laboratory-based interventions may improve cortisol regulation (see Slopen, McLaughlin, & Shonkoff, 2014, for a review) there are important considerations when employing cortisol as an outcome measure in school-based intervention research. One is timing; because of the diurnal pattern of cortisol, timing of collection is critical. Several studies in this review cite school scheduling difficulties as they relate to timing of cortisol collection, particularly school start-times (Hatfield & Williford, 2016; Lupien et al., 2013) and limited timeframes for intervention delivery and measurement (Roden et al., 2016). Other intervenors found it necessary for teachers to modify students' schedules related to physical activity and snack times (Schonert-Reichl et al., 2015; Butzer et al., 2015).

Another challenge in measuring cortisol in schools to provide a picture of stress dysregulation is that researchers are not able to get a full diurnal picture of cortisol, which would require samples immediately upon awakening and at bedtime (Slopen et al, 2014). Since CAR needs to be measured very shortly after awakening, it cannot easily be

measured in school-based research, unless collected at home (presumably) by a parent. In laboratory research, parents are often given instructions to collect these samples at home, but problems related to timing accuracy and compliance have been noted (Michels et al., 2012). In the studies reviewed here, only two out of the 11 that measured salivary cortisol (including the one study that measured alpha amylase) measured CAR (Lindblad et al., 2006; Sibinga et al., 2013), however it was unclear who collected the awakening samples, so it is difficult to determine if or how collection methods influenced results. Schonert-Reichl et al. (2015) mentioned the importance of CAR, however chose not to collect it to lessen the burden on children's caregivers.

Blood Pressure

Blood pressure is an indicator of autonomic nervous system (ANS) activity, specifically the sympathetic branch of nervous system (SNS) function, and is linked to autonomic arousal regulation (Hagins, Haden, and Daly, 2013). ANS activity and the aforementioned HPA axis system reflects the two physiological bodily systems most related to the human stress response (Kramer et al., 2012). When a stressor occurs, it triggers a *fight or flight* response from the SNS, which stimulates release of epinephrine (also known as adrenaline), inducing increased blood pressure, among other things.

Increased blood pressure in response to ongoing stressors can contribute to adverse physical health outcomes later in life. There is evidence that increased blood pressure in response to a stressor is associated with future risk of hypertension in adolescents (Matthews, Salomon, Brady, & Allen, 2003) and adults (Matthews, Woodall, & Allen, 1993; Matthews et al, 2004). Anxiety has shown to increase blood pressure in

youth (Howell, Rice, Carmon & Hauber, 2007), and elevated blood pressure has been related to psychosocial and chronic stress factors such as anxiety (Howell, Rice, Carmon, & Hauber, 2007) and poverty/low SES (Chen, Matthews & Boyce, 2002), as well as physical health issues such as obesity (Cai et al., 2014) and hypertension (Ingelfinger, 2014). Stress reduction techniques that reduce perceived stress may influence blood pressure levels (Barnes, Davis, Murzynowski, & Trieber, 2004).

Blood pressure measurement, reliability and validity. Blood pressure can be examined in a resting state, ambulatory (over 24 hours) or in response to a stressor (measuring reactivity). Resting blood pressure provides a snapshot picture of heart health. Ambulatory blood pressure (ABP) is blood pressure monitored in regular intervals, usually for 24 hours while the participant is participating in normal daily activities throughout the day and night. Ambulatory blood pressure may provide a more comprehensive picture of health than resting blood pressure, as well as a more accurate representation of normal BP functioning and allows for monitoring of changes in blood pressure during activities such as exercise or sleep.

Blood pressure can also be examined in response to a stressor. Similar to cortisol reactivity, blood pressure measured in response to a stressor provides valuable information on how well an individual's stress response system reacts to daily stressful events (Meininger et al., 1999). Chronic stress reactions have been associated with higher blood pressure acute reactivity, and therefore increased risk for cardiovascular disease via hypertension (Gasperin, Netuveli, Dias-da-Costa & Pattussi, 2009).

The gold standard for blood pressure measurement is use of a mercury sphygmomanometer and the Korotkoff sound technique (Pickering, 2005) although this method has been slowly phasing out in favor of safer (mercury-free) and more user-friendly devices, such as blood pressure cuffs used with electronic monitors (Ogedegbe & Pickering, 2010). In fact, all three studies reviewed here that examined resting BP used electronic devices, although accuracy and reliability of devices should be considered (Ogedegbe & Pickering, 2010). Overall, these devices are relatively easy to use, easy to transport, and is relatively inexpensive, although some basic training may be necessary. Measuring blood pressure in children requires smaller, child-size cuffs (depending on age and size) for accurate measurement (Urbina et al., 2008)**.**

Measuring ambulatory blood pressure requires the use of a belt-like device around the waist or shoulder and attachment of a small electronic device that produces BP readings (SpaceLabs, 2017). Reliable and valid devices exist, however they may be less accurate when the participant is engaged in physical activity (Ogedegbe & Pickering, 2010). Overall, ambulatory BP may be a better predictor of risk (Gregoski et al., 2011), however it may be more cumbersome to implement in school-based intervention research and would likely be even more challenging with young children.

Sensitivity – Blood Pressure. Resting blood pressure was measured in three studies. Hagins, Haden, & Daly (2013) and Roden, Zepf, Kreutz, Grube, & Bongard (2016) examined blood pressure in response to a stressor to determine effects of an intervention, a yoga program and an instrumental music training program, respectively. Hagins et al. measured BP twice (averaged) pre-stressor, once during each of two stress

conditions, and twice (averaged) post-stressor. Roden et al. measured BP three times (averaged for a mean) pre-stressor, and twice (averaged) after each of two stress conditions (two tasks in a computerized competitive reaction time game). Neither intervention found significant effects on blood pressure. Sieverdez et al. (2014) examined BP before and after a yoga intervention, monitoring BP over a 10-minute timeframe and averaging the last three (at 6, 8, and 10 min.) collections for pre-intervention and post-intervention and found effects with a sub-group of pre-hypertensive children.

Ambulatory blood pressure (ABP) was used in three studies that examined effectiveness of a breathing awareness meditation program (Barnes et al., 2008), the Williams Life Skills program (Barnes et al., 2012), and a breathing awareness meditation and Botvin Life Skills program (Gregoski et al., 2011). All three measured BP over a period of 24 hours and used a SpaceLabs ambulatory monitoring system. BP was measured and recorded every 30 minutes while participants were in school and asleep and every 20 minutes during awake, non-school hours. Barnes et al. (2008) and Gregoski et al. (2011) found effects at certain times of day with pre-hypertensive adolescent samples, however Barnes et al. (2012) did not find significant effects in a normotensive population. Although more research needs to be conducted, initial evidence suggests that resting blood pressure as a physiological measure of stress may not be sensitive to school-based intervention effects in normotensive children.

Heart Rate and Heart Rate Variability (HRV)

Heart rate is an indicator of sympathetic and parasympathetic nervous system activity, which are systems involved in the fight or flight response, as well as overall

emotional arousal (Vries-Bouw et al., 2011) or dissociation (Perry, Pollard, Blakely, Baker, & Vigilante, 1995). Stimulation in the sympathetic nervous system, which occurs in response to stress, increases heart rate. Resting heart rate, which tends to decrease as we age, is sensitive to the effects of stress, with higher resting heart rates indicating higher stress levels. For example, stressors such as exposure to road-traffic noise were linked to higher resting heart rate in preschool children (Belojevic, Jackovljevic, Stojanov, Paunovic, & Ilic, 2008). Sometimes, lower resting heart rates are associated with trauma, specifically a dissociation response which is most often seen in females (Perry, 1997).

While resting heart rate simply looks at number of beats per minute, heart rate variability (HRV) is "the variation over time of the period between consecutive heartbeats" (Acharya, Joseph, Kannathal, & Suri, 2006). HRV is an indicator of the autonomic nervous system and is an indicator of how well the body responds to environmental and physiological stimuli (Michels et al., 2012). Medium and high HRV coherence are physiological indicators of autonomic control and relaxation (due to dominance of the parasympathetic nervous system, which promotes "rest and digest" as opposed to "fight or flight"; Bothe, Grignon, & Olness, 2014). Lower HRV has been associated with chronic stress in children, however research is still in its infancy (Michels, 2013). HRV has been associated with negative emotions (Michels) including anxiety (Chalmers, Quintana, Abbot, & Hemp, 2014).

Resting heart rates have been associated with behavioral and mental health issues. For example, higher resting heart rates have been seen in individuals with anxiety and

social phobia (Kramer et al., 2012), depression and PTSD, among others (Raine, 2002). Low resting heart rate, as well as low heart rate during a stressor, have been correlated with antisocial behavior in children and adolescents (Ortiz & Raine, 2004) and in response to trauma (Perry, 1997).

HRV has been associated with attentional control and emotion regulation. High vagal tone, as measured through HRV, has been associated with greater self-regulation and behavioral flexibility (Thayer & Lane, 2000). Low heart rate variability is viewed as a disease marker and associated with increased mortality (Thayer & Lane, 2007). HRV has recently been noted as a useful physiological stress marker, and may be associated with threat appraisal (Thayer, Ahs, Fredrickson, Sollers, & Wager, 2012).

Cardiovascular responses to stress may be differentiated by gender. Perry (1997) found that pre-adolescent males who experienced trauma had higher heart rates when discussing a traumatic event (compared to resting heart rate), while females' heart rates tended to decrease. This is likely due to a dissociative (or freeze) response to trauma, which is more common in females, whereas a hyperarousal response (fight or flight) is found more often in males (Perry, 1995). These changes were associated with a difference in behavior as well, with males showing more externalizing symptoms and females showing more internalizing symptoms, likely due to a neurobiological sensitization around either hyperarousal or dissociation.

Heart rate and HRV measurement, reliability, and validity. Heart rate and heart rate variability are physiological markers of stress and health in children and adults (Thayer et al., 2012; Vries-Bouw et al., 2011) and are also measured in resting conditions

or in response to a stressor. Both heart rate and heart rate variability decrease with age, particularly between the ages of five and ten (Finley, Nungent, & Hellenbrand, 1987). Differences exist between daytime and nighttime hours as well, which has implications for data collection.

Heart rate can be measured several ways, including blood pressure cuff (*resting and/or ambulatory, as described above*) and fingertip pulse oximeters. Similar to blood pressure, considerations must be made relative to equipment size and error readings due to movement when working with children. Devices that are FDA approved for children are specifically designed to minimize movement-related inaccuracy, although children will need to be encouraged to remain still during the reading. Fingertip pulse oximeters may have reliability issues related to issues such as nail polish, body temperature and accuracy due to participant movement and/or calibration issues (Fouzas, Priftis, & Anthracopulos, 2011) but may be easier to use in a school setting.

Sensitivity - heart rate/HRV. Four studies examined resting heart rate (Hagins, Haden, & Daly, 2013; Osbourne, Kenny, & Cooksey, 2007; Roden et al., 2016; Sieverdez et al, 2014) with one specifically looking at reactivity to a stressor (Hagins, Haden & Daly, 2013) and none found any significant changes in the intervention group (all but Roden et al. had randomization). Two studies examined ambulatory heart rate (Barnes et al., 2008 & Gregoski et al., 2011) and both found significant effects for a breathing meditation intervention on heart rate during school hours, with Gregoski et al. (2011) also finding significant changes in 24-hour HR. This suggests that examination of resting heart rate at one point in time may not reflect changes that may be occurring. Another

possibility to consider is a possible "white coat effect," where office/lab readings are higher due to the stress of the setting (Flynn et al., 2014). This may be especially prevalent in children (Briasoulis et al., 2016; Matsuoka, Kawamura, Honda, & Awazu, 2002), however the more natural and casual nature of a school-based setting may minimize this.

Two studies examined HRV (Bothe, Grignon, & Olness, 2014; Bradley et al., 2015) and both found intervention effects. Bothe et al. found no significant change after a four-month breathing, stretching and imagery intervention at post-intervention, but found improvement in HRV coherence at a one-year follow-up. The authors stated that 14 out of the 15 children reported that they continued to use some or all of the intervention techniques, so similar to Lozada et al. (2014) who found follow-up cortisol effects, increased duration of the intervention may have facilitated physiological effects. Bradley et al. (2015) examined TestEdge, an emotion self-regulation program given two times per week for approximately four months, and studied HRV in response to a stressor (Stroop color-word conflict test). They found improvements in several markers of HRV in the intervention group, as compared to a randomized control group, most notably heart rhythm coherence, which indicates a greater ability to relax. More research is needed, but HRV appears to be a promising measurement tool to examine effects of school-based intervention research and based on these limited studies, may be more sensitive to intervention effects than resting heart rate.

Feasibility considerations – blood pressure and heart rate. Physiological measures may present unique challenges in some children, for heart rate and blood

pressure measurements require levels of compliance that may prove challenging, especially for young children. In one study measuring HRV (Bothe, Grignon & Olness, 2014) the children were required to remain "quiet and still" for ten minutes; this may be difficult for most children and may not be feasible at all with younger children. Relative stillness and quiet are required for accurate reading of both resting blood pressure and pulse rate, as well. As per guidelines from the American Heart Association (Pickering, 2005) children should sit in place for at least five minutes before blood pressure is taken, and should not have consumed food or drink within 30 minutes of the reading (Pickering, 2005).

Although ambulatory heart rate may be a more comprehensive measure of sympathetic activity, it requires equipment that may not be easily accessible in a school setting (as described above). The most accurate method of measuring HRV is through the use of electrocardiogram (ECG), however other less invasive techniques exist such as fingertip heart rate monitors (Bothe, Grignon & Olness, 2014) and optical earlobe sensors (Bradley et al., 2015). HRV analysis software is also required.

Measuring HRV in a school setting presents equipment-related challenges due to cost. Recent advancements in technology allow for HRV measurement with equipment other than standard electrodes, which would not be feasible in most school settings and may be uncomfortable for children, especially younger ones. There is evidence to suggest that photoplethysmography, or blood flow from the finger, may be used to reliably measure HRV (Lu et al., 2007) and therefore allows use of electrode-free portable devices, such as pulse oximeters (finger monitors) or even cell phones (by way of resting

a finger on the camera lens). Ambulatory blood pressure, in addition to equipment, requires at-home monitoring. Selection of equipment requires careful consideration (i.e. cuff size, sensitivity to movement, etc.) when being used with children (Urbina et al., 2009) and may also require procedures to acquaint the children with the equipment.

Both studies that measured HRV used biofeedback equipment, which can be relatively expensive but appears to be incorporated easily into an educational context for both younger children and adolescents. Providing that the researcher has access to the analysis software, the actual measurement sensors are relatively small and portable. Further examination of the effectiveness and feasibility of HRV in school-based intervention research is worthwhile.

Sodium Handling

Two studies (Barnes et al., 2008; Gregoski et al., 2011) examined increased sodium handling, an indicator of behavioral stress (Gregoski et al., 2011). After a stress event commences, the body's ability to restore sodium balance indicates normal sodium handling (Barnes et al., 2008). Because stress induces sympathetic nervous system activation and this triggers in increase in sodium retention, it is expected that a post-stress response would induce increased sodium excretion. When this doesn't happen as expected, a person is at higher risk of complications such as hypertension and cardiovascular disease (Reaven, Abbasi, & McLaughlin, 2004). Studies have demonstrated that African-Americans are particularly at risk for sodium retention (Nesbitt & Victor, 2004). It is difficult to assess the sensitivity of sodium handling for use in school-based intervention research, since it was only used in two studies. One study

showed a significant decrease in urine sodium excretion and content (Barnes et al., 2008) and the other (Gregoski et al., 2011) showed no change. They examined a similar breathing awareness meditation and the collection methods they described were similar. More research is needed here.

Sodium handling measurement. These studies required participants to provide two urine samples at home at bedtime and waking using take-home containers, where they analyzed urine volume, sodium content and sodium excretion rate. Analysis of this collection method proved somewhat challenging, as both studies showed a large amount of missing data for this variable. For example, in Barnes et al. (2008) only 39 of 66 participants were analyzed due to attendance at less than 70% of the sessions, and the authors' preliminary analysis showed that a certain dosage of the intervention (in this case, a breathing awareness meditation) was necessary to show an effect. These studies examined interventions that target stress-reduction in youth who are at risk for hypertension, specifically African-American adolescents, who may be particularly at risk for ill health-effects associated with stress due to issues such as racism and status inequality (Clark, 2001).

Feasibility considerations – sodium handling. Targeting populations who may be particularly at-risk for deleterious stress effects is critical, but this collection method may be infeasible with many school-based populations. It requires urine collection at bedtime and at wake-up, and although collection methods seem straightforward, compliance may be an issue, although this wasn't described in either of the studies that measured it. More research is needed to assess the utility of this measure.

Skin Conductance

One identified study examined skin conductance (Fishbein et al., 2016), which is a physiological indicator of increased arousal in the autonomic stress response system. Skin conductance, or electrodermal activity, examines skin response to small electrical currents, reflecting sympathetic activity. Sweat glands induce electrodermal activity in response to a stressful event, therefore producing higher SNS arousal (El-Sheikh, Keller, & Erath, 2007). Higher skin conductance in response to a stressor or cognitive task is considered a positive response, indicating an individual's ability to allocate resources to information processing (Fishbein et al., 2016). This is related to the *fight or flight* response mentioned earlier, where the body increases sweat to cool itself and prepare for the exertion necessary to deal with a perceived stressor or threat.

It is difficult to assess sensitivity to intervention effects since only one study used this and the results were not significant, although the authors reported significance at the trend level ($p<.10$). More research is needed.

Skin conductance measurement, feasibility and reliability. Skin conductance requires electrode measurement, where an undetectable electric current passes through the electrodes and measures activity. Fishbein et al. (2016) used electrodes attached to two fingers on the non-dominant hand to the chest in a study of older adolescents (mean age = 16.7) and this collection lasted about 10 minutes. This type of measurement procedure may not be feasible or practical in some school settings and may be more difficult with younger children. More research is needed. Similar to heart rate and blood pressure equipment, reliability of the measurement apparatus should be considered.

Alpha Amylase (AA)

Salivary alpha amylase has been identified as an indicator of ANS dysregulation and has been linked to anxiety-related disorders (Schumacher, Kirschbaum, Fydrich, & Strohle, 2013). A stressor would be expected to induce higher levels of alpha amylase in less than five minutes (as opposed to cortisol, which takes approximately 20 minutes; Robert-Mercier, Dehoux, Longrois, & Guglielminotti, 2014). Salivary AA follows a diurnal pattern where levels decline after the first hour after awakening and increases throughout the day, with peak levels in the late afternoon or early evening (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007). An altered awakening response has been identified and linked with mental health problems (Ghiciuc et al., 2011). For example, PTSD has been associated with an increase, rather than a decrease in awakening (Thoma, Joksimovic, Kirschbaum, Wolf, & Rohleder, 2012).

Alpha amylase measurement, feasibility, and reliability. One study examined salivary alpha amylase (Sieverdez et al., 2014), a biomarker of sympathetic nervous system functioning that can be measured in blood or saliva. Although in this study salivary AA was measured pre-post intervention, it can also be measured with a stressor protocol. No changes were found in alpha amylase as an outcome measure for a yoga intervention with seventh graders, so it is difficult to assess sensitivity to intervention effects.

When assessing the reliability and validity of alpha amylase, like cortisol, specificity, accuracy and precision of the assay need to be considered, as well as collection methods, storage considerations and sampling conditions. Collection strategies

are similar to cortisol, with oral swabs or the passive drool method most common. Samples can remain unrefrigerated for about three weeks, however refrigerator or freezer storage is preferable (van Stegeran et al., 2006). It is important to select an assay that is highly sensitive and specifically validated for measurement of alpha amylase, as well as to follow manufacturer instructions for collection and handling of saliva samples (Salimetrics, 2017).

Like cortisol, sampling conditions as they relate to timing of collection are critical due to the diurnal pattern. Collections should occur at the same time each day. When assessing reactivity to a stressor, a typical cortisol response would be present in saliva approximately 5 minutes after the stressor (Robert-Mercier et al., 2014).

Although alpha amylase may be a marker of chronic stress in high-risk populations, including children (Nater & Rolheder, 2009), more research is needed to assess its utility in intervention research.

Conclusions and Future Research

This review provides an overview of biological stress measures that have been used with children in school settings and describes the basic physiology associated with each, as well as measurement and feasibility considerations for use in schools. Although initial evidence suggests that some biomarkers can be used to show intervention effects in some populations, more research is necessary. Many of the studies reviewed here target non-clinical populations. While most, if not all, children can benefit from relaxation and coping strategies, the treatment effects may not be as significant and may account for some of the non-significant results.

This review reveals that researchers are recognizing the importance of using physiological measures of stress and that many can be relatively easy to use in school settings, although feasibility issues do exist. I examined studies published over the last 10 years, the majority of the studies were published in the last four years, indicating a trend toward use of these measures in schools. Future studies would benefit from continuation of this trend and this review provides researchers an overview of considerations for implementation of such measurement strategies.

More research should be conducted with all feasible physiological stress-measures to ensure we are targeting the multiple physiological systems that play a role in stress-response. Overall, it is still unclear as to whether physiological measures respond to interventions as well as psychosocial measures (Adam et al., 2010), as well as how biological measures correlate with psychosocial measures and with each other (Galantino, Baime, Maguire, Szapary, & Ferrar, 2005).

Many challenges and opportunities exist in school-based intervention research for stress. In terms of feasibility, salivary cortisol, heart rate, blood pressure, and alpha amylase seem portable and easy to measure in schools. Measures such as ambulatory blood pressure/heart rate and HRV require specialized equipment that some researchers have identified as being more challenging to transport (Osbourne, Kenny, & Cooksey, 2007). Measurement methods that use electrodes, such as skin conductance and some HRV methods may not be well-received by schools and/or parents and may be stressful when used with younger children, thus inserting errors due to method. Measurement of sodium handling by way of at-home urine screening, while an especially important

biomarker for minority children and adolescents who are at an increased risk of hypertension, may suffer from missed collections, and therefore missing data. More research with varied populations is needed.

Monitoring of intervention dose and duration and how they relate to physiological stress responses is important. Some challenges exist here. Many of these interventions have at-home practice components, and examining dose response in these situations poses challenges (i.e. Sioverdez et al.). Dose and duration vary greatly across studies and this likely affects physiological changes and intervention effects. In addition to number, timing, and duration of sessions, Osbourne, Kenny, and Cooksey (2007) discuss how treatment adherence (active engagement, compliance) can influence outcomes; they offer a method to assess adherence by way of a treatment adherence score measured by therapist judgment and objective measures. Osbourne et al. (2007) discuss that even the way students are dressed can influence engagement in interventions. Several studies indicated that the dosage needed for a change in physiological stress response is unknown (i.e. Fishbein et al., 2015; Lindblad, Hogmark, & Theorell, 2006), so more needs to be done to assess this with various types of stress intervention.

Logistical issues such as classroom schedules, child arrival times, and school absences were noted as issues in several of the studies (i.e. Fishbein et al., 2015; Hatfield & Williford, 2016). As mentioned, collection timing of physiological measures is often critical, requiring either specific collection times or replication of previous collection times, making variations in day-to-day schedules problematic. Child absences can influence dose of the intervention, as well as missing collection data. Other

considerations include mealtimes and physical activity, which may influence a child's physiological state. For example, cortisol should be collected after a child has had nothing to eat or drink for 60 minutes (according to many protocols), so collections often need to be critically placed between breakfast, lunch and snacks, which requires a great deal of flexibility from the school staff. While these are challenges in all school-based research, timing of physiological measures is critical and provides a unique challenge in interpreting results regarding exposure and/or continuity of treatment protocols as well as measurement interpretation.

Stress intervention research supports the first of the Social Work Grand Challenges, to "ensure healthy development for all youth" (American Academy of Social Work and Social Welfare, 2016). Stress has a significant impact not only on physical health and psychosocial functioning, but directly related to academic achievement. Brain regions related to executive function (cognitive flexibility, working memory, and self-control) and stress regulation, which in turn affects cognitive development and susceptibility to learning disorders are predominantly at risk (Blair, 2010; Bradshaw et al., 2012; Perry, 2009). A review by Carrion and Wong (2012) noted a correlation between traumatic stress and poor school performance, particularly lower reading achievement, decreased verbal IQ and more school absences. Lipina and Posner (2012) cite attention, literacy and numeracy, which are key to success in school, as particular areas that are often deficient as a result of stress associated with low SES. This, in addition to the brain damage that occurs via chronic stress that leads to various social-emotional problems, leaves children in low-SES environments particularly at risk for the

ill effects of chronic stress (Hackman, Farah, & Meaney, 2010). Because schools in these communities are often under-funded, they often lack the necessary resources to bridge the gaps. Greater attention should be given to children in lower-income school districts.

Finding ways to incorporate physiological measures into school-based intervention research is critical, especially with younger children where self-report of stress and stress-related symptoms is challenging. It has been suggested that younger children may under-report anxiety to avoid treatment and/or to be viewed upon more favorably (Glennon & Weisz, 1978). Also, younger children may not be able to identify complex or abstract features associated with anxiety (Luby, Belden, Sullivan, & Spitznagel, 2007) or they may not be able to verbalize anxiety or stress. For these reasons, incorporation of physiological measures seems advisable.

Early intervention and prevention are key aspects of stress intervention. We know that interventions that change neural processes are best performed during periods of development when brain plasticity is highest. It was once thought that once a person reached early adulthood, the brain was fully formed and no longer amenable to changes, although it is now clear that the human brain is malleable throughout the lifespan (Bavelier, Levi, Li, Dan, & Hensch, 2010). On the other hand, researchers document that early, preventative interventions for stressed children are significantly more effective than reactive services in terms of reducing the damage to the brain caused by stress (Perry, 2009; Thompson, 2014). School-based interventions, especially in the early years, are necessary to tackle this glaring health issue and ensure healthy development of all youth

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Appendix A

Table 1-A. School-Based Intervention Studies Using Stress Biomarkers

Study	Sample	Setting	Methods	Intervention	Biological Measures	Outcomes
Barnes, Pendergrast, Harshfield, & Trieber, 2008	Pre-hypertensive African American 9th graders (N=66)	Public High School; US	Random assignment; health education control group	Breathing Awareness Meditation; 10 min. at school & 10 min. at home daily for 3 months; teacher-led	Ambulatory BP; Ambulatory PR; Sodium Handling	Significant change in systolic BP (ns for diastolic) during school hours and at night; significant change in PR during school hours; significant decrease in 24-hr urine sodium excretion rate and content
Barnes, Johnson, Williams & Williams, 2012	Normative 14-17- year-olds; 86% African-American (N=159)	Public High School; US	Random assignment; single-blind; health education control group	Williams Life Skills anger & stress management workshop; 12 50-min. sessions; teacher-led	Ambulatory BP	No significant changes in BP
Bothe, Grignon & Olness, 2014	Normative third graders (N=14)	Elementary School; US	Prospective longitudinal study; control group (teacher-led book reading)	Stress Management program (breathing, stretching, imagery);2 30-min. teaching sessions, then 10 minutes daily for 4 months; teacher-led	Heart Rate Variability	No change post-intervention for HRV, but significant at 1-year follow-up
Bradely et al., 2010	10th graders w/test anxiety (N=136)	Public High School; US	Quasi-experimental controlled pre-post experiment	TestEdge (emotion & self-regulation program)	Heart Rate Variability (response to stressor)	Significant improvement in several markers of HRV
Butzer et al., 2015	Normative 2nd and 3rd graders (N=36)	Elementary School; US	Pre-post test; no control group	Yoga 4 Classrooms (themed discussion, breathing, yoga postures); 30 min. weekly for 10 weeks; certified instructor-led	Salivary Cortisol	2nd graders showed a sig. decrease in cortisol post intervention; no acute change after 1 session; both groups showed a significant decrease after ANT; no short-term 1 session change

Table 1-A. Continued.

Study	Sample	Setting	Methods	Intervention	Biological Measures	Outcomes
Dewi et al., 2015	13-15- year-olds with conduct disorder (N=103)	Junior high schools; Indonesia	Quasi-experimental	Mindfulness; 8 weeks (durations specifics unclear); treatment as usual as control; Student counselor-led (psychiatrist supervision)	Serum cortisol	Statistically significant decrease in cortisol and score on TCD-AM for intervention, compared to control
Fishbein et al., 2015	At-risk 9-12th graders (N=69)	Non-traditional (at-risk) Public Middle & High School; US	Pilot Randomized controlled trial; control – school schedule as usual	Mindful Yoga; 20 sessions; 50 min. classes 3x/week over 7 weeks. Led by Yoga instructor.	Skin Conductance	Marginally significant (P<.10) change in skin conductance (stress reactivity).
Gregoski, Barnes, Tingen, Harshfield, & Trieber, 2011	Pre-hypertensive African-American adolescents (N=166)	Public High School; US	Randomized controlled trial; Health Education control group	Breathing Awareness Meditation (BAM) 10 min 2x/day, Botvin LifeSkills Training (BLT) 50 min. 1x/week; all 3 months. Teacher Led.	Ambulatory Blood Pressure; Ambulatory Pulse Rate; Sodium Handling	Significant change in SBP during school and 24-hour, DBP during school and 24-hour, and HR during school and 24-hour; No change in sodium handling; Greatest overall increases with BAM treatment.
Hagins, Haden, & Daly, 2013	Normative 6th graders (N=30)	Public Middle School; US	Pilot Randomized Controlled Trial; Physical Education class control	Yoga; 50 min. 3x/week for 15 weeks; teacher-led (teachers w/yoga certification)	Blood Pressure; Heart Rate (Stress Reactivity)	No significant changes between groups on any measures.

Table 1-A. Continued.

Study	Sample	Setting	Methods	Intervention	Biological Measures	Outcomes
Lindblad, Hogmark, & Theorell, 2006	Normative 5th and 6th graders (N=60)	2 schools in Sweden	Pilot controlled quasi-experiment	Music Education Intervention (concentration, music activity and relaxation) 1 hour each week over school year; Data intervention (learning to use computers) and walking control	Salivary Cortisol	Significantly lowered afternoon cortisol levels at end of school year in intervention group but not compared to control groups.
Lozada, Carro, D'Adamo, & Barclay, 2014	Normative 2nd & 3rd graders (N=44)	Private school; Argentina	Pilot Randomized (classroom level) quasi-experimental controlled design; regular classes control	Mind-body integration (breathing and tai Chi, cooperative games, & reflection); 1x/week for 10 sessions (60 minutes each); researcher led (w/teacher)	Salivary Cortisol	Cortisol decreased in experimental group at 5-month follow-up compared to control group (ns post-intervention).
Lupien et al., 2013	Normative 11-13- year-olds (N=504)	2 Private high schools in Montreal, Quebec, Canada	Randomized, controlled design?	DeStress for Success Program (focused on identifying and coping w/stress); Five 40-minute workshops over 1 month; researcher-led	Salivary Cortisol	NS decrease in cortisol following intervention; students who reported more anger presented higher cortisol levels and significant decreases were found in that sub-group who received intervention.
Osbourne, Kenny, & Cooksey, 2007	12-16- year-olds with music performance anxiety (N=23)	Selective High School (competitive music auditions) in Sydney, Australia	Randomized controlled design; behavioral exposure only control	Cognitive behavioral program (psychoeducation, goal setting, cognitive restructuring, relaxation & behavioral exposure)	Resting Heart Rate	NS for resting heart rate.

Table 1-A. Continued.

Study	Sample	Setting	Methods	Intervention	Biological Measures	Outcomes
Roden, Zepf, Kreutz, Grube, & Bongard, 2016	Normative 7-8-year-olds (N=34)	Elementary schools in Germany	Quasi-experimental; natural science training program control	Instrumental music training program; weekly 45 min. sessions; teacher-led	Resting heart rate, blood pressure and salivary cortisol (stress reactivity)	No differences in any physiological measures.
Schonert-Reichl et al., 2015	Normative 9-11- year-olds (N=99)	Elementary school in Canada	Randomized-controlled trial; business as usual social responsibility program control	MindUP (Social Emotional Learning Program w/Mindfulness); 12 sessions 1x/week 40-50 min for 4 months	Salivary cortisol	Significant change in post-intervention morning cortisol (not pre-lunch or afternoon)
Sibinga et al., 2013	Low income 11-14- year-old African-American males (N=41)	Application—based middle school for males with academic promise & financial need; US	Randomized-controlled trial; Health Education control	Mindfulness-Based Stress Reduction; 12 weekly 50-min. sessions	Salivary cortisol	No change in cortisol between groups
Sieverdes et al., 2014	Normotensive and pre-hypertensive 7th graders (N=31)	Science and math charter school; US	Randomized controlled trial; music or art program control	Hatha Yoga; 90 min. sessions every other school day; yoga instructor-led	Resting blood pressure; resting heart rate; salivary cortisol and alpha amylase	Significant reduction in BP in the pre-hypertensive sub-group; no group differences in alpha-amylase, cortisol or heart rate.
Stueck et al., 2016	Normative female 4.5-6.5-year-olds (N=10)	Child care center in Germany	Pre-post test design; no control group	TANZPRO-Biodanza (dance, movement and non-verbal communication); 10 sessions weekly, 45-60 min.	Salivary Cortisol	Significant difference in pre-post comparison for cortisol; significant decrease in cortisol for children with high and medium baseline levels (ns low baseline).
Yoo et al., 2016	Normative 5th graders (N=42)	Elementary School in South Korea	Pre-post test non-equivalent group comparison	Mind-subtraction meditation; 8 weeks, 4x/week for 30 min.; certified instructor-led	Salivary Cortisol	Significant between-group differences in cortisol.

Chapter III
A Deep Breathing Intervention for Stress Reduction in 5-Year-Old Children

Abstract

This study examines the efficacy of a deep breathing intervention for stress reduction in 5-year-old children using a multiple-baseline-across-individuals design. Five children in pre-kindergarten or kindergarten received a 5-minute deep breathing intervention daily for four weeks. I examined physiological stress via salivary cortisol and pulse rate, and perceived stress via a faces scale developed for this study. Although the deep breathing intervention did not significantly alter physiological stress levels in four out of five children, I found that deep breathing may be beneficial for stress reduction in some children and that five-year-olds are able to participate in a deep breathing intervention. In terms of practical significance, this work provides teachers and clinicians with a scripted deep breathing protocol that can be used in the classroom in an individual or group setting. Limitations for stress measurement with young children and barriers to intervention delivery in a school setting are discussed.

Although people often see childhood as a carefree time, children do experience stress and its harmful consequences. Toxic stress is especially problematic, defined by the National Scientific Council on the Developing Child as a "strong, frequent, or prolonged activation of the body's stress response systems in the absence of the buffering protection of a supportive, adult relationship" (Shonkoff et al., 2012, p. e236). Toxic stress produces the most negative impacts on early brain development and dysregulation of the physiological stress response (Shonkoff et al., 2012). Impairment of brain structures has been noted in studies related to chronic toxic stressors such as child abuse and neglect, poverty, and exposure to domestic and community violence (Blair & Raver, 2012; Blair, 2010; Bremner & Vermetten, 2001).

Young children experiencing high levels of physiological stress may develop behavioral and mental health problems, as well as subtle brain damage due to their bodies' efforts to adapt to that stress (Blair, 2010; McEwen, 2000). Due to high levels of brain plasticity in young children, toxic stress in the early years of life may have particularly damaging and long lasting effects (Bremner & Vermetten, 2001), but this higher plasticity may also provide opportunity for intervention (Bradshaw, Goldweber, Fishbein, & Greenberg, 2012).

However, many children experience potentially harmful types of stress, even without these traumatic experiences. Increased incidents of bullying (U.S. Department of Education, 2016) and implementation of high-stakes standardized testing (Duckworth, Quinn, & Tsukayama, 2012) are examples of ongoing stressors that many children experience. These types of stressors, combined with less emphasis on activities that may

reduce stress, such as physical education and recess (Slater, Nicholson, Chriqui, Turner, & Chaloupka, 2012), make the introduction of coping strategies valuable.

Development of coping skills in childhood is linked to future psychological adjustment, particularly related to common daily stressors (Rutter, 1994). Dubow and Tisak (1989) demonstrate that a "toolbox" of coping skills can influence young children's responses to negative events. The use of both "problem-based" (efforts to change the stressful situation) and "emotion-focused" (efforts to manage the negative emotions created by the stressor) strategies by young children can be effective in reducing stress (Weisz, McCabe, & Denning, 1994).

Studies show that different age groups rely on different coping strategies. Older children, for example, tend to use more emotion-focused strategies and are more flexible, alternating between this and problem-based coping (Compas et al., 1991). Younger children tend to use more problem-based coping, however a study examining awareness of coping strategies of children experiencing stress suggested that although use of cognitive strategies increased with age, 5-6-year-olds did indicate use of some cognitive strategies, suggesting they are able to do so (Altshuler & Ruble, 1989). Both temperament and socialization have been identified as contributing factors in the development of coping (Skinner & Zimmer-Gembeck, 2007).

Interventions provided to stressed children and their parents would be desirable, but such interventions require significant investments of time and resources by parents. Many families of stressed children have significant problems that limit these resources. One way for social workers to address this problem is to develop simple interventions

and measurement strategies that may be delivered in settings such as schools. Although there are challenges to developing such interventions (such as issues of age-appropriate valid measurement tools), recent advances in neuroscience research suggest that exploration of this strategy is worthwhile.

Increased understanding of the physiological and behavioral stress response and awareness of the connections between "mind and body" have prompted a plethora of interventions designed to reduce subjective distress and stress-related deficits (i.e., emotion regulation) through processes that alter physiology directly (Slopen, McLaughlin, & Shonkoff, 2014; Bradshaw, Goldweber, Fishbein, & Greenberg, 2012). These interventions may include purposive physical movement, such as yoga (Chong, Tsunaka, & Chan, 2011; Sharma, 2014), tai chi (Wang et al., 2010) and deep breathing (Brown & Gerbarg, 2005) as well as activities involving cognitive participation, such as guided imagery (Utay & Miller, 2006), meditation and mindfulness (Chiesa & Serretti, 2009) which may reduce physiological reactivity and/or perceived distress. For example, meditation has been shown to increase brain activation in the medial prefrontal cortex in adults, which suggests the possibility of improved emotion regulation if done over time (Holzel et al., 2007; Farb et al., 2007). Deep, slow breathing improves autonomic functioning by increasing parasympathetic activity and decreasing sympathetic activity, that is improving vagal tone and therefore decreasing heart rate and blood pressure (Mohamed, Hanafy, & El-Naby, 2013; Pal, Velkumary & Madanmohan, 2004), common markers of physiological stress.

Deep breathing is incorporated in manualized treatments such as Mindfulness-

Based Stress Reduction (MBSR; Chiesa & Serretti, 2009) and Trauma-Focused Cognitive Behavioral Therapy (TF-CBT; Cary & McMillen, 2012) in clinical settings. Recent work has highlighted breathing techniques in combination with other mind-body practices in children to improve mental health-related outcomes, particularly in schools *(*Barnes, Davis, Murzynowski, & Treiber, 2004; Bothe, Grignon, and Olness, 2014; Joyce, Etty-Leal, Zazryn, Hamilton, and Hassed, 2010; Mendelson et al., 2010*;* Powell, Gilchrist, & Stapley, 2008; van de Weijer-Bergsma, Langenberg, Brandsma, Oort, & Bogels, 2012) although most studies examine this work with adolescents and older children.

Fewer studies examine mind-body techniques with children younger than eight. In 2005, Semple, Reid, and Miller conducted a within-subjects, pre-post-test, six-week feasibility study of mindfulness in seven- and eight-year-olds to determine its clinical promise and if it can be taught to younger children. They selected a sample of children who were not clinically referred but were determined to experience some impairment of functioning due to anxiety. The intervention included breathing, meditation and mindful eating. Pre-post-test measures included the Child Behavior Checklist (CBCL) - Teacher Version (Achenbach, 1992), the Multidimensional Anxiety Scale for Children (self-report; March, 1997), and the State-Trait Anxiety Inventory for Children (self-report; Spielberger et al., 1970). The authors indicated that the self-report anxiety measures were problematic for two reasons and did not report the results. First, contrary to the clinical observations and teacher reports of anxiety in the selected children, the children themselves reported experiencing little anxiety. Second, they discovered that the

measures were unsuitable for the age of the children (the nominated children were slightly younger than their original target age group), which also may account for the anxiety reporting. It has been suggested that younger children may under-report anxiety to avoid treatment and/or to be viewed upon more favorably (Glennon & Weisz, 1978). Also, younger children may not be able to identify complex or abstract features associated with anxiety (Luby, Belden, Sullivan, & Spitznagel, 2007). This study sparks some interesting questions as to how to assess constructs such as stress and anxiety, younger children's understanding of terms (for example, in this study children didn't understand the words "jittery" or "tense"), and whether self-report measures are appropriate for younger children. A Feely Faces Scale was developed by the authors to assess the child's mood before and after the intervention, and was used in addition to the CBCL. This scale consisted of three 5x7 grids, where children placed stickers in a one to five scale, indicating their feelings at a point in time (1= I don't feel good; 5= I feel great). They were used before and after the intervention, as well as once to assess class satisfaction. The authors indicated some variability issues with this scale, with some children showing little or no variability in pre-post-test mood ratings, which may highlight the issue of younger children's self-report if the intervention is, in fact, evoking change. Teachers reported improvements in academic functioning and reductions in clinical symptoms, based on the CBCL, for four of the six children.

A pilot study by Ball, Shapiro, Monheim and Weydert (2003) examined the use of guided imagery with children five to 18 years old experiencing abdominal pain. Guided imagery is "a technique that combines facets of relaxation, imagery, and hypnosis" (Ball,

Shapiro, Monheim, and Weydert, 2003, p. 528). The children learned relaxation techniques, such as deep breathing, and guided imagery; children who received the intervention reported a 67% decrease of abdominal pain during treatment, as measured by the Faces Pain Scale (Bieri et al., 1990), which was completed by the child and parent separately. In this study, younger children seemed able to self-report pain using a visual measure, however it is not clear how reporting would translate to a construct such as stress.

Although many studies examine mind-body interventions with children with asthma, usually focusing on the physical nature of the illness, one study examined anxiety in a sample of 48 children with asthma, ages six to 14 (Chiang, Ma, Huang, Tseng, & Hsueh, 2009). This was a two-group experimental design, with children and parents in both the experimental and control groups receiving a 12-week self-management program on asthma-related issues, but only the intervention group received relaxation-breathing training, which included muscle-relaxation and deep breathing. Participants were encouraged to practice, and indicated practice at least three times per week for 30 minutes. In addition to physical asthma-related variables, anxiety and perceived health status were examined by the Chinese Children's Anxiety Scale (Lin et al., 1973), the General Anxiety Scale for Chinese Children (Sarason et al., 1960; test/retest=.78), and four items adapted from the Perceived Health Status Scale (Kaplan & Camacho, 1988; alpha=.71). Total anxiety and general tendency for overall anxiety were improved and no change was noted in trait anxiety (which tends to be a stable trait). The authors noted that the measurement tools were self-reported and took a lot of time and

effort from the children. It is unclear what role parents had in the completion of these measures, since it is assumed that the younger children in this study (as young as 6) would not be able to complete them without assistance.

Attention to developmental stage, as well as levels of cognitive and social-emotional skills need to be considered when tailoring treatment programs to young children (Freeman & Garcia, 2008). The concrete-operational stage (Piaget, 1962) is thought to occur at around age 7, which may make it challenging for younger children to participate in techniques that require recognition of attitudes and beliefs (Grave & Blissett, 2004). Because of this, play therapy is often used with younger children, where the primary mode of communication between the child and the treatment provider is play, rather than verbal communication. The empirical evidence for the effectiveness of play therapy is limited and is plagued by poor research designs, small samples, and inability to generalize results (Bratton, Ray, Rhine, & Jones, 2005).

In an attempt to isolate one technique that seems to be an integral part of relaxation and mind-body protocols, deep breathing as a sole intervention was examined. Deep breathing may be effective for a variety of stress-related health issues, although there is not sufficient research to produce definitive evidence, particularly in children.

Breathing practices include "voluntary changes in the rate, pattern and quality of respiration" (Brown, Gerbarg, & Muench, 2013). Deep breathing improves the flow of blood and oxygen to the brain (Mohomed, Hanafy & El-Naby, 2014; Huang, 2011; Telles & Desiraju, 1991). This technique balances the body's carbon dioxide and oxygen levels to induce a state of relaxation (Larson, El Ramahi, Conn, Estes, & Ghibellini, 2010).

Deep breathing is a practice where breathing comes from the diaphragm, which prompts rising of the abdomen, rather than the chest (Consolo, Fusner, and Staib, 2008). It is often referred to as "diaphragmatic breathing", or, with children, as "belly breathing". There are many variations of deep breathing, for example yoga breathing (Dissanayake, Liyang, & Senanayake, 2014), which incorporates slow, deep breathing paired with certain body positions, and elevator breathing (Larson et al., 2010), which combines deep breathing with visualization (as if the "elevator" is riding through the body). Breathing exercises may counteract irregular breathing patterns of those who are experiencing stress and/or anxiety (Park, 2013).

Breathing interventions have shown both short- and long-term effects for stress reduction. Short-term relaxation effects have been reflected in reduced blood pressure (Di Marco, Zheng, & Murray, 2012; Mohamed, Hanafy, & El-Naby, 2013), heart rate (Mohamed et al., 2014), and improved vagal tone, a marker of parasympathetic nervous system activity in adults. (Laborde et al., 2016). Long-term changes over time have been noted as well, by way of lowered blood pressure and heart rate after yoga breathing for 15 minutes twice per day for one month (Dissanayake, Liyange, & Senanayake, 2014). Alteration of neural activity may be predicated on a certain amount of repetition, so a focus on dose and intensity of treatment is paramount (Greenberg & Harris, 2012). The influence of how duration, dose and frequency influence overall outcomes needs further exploration, but according to Baker (2012), a minimum of three to four weeks' practice is recommended for meditation and breathing techniques for the most beneficial physiologic changes. It is important to keep in mind, however, that modified treatment

protocols for children need to weigh these issues with developmental limitations, especially with younger children. For example, Semple et al. (2005) found that it was challenging for seven to eight-year-olds to practice breathing meditation for more than three to five minutes, as opposed to the 20-40 minute sessions that are typical for adult interventions. White (2012) cited intervention adaptations such as reduced length of homework, reduced teacher/learner ratio, shorter intervention sessions and more repetition as important elements when modifying interventions for children.

The issue of duration, dose and frequency of intervention is also important as it relates to at-home practice (a core component of many of these techniques). Although increased practice of skills may lead to improved outcomes, some have found this difficult to implement and measure due to lack of cooperation of participants (Chiang et al., 2009). It is possible that home practice of mind-body skills may be more difficult with children and may need to be modified. Duration, dose and frequency, as well as specific intervention components, should be reported more thoroughly for replication and clinical use.

A search of deep breathing literature with children indicates it may be a viable treatment option for stress-related conditions, however research is limited and inconclusive. Marwa, Mohammed, and Mohammed (2014) examined the physiological effects of deep breathing in 70 children, ages three to ten (39% between ages three and six) receiving chemotherapy. A deep breathing exercise improved respiratory efficiency and lowered pain intensity, as measured by a respiratory efficiency questionnaire (including pulse rate and respiratory rate; blood pressure was not changed) and pain

facial assessment scale. The authors used a pre-post-test design with no control group and only examined short-term changes (one session). Detailed information on methods or duration of the breathing exercise was not provided.

No deep breathing studies with younger children (under the age of eight) were found that examined variables other than pain, and few examining other variables were found exploring deep breathing as a sole intervention in children or adolescents.

Akinsola and Nwajei (2013) examined 72 high school students, ages 13-19 (who scored above the mean on test anxiety out of an original sample of 420) in an experimental study and found that eight 10-minute sessions of deep breathing reduced overall anxiety, depression and test anxiety. Pairing the deep breathing with cognitive restructuring was even more effective. They used anxiety measurement tools such as the State-Trait Anxiety Inventory (Spielberger, Gursuch, & Lushene, 1970), a test anxiety inventory (Spielberger, 1980), and a symptoms distress checklist (Derogatis, Lipman, & Covi, 1977). These authors also found a positive relationship between trait anxiety, test anxiety and depression, and higher levels of test anxiety in females compared to males.

Gains and Barry (2008) showed preliminary evidence that relaxation breathing may reduce impulse control behavior in male adolescents by examining six adolescents in a juvenile justice residential program. They used a single-subject-multiple-baseline-across-subjects design and measured daily behavior scores and daily inappropriate language checks. The initial treatment phase was 14 days, with two days of withdrawal and then another 12-16 days of deep breathing. They found inconsistent results, but noted some benefits to some participants in different treatment phases, as indicated by visual

trends in a separate chart for each subject. Three of the six subjects noted improvements in impulse control, as measured by language checks, during the second half of the treatment phase. Only one of the three showed improvement in both measured variables over the second phase of treatment. In a fourth subject, impulse control was improved over the first two weeks of treatment, but gains were lost during the second half. A fifth subject showed slight improvement in overall daily behavior. Observer reliability was noted as a flaw, as participant self-report was used as the sole informer of treatment compliance and performance on dependent measures.

In a study with 59 younger children, in 3rd -6th grade, music performance anxiety was examined and measured using a music anxiety performance inventory for adolescents (MPAI-A) (Su et al., 2010). Prior to a public music concert where children played either piano or violin, the children received relaxation breathing training for 10 minutes, twice per week for two months. Authors reported that the intervention was effective short-term (10 minutes prior to exam) for reducing music performance anxiety, but not long-term (measured at two months, one month, and 30 minutes prior to performance). This intervention was measured in multiple time points, but no control group was used. They used a measure validated initially for ages 12-19, and later from age 11-13, but it was not a noted valid measure for third through fifth grade students. This may speak to the limited stress-related measures available for younger children.

Laborde et al. (2017) examined the effects of one session of slow, paced breathing on vagal tone after a cognitive stress test in 16 adolescents between ages 15 and 19 who were diagnosed with an intellectual disability. This intervention was compared with a

passive relaxation (audiobook) control condition, and while vagal tone, measured via a heart rate variability (HRV) device, did not differ at baseline, it was higher in the breathing condition compared to the audiobook during a cognitive stress test, suggesting enhancement of vagal tone, which is an indicator of improved stress management during a stressor. However, since it was only implemented in one session, only short-term changes were examined.

The current study tested a simple, short, noninvasive intervention in a non-clinical setting using practical and inexpensive proxy variables for physiological and perceived stress in young children. Specifically, the study examined whether a four-week, daily deep breathing intervention can be effective in reducing physiological and perceived stress levels in five-year-old children.

This research contributes a missing, fundamental element to our knowledge about who can benefit from this type of intervention. For example, although children as young as five have participated in deep breathing interventions to reduce pain, with positive outcomes, we do not know if children this young can benefit from a deep breathing intervention for stress reduction, since no known studies have targeted this. Deep breathing is used as a component of Trauma-Focused Cognitive Behavioral Therapy for preschoolers (Hendricks, Cohen, Mannarino, & Deblinger, n.d.), however, the effectiveness of these protocols are not examined separately from the context of other treatment components. There are also fundamental questions about the appropriate measurement of stress in young children. We do not know, for example, if children this age can reliably report their well-being (Rebok et al., 2001). Perceived stress measures

for younger children below reading age are virtually non-existent, and while several feeling faces-type scales exist, most are designed and validated to measure pain.

Deep breathing can be implemented briefly, which is particularly important in school-based settings to minimize disruption to learning. Although the limited number of studies and methodological limitations make it difficult to conduct an overall assessment of the deep breathing literature with children, deep breathing has been effective with adults and adolescents for reduction of stress and stress-related symptoms (Dissanayake, Liyange, & Senanayake, 2014; Hedge et al., 2012; Su et al., 2010) with the few aforementioned studies showing effectiveness in younger children.

A survey of a national sample of school psychologists found that due to constraints and demands of providing school-based services, interventions tended to be relatively brief in terms of length and number of sessions (Prout et al., 2005). Clinicians in schools often have to perform counseling/therapy in as little as 30 minutes. Deep breathing could be executed relatively quickly and added easily to many current treatment protocols. This practical concern, in addition to the recent increased focus on student well-being in education (Waters, 2011), makes deep breathing a potentially desirable school-based intervention.

Methods

The study used a single-subject multiple-baseline-across-individuals design. Single-subject designs are characterized by systematic, repeated measurement of participant outcomes at frequent, pre-designated times under different conditions (e.g., baseline and intervention). Single-subject designs are especially appropriate for

developing interventions and improving quality of services (Kazdin, 2011). A single-subject design can show how an intervention changes behavior over time and may show differential treatment effects in individual participants (Thompson, 1986). It can also offer an opportunity to pilot new intervention and/or measurement techniques that can be more easily modified than with a larger group. They are a starting point for future experimental or quasi-experimental studies on deep breathing interventions for this age group.

A multiple-baseline across individuals design begins with baseline observations of the same problem or behavior for two or more individuals at the same time in one setting (Orme & Combs-Orme, 2012; Kazdin, 2011). Baseline is followed by implementation of the intervention with one individual after achievement of a stable baseline, while baseline conditions remain in place for the other individuals. If the intervention is producing its desired effect, it is expected that the behavior or condition of the individual receiving the intervention will change, while those in the baseline condition will remain stable. The intervention is then applied to the remaining individuals (Orme & Combs-Orme, 2012; Kazdin, 2011).

Baseline data allow for assessment of stress variables before the intervention, as well as prediction of what may be expected if no intervention were implemented. I collected measures for four children during two baseline phases (two children per phase) lasting two to four weeks (a fifth child was added after the study began) and an intervention phase lasting four weeks (with the additional child having three weeks of intervention). I collected repeated measurements at approximately the same time each

day for each child on Monday through Friday for each week of baseline and intervention. The baseline acted as a control for each participant and measurements during intervention were compared to measurements during baseline to determine possible intervention effects. I expected change to occur in the children receiving the intervention, while the children not receiving the intervention would remain stable.

Participants

Participants were pre-kindergarten and kindergarten children at a private school in the Southeast US. After receiving letters with a written description of the study, its potential benefits and risks, and the three selection measures, consenting parents signed forms and completed questionnaires and the selection measures. In addition to parental consent, each child provided verbal assent to participate. Teachers also consented to participate and provide the intervention to the children. Thirteen of twenty-six age-eligible children consented to participate. This study was approved by the Institutional Review Boards of the University of Tennessee (Knoxville, TN) and Centenary University (Hackettstown, NJ).

I selected participants for the intervention based on scores on three measures completed by parents for this study - the Coddington Life Events Scale (CLES; Coddington, 1972), the Children's Hassles Scale (CHS; Kanner, Feldman, Weinberger, & Ford, 1987), and the Pediatric Emotional Distress Scale (PEDS; Saylor, Swenson, Reynolds, & Taylor, 1999). Some of the participant stressors identified in the initial screening tools included: parental divorce, parental illness, death of grandparent,

transition of older children out of home, close friend changing schools, birth of a sibling, personal illness and remarriage of a parent.

Participating children had full command of English and at least low-average cognitive ability (determined by a developmental screening tool given to students at the school at admission). They did not have mental health diagnoses related to psychotic features. Children were excluded from the study if they have had any moderate to severe asthma-related attacks during the past six months. Although deep breathing has been shown to be an effective intervention for asthma-related illness (Ernst, 2000), different procedures might be indicated for a child with severe asthma symptoms.

I selected the four children (two in pre-kindergarten and 2 in kindergarten) who had the highest combined scores from all three measures and who met all inclusion/exclusion criteria for the intervention. I added a fifth child (in pre-kindergarten) after baseline began due to excessive school absences with one participant**.** None of the participants had received any formal mind-body training prior to participation in the study.

Measurement

Selection measures. The Coddington Life Events Scale (CLES; Coddington, 1972) is a self-report (assisted self-report in this study) measure that examines the frequency and recency of stressful life events. The purpose of this measure is to identify potentially stressful life events the child has recently experienced. The preschool version (30 items; designed for ages five and under) was used. Although the measure is designed as a self-report or assisted self-report, the manual suggests that the preschool version may

be completed by a parent or read to the child by a parent when the child has below a fourth-grade reading level, which will be done in this case. The estimated completion time is about 10-15 minutes.

The CLES contains both positive and negative life events (although the majority are negative) and the respondent is asked to indicate how many times (zero, once, or twice or more) each event has occurred during the last 0-3 months, 4-6 months, 7-9 months, or 10-12 months. Both event severity and timing (with the idea that effects of an event diminish over time) account for the score for each item, which are then calculated to Life Change Units (LCU) for each timeframe and then totaled for a cumulative score. The 75th percentile is identified as the cutoff point for children whose stressful life events may place them at higher risk of physical and/or emotional health problems.

The CLES was normed on a community sample of 3,617 children and parents. Reliability statistics were drawn from the adolescent version of the measure. Test-retest reliability was examined with 120 high school students at three time points over three months ($r = .69$; Blount et al., 2008). Blount et al. (2008) stated that potential exists for the CLES to be used to identify children in need of treatment. The predictive validity of the scale is still in question, with some studies indicating that the CLES is useful for identifying clinical vs. non-clinical populations, while others have not (Blount et al., 2008).

The Children's Hassles Scale (CHS; Kanner et al., 1987) measures daily hassles that may affect emotional adjustment, which have been identified as an even stronger predictor of well-being than stressful life events (Lazarus & Folkman, 1984). The CHS is

a subscale of the Children's Hassles and Uplifts Scale (Kanner et al., 1987). Hassles are defined as the "irritating, frustrating, distressing demands that to some degree characterize everyday transactions with the environment" (Kanner, 1981, p.3). This measure is indicated as a self-report measure for participants ages 8-17 years; however, it has been used with children as young as five (Creasey, Mitts, and Catanzaro, 1995; Michels et al., 2012) in both research interview and parent-assisted (assumed, although identified as a self-report) format. Participants identify which of 25 hassles have occurred during the past month (a severity index can also be used, but was not for this study, where the children are asked if the hassle "didn't feel bad", "felt sort of bad", or "felt very bad"). The score is determined by the frequency of daily hassles, and rated on a 0-100 scale. The measure has shown good internal consistency ($\alpha = .85$ and above; Blount et al., 2008). Predictive validity indicates more frequent hassles are correlated with greater emotional distress and perceived interpersonal problems, in a study with 12-year-olds (Kanner et al., 1987). Von Weiss et al. (2002) found that in children ages 8-17 with rheumatic disorder, 25% of the variance in trait anxiety scores were due to daily hassles, providing further support for validity of the CHS. In a sample of 6-14-year-old children, Abela and McGirr (2007) found that that test/retest (.54) and parent/child reliabilities ($r = .66$, $p < .01$) were similar in the 6-7 age group compared to the rest of the sample. Creasey et al. (1995) found an internal consistency of $\alpha = .85$ in children 5-6 years old.

The Pediatric Emotional Distress Scale (Saylor et al., 1999) is a 21-item scale that identifies symptoms and behaviors common to children who have experienced stress and/or trauma. Parents report behaviors on a 1-4 likert scale (1-almost never, 2-

sometimes, 3-often, 4-very often) and on four items related to trauma, which allow parents to indicate specific types of traumatic experiences. This measure is indicated as appropriate for children ages two to ten. Total scores are shown to have good internal consistency (alpha .85) in a sample of 475 children (Saylor et al., 1999). Test-retest and inter-rater reliability were found to be acceptable (Saylor et al., 1999).

Outcome measures. The dependent variables were physiological stress measured by salivary cortisol and pulse rate, and perceived stress measured by a faces scale developed for this study. Multiple measures can be more informative than a single measure, in that effects can be examined over several domains (Kazdin, 2011). Also, research has consistently shown poor agreement across informants (i.e. parent, teacher, etc.) with regard to children's behavioral or emotional functioning (Frick, Siverhorn, & Evans, 1994; DeLos Reyes & Kazdin, 2005), thus highlighting the importance of physiological measures.

The research team collected eight salivary cortisol measures for each child, the first four in the morning and afternoon before intervention. Two samples were taken on two days approximately 48 hours apart (for example, on a Wednesday and Friday morning and afternoon). The two morning samples were averaged, as were the two afternoon samples, and calculated into one morning pre-test concentration and one afternoon pre-test concentration. The remaining four were taken at the end of intervention (post-test) in the same manner, since I was only examining long-term effects of the intervention, as opposed to short-term effects. Specific times were chosen based on the

convenience of the school and selected participants (for example, preschool children arrive at various times throughout the morning).

We collected cortisol at the same time of day for each child, in the morning and afternoon (which varied across children) using Salimetrics Children's Swabs. The straw-shaped, spongy swab was placed under the child's tongue and held for 90 seconds, then immediately placed in a test tube designed for saliva testing. The teachers practiced with each child prior to the first collection, demonstrating how the saliva would be collected and using the collection tool on themselves, and then allowed the children to do the same. This is a non-invasive, but effective way to measure salivary cortisol in youth. It is important that the timing is consistent, due to the diurnal rhythm of cortisol, with highest levels produced in the morning and declines throughout the day. Salivary cortisol samples were transported by the student researcher or another doctoral student to a lab where they were stored in a freezer at -70 degrees C until analysis.

We measured pulse rate daily at the same time during baseline and intervention (immediately before and after the intervention) using an FDA-approved pediatric fingertip pulse oximeter, a non-invasive, easy-to-read digital monitor. FDA-approved devices meet reliability standards of +/- 2 bpm equating to 80-99% accuracy. We placed the device on the child's right index finger and instructed him or her to remain as still and quiet as possible until the reading was displayed. This was repeated after a five-minute rest period during baseline and after the deep breathing in the intervention phase, in order to ascertain short-term effects of the intervention.

Children reported stress using a single-item faces scale adapted for this study from the adult Faces Anxiety Scale (McKinley, Coote, & Stein-Parbury, 2003), where the children had five faces to choose from to indicate which was closest to how they felt in that moment. We adapted a script for this measure from the Children's Anxiety/Fear Scale (McMurtry, Noel, Chambers, & McGrath, 2010). The scale was chosen due to the clarity of the faces and non-inclusion of numerical or word anchors and based on information from the pre-school teachers that including more than five faces would likely be overwhelming for the children.

Several studies have found face scales to be the most effective tool for self-report in young children, although virtually all of this research has been related to pain and/or pain-related anxiety/fear or procedural distress (Elfering & Grebner, 2012; Kuttner & LePage, 1989; Woodgate & McClement, 1998). There are no known self-report measures for perceived stress for this age group, and because most five and six-year-olds have limited reading ability (Laerhiven, van der Zagg-Loonen, & Derkx, 2004), in addition to attentional limitations, questionnaires are likely inappropriate. Face scales used to measure pain in children have been used with children as young as three (Chambers, Giesbrecht, Craig, Bennett, & Huntsman, 1999). Harter and Pike (1984) report that a picture-based assessment tool is more understandable, interesting, and sustains a young child's attention, which produces more meaningful responses. Pictorial scales are more interpretable for young children than other types of scales such as numerical scales (Bosenberg et al., 2003) and/or visual analogue scales (Shields, Palermo, Powers, Grewe, & Smith, 2003).

Because perceived stress can only be measured by self-report, it is important to know whether children this young can report this. Internal states may not be fully measured by observation (Wright, Eisner, Stewart, & Finley, 2010), so although self-reports are not often used in children younger than seven (Lagattuta, Sayfan, & Bamford, 2012), they may provide a more accurate picture of children's psychosocial stress. And although parent report measures are common when working with younger children, studies have shown that parents underestimate constructs such as worry and anxiety in their children, and their reports often do not correlate with their children's self-report (Lagattuta et al., 2012).

Although no studies were found measuring "stress" with this type of scale, a couple of studies showcased younger children's ability to identify subjective feelings. For example, in a study examining emotional competence, five- and six-year-olds were able to use a five point "smiley face" Likert scale to rate peer likability (Denham et al., 2003). Three- and four-year-olds in this study were able to identify happy, sad, angry and afraid expressions represented by puppets; specifically, they were able to predict the feelings of puppets based on an emotion-laden story.

To ensure that the children selected for this study were able to interpret the feeling-faces, I enlarged the faces and pasted them on small wooden statues, asking the children to choose three different pairs of faces and tell which face was feeling "more bad." All 13 children initially screened were able to accomplish this without prompting.

The children selected for the intervention completed the measure daily, before and after the intervention (and in baseline before and after the five-minute rest period). The

data collector read a script to assist the child in assigning meaning to each face, which was blown up on letter-sized paper and laminated. The faces were laid out in order on the floor and the child was asked to step on the face that best indicated how they were feeling. Each face was assigned a numerical value for scoring purposes (0-4) with lower scores indicating lower perceived stress (0= least anxious face and 4= most anxious face).

Procedures

Data were collected by one of two female PhD students with whom the children were unfamiliar prior to the study, in an area near, but separate from the child's classroom, while each child's teacher performed the intervention using a script. During baseline, each child had a five-minute rest period prior to measurement, with the child seated and performing a sedentary activity of the child's choice, such as having a story read to them or playing with legos/blocks**,** since it would be difficult for a five-year-old to be seated unengaged for five minutes**.** After pulse rate and perceived stress measures were collected, the child had another five-minute rest period to mimic the timeframe of the intervention, and the measures were taken again.

In the intervention condition, each child had a five-minute rest period before pulse rate and perceived stress measures were taken, and then the intervention and stress measures were administered. The first day of intervention began with the teacher's explaining in simple, age-appropriate language the purpose of the study. The word "stress" was not used, since it is unclear how young children may interpret this term. Teachers used the term "belly breathing," and used a script dictating 3 counts in and 7 counts out. In addition to this 5-minute daily individual intervention, the teachers offered

reminders to practice the deep breathing skill during the school day in situations where they feel it could be useful for the child. Students received daily practice sheets to note their practice at school and at home, however these were not used consistently and were not included in any analyses.

The brief nature of this deep breathing intervention is congruent with the age and developmental stage of the children in the study. Although many structured mind-body interventions are based on research with adults and older children, they must be modified for use with younger children. For example, researchers examining a mindfulness intervention with seven- and eight- year-old children with anxiety found it challenging for children to sit and perform a breathing meditation for more than three to five minutes at a time (Semple, Reid, & Miller, 2005). One standard that has been identified is implementing no more than one minute of practice per year of age (Saltzman & Goldin, 2008), which was used in the intervention for this study.

Treatment Fidelity. Each teacher who provided the intervention participated in two 90-minute training sessions. Teachers observed the PI reading the script, and then practiced it with the PI on two separate occasions before the intervention. The PI then observed each teacher practicing the script twice. If a teacher did not follow the script exactly, we practiced it until his/her delivery was accurate.

To further ensure treatment fidelity, the PI listened in on at least one intervention session for each teacher, and teachers completed fidelity checklists immediately after all sessions.

Data Analysis

Because single-subject design most often relies on visual analysis to determine change (Orme & Combs-Orme, 2012), I initially analyzed perceived stress levels and pulse rate by graphing each participant's data over baseline and intervention phases using conventional single-subject design line graphs. I visually assessed and interpreted data by evaluating changes in level, trend, and variability to determine if changes were occurring, which direction, if any, the changes were occurring, and the pace at which the changes were occurring (Orme & Combs-Orme, 2012). It was hypothesized that pulse rate would lower both immediately after the intervention, and across time after the intervention commenced.

In addition to visual analysis I also conducted statistical analyses using the "R" package designed for analysis of single-subject design data (Auerbach & Zeitlin, 2014). To test statistical significance between baseline and intervention phases, I used the Conservative Dual Criterion (CDC; Fisher, Kelley, & Lomas, 2003) approach because it works well with positive autocorrelation in the range typically found in single-subject design research and because it can be used when there is a trend during either baseline or intervention. I calculated the Improvement Rate Difference (IRD; Parker, Vannest, & Brown, 2009) to examine effect sizes. This method calculates the smallest number of data points required to eliminate all overlap between the phases, therefore expressing the difference in outcome between baseline and intervention (Parker et al., 2009). The maximum IRD is 100%, which would indicate that all intervention data points are improved from all baseline data points. An IRD of 50% would indicate only a chance-

level improvement. A zero would indicate no improvement and a negative IRD would suggest deterioration (Parker et al., 2009).

Saliva samples were assayed in duplicate, on one plate, using an enzyme immunoassay, which was performed according to manufacturer instructions (Salimetrics, State College, PA). I compared pre- to post-test samples at the group level using a paired-samples *t*-test. The lower detection limit of the assay was .007 (ug/dL), with a mean intra-assay coefficient of variation of 11%.

Results

Daily pulse rate change during baseline and intervention for all five participants are displayed in Figure 1**.** All five children demonstrated pulse rates within normative ranges for five-year-old children throughout baseline and intervention, with the exception of a few low readings (under 60 BPM). Thus the expectation would be that the data would remain moderately level, and overlap of data points between baseline and intervention was prevalent. I examined both short-term (immediate changes before and after intervention) and longer-term changes (over time, during the course of intervention) for pulse rate. Figure 1 highlights pulse rate before and after the intervention, and in baseline before and after the five-minute rest period. Figure 2 shows the difference in pulse rate from time 1 (before intervention) to time 2 (after intervention) in both baseline and intervention phases, highlighting short-term changes per session. Thus, a value of zero would indicate no change, numbers above zero indicate a reduction in pulse rate and values below zero indicate an increase in pulse rate.

Daily perceived stress change during baseline and intervention for all five participants are displayed in Figure 3. Interpretation was challenging, as there was little variation in some cases. In others the variation tended to fall on extreme ends of the scale, which is noted in the literature as a limitation of using scales with young children (von Baeyer, 2006). Figure 4 shows the difference in perceived stress from time 1 to time 2 in baseline and intervention, highlighting short-term changes per session. Like pulse rate, a value of zero would indicate no change, numbers above zero indicate a reduction in pulse rate and values below zero indicate an increase in pulse rate.

Table 1 shows salivary cortisol concentrations for morning (or mid-morning) and afternoon collections for each child, as well as normative cortisol values for age and time of collection (McCarthy et al., 2009). All concentrations are reported in micrograms per deciliter. A paired-samples *t*-test was conducted to compare salivary cortisol levels pre- and post-intervention. There was no statistically significant difference in salivary cortisol between pre- (M= .1490, SD= .083) and post-intervention morning levels (M=.1480, SD= .081; t(4)= .082, p = .939) or between pre- (M= .099, SD= .048) and post-intervention afternoon levels (M= .096, SD= .024; t(4)= .133, p = .900). I also calculated pre-post intervention differences in diurnal slope, by computing the difference between morning and afternoon cortisol levels. There was no statistically significant difference in diurnal slope between pre- (M=.0500, SD=.0516) and post-intervention levels (M=.0520, SD=.0676; t(4)=-.089, p=.933). These results suggest that the intervention did not have an effect on morning or afternoon cortisol levels, however there was not enough power to

detect change given the small sample. Also, all concentrations were within normal ranges, so it may have been difficult to demonstrate intervention effects.

Colin, Christopher, Anna, and Jake all began baseline together and after two weeks of baseline, Colin and Christopher began the intervention, while Anna and Jake continued in baseline for another two weeks. In week five, when Anna and Jake began the intervention, Sarah was added and began baseline for one week. Sarah was only able to participate in the intervention for three weeks, instead of four, due to the school calendar.

Colin

Colin was a five-year-old male kindergartener. He was a talkative, energetic child who displayed some hyperactive behavior and anxiety symptoms during data collection, especially in the first week of baseline. His teacher reported that he was "stressed all the time".

Colin's teacher reported observing him using the deep breathing as a calming technique on a few occasions, stating that if he was getting "riled up" or "acting out" the teacher was able to redirect him to use deep breathing. When asked (during a class activity) what was the most important thing he learned in kindergarten, he responded "deep breathing." On several occasions, particularly when writing stories, he would mention "learning how to deep breathe." The teacher stated he seemed to enjoy the intervention and there was never a time when he didn't want to do it.

Colin completed baseline measurements for two weeks (8 sessions) and participated in the intervention for four weeks (18 sessions). He achieved a stable

baseline for pulse rate, and an immediate onset of increased variability in pulse rate occurred after the deep breathing intervention was implemented and continued throughout the four weeks.

There was considerable overlap between phases as indicated by the IRD (50%) and the conservative dual criterion approach (CDC) was not statistically significant for pulse rate. His pulse rate increased rather than decreased on most occasions. Colin exhibited hyperactive behavior during some of the sessions, possibility due to being required to remain seated and still. The teacher hypothesized that transitions in and out of the classroom may have been difficult, particularly since he was often concerned about his toys being played with and/or broken by others in the class during his absence. Colin's teacher stated that "end of the year emotions" were at play, especially since he will attend a different school for first grade, which may have increased his stress levels toward the end of the intervention.

For perceived stress, the CDC was not statistically significant and the IRD was 0. There was some slight variability in Colin's perceived stress during baseline phase, which may have been related to his initial discomfort with the data collection procedures. There was no variability in perceived stress during the intervention phase.

Christopher

Christopher was a five-year-old male kindergartener. He appeared happy, quiet, and even-tempered. Like Colin, he always participated in the intervention enthusiastically. His teacher reported that he would use the deep breathing when prompted when he was upset, but neither teacher witnessed him using it on his own.

Christopher was reported by teachers to get frustrated when he wasn't getting his way, and it was unclear if he was able to make the connection between his frustration and the utility of deep breathing in that moment on his own. Christopher's teacher reported that he initially had difficulty breathing out of his mouth, but after a demonstration using a piece of paper, he was able to navigate the deep breathing successfully.

Christopher completed baseline measurements for two weeks (9 sessions) and participated in the intervention for four weeks (18 sessions). Baseline pulse rate showed a moderate amount of variability, and this variability continued into and through the intervention phase. It appears that pulse rate increased at the start of intervention. While it appears that the intervention did not produce an immediate effect, there is a decreasing trend over time, especially during the last two weeks of the intervention, possibly suggesting that a longer intervention period may have produced a continued decline. However, there was considerable overlap between phases as indicated by the IRD (28%) and the CDC was not statistically significant for pulse rate.

An examination of short-term effects for pulse rate showed that in 11 out of 18 sessions, Christopher's pulse rate decreased after the intervention. During baseline, his second pulse rate measure decreased 3 out of 9 sessions. Therefore, deep breathing may have had an immediate effect on pulse rate in the predicted direction.

Christopher had no variability in perceived stress for either the baseline or intervention phases, and he often asked for a face that was "even more happy" than the happiest face.

Anna

Anna was a five-year-old female pre-kindergartener. Anna's presentation was inconsistent; at times she was very enthusiastic and energetic and at other times she appeared lethargic and sad. Anna was playful and would often "hide" under a table, wanting to surprise the teacher who gave her the intervention. Anna appeared to have an emotional response to the main data collector, telling her on one occasion "you are my best friend" and on many occasions not wanting her to leave. The teacher stated that she was able to follow the intervention well, but he felt he needed to breathe along with her, rather than count.

Anna completed baseline measurements for four weeks (13 sessions) and participated in intervention for four weeks (17 sessions). Baseline data showed some variability and were difficult to interpret, due to 7 out of 20 missing data points. Variability continued into and through the intervention phase.

Despite variability, visual and statistical analyses indicate that Anna may have benefited from the intervention, as evidenced by an immediate decrease in pulse rate at the start of the intervention phase. Because a stable baseline was not present, it is difficult to know if these results were achieved due to the intervention or other factors. The CDC showed a statistically significant improvement for pulse rate, and the IRD was 60%.

Anna showed some variation in perceived stress, although her choices tended to fall on the extreme ends of the scale. Upon visual inspection, it seems that in the initial weeks of the baseline phase, she indicated feeling less stressed than the latter part of the baseline phase. The beginning of the intervention phase showed greater perceived stress,

which seemed to improve over time during intervention. This may have been reflective of significant family stressors that were present during this time. It is possible that once Anna became more comfortable with the data collector, she was able to identify more negative feelings more comfortably. The CDC showed a statistically significant improvement for perceived stress and the IRD was 26%.

Jake

Jake was a 5-year-old male pre-kindergartener. Jake was an engaging child who was enthusiastic, energetic and friendly. Jake exhibited some hyperactive and oppositional behavior during data collection and intervention. Jake's teacher stated that he was able to learn the deep breathing technique easily and was able to use it when prompted. He demonstrated the technique to his mother when she came to pick him up, and mom reported to the teacher that he had used deep breathing at home. Jake's teacher stated that she thought he enjoyed the intervention, saw it as special and especially liked the extra attention he received. He is one of two children who proudly and confidently explained it to his pre-kindergarten class when the teacher began to implement it with all the students.

He completed baseline measurements for four weeks (18 sessions) and participated in the intervention for four weeks (14 sessions). Baseline pulse rate showed a moderate amount of variability, and this continued into and throughout the intervention phase. There was considerable overlap between phases as indicated by the IRD (37%) and the CDC was not statistically significant for pulse rate.

Similar to Colin, it is possible that it was difficult for him to be seated for the intervention period. His perceived stress reports tended to fall on the extreme ends of the scale. On several occasions, this child would choose "face 4," which reflects feeling bad and then say, "just kidding" or "I'm kidding" and then choose another face. On one occasion, his teacher asked "Is that how you are really feeling?" when the child stepped on a "sad" face and the child then moved to a different face. The CDC was not statistically significant for perceived stress and the IRD was 0.

Sarah

Sarah was a five-year-old female pre-kindergartener. She appeared somewhat apprehensive and very quiet. She did not have unprompted verbal interaction with either data collector until the last two weeks of the intervention. Sarah's teacher reported her as anxious, having difficulty with separations, and needing time to build trust. Her teacher stated that she was initially unsure that Sarah would participate in the deep breathing intervention, but feels it was helpful that another child in the class began data collection before her. Sarah's teacher reported that she was "regularly using it (deep breathing) at home". Her mother reported to the teacher giving her prompts and seeing success in helping her recovery quickly when she became anxious, nervous, or upset. Sarah is one of two children who proudly and confidently explained the deep breathing technique to her pre-kindergarten class when the teacher began to implement the deep breathing with all students in the class.

Sarah completed baseline measurements for one week (5 sessions) and participated in the intervention for three weeks (15 sessions). The baseline phase was

relatively short and showed a moderate amount of variability for pulse rate. This variability continued into and through the intervention phase. There was considerable overlap between phases as indicated by the IRD (20%) and the CDC was not statistically significant for pulse rate.

Sarah had the most consistent variation in perceived stress and more often would choose a more "stressed" face after the intervention. The impression from the data collector is that she may have felt that it was necessary to choose a different face each time, although she was reminded that she could choose the same face or a different face. Behaviorally, there was nothing to suggest that the intervention made her feel worse or uncomfortable, so interpreting this outcome is challenging. The CDC showed a statistically significant change and the IRD was 27%.

Discussion

Overall, one out of five children showed significant change in both pulse rate and perceived stress, in the expected direction. One child showed a significant change in perceived stress only, but visual analysis concluded that this was not in the expected direction. Three out of five children showed no change in either pulse rate or perceived stress. There was no pre-post intervention change in cortisol for the group as a whole.

Although the findings related to physiological and perceived stress are inconclusive, this study contributes important findings related to feasibility of a deep breathing intervention with five-year-olds. In terms of practical significance, this work provides clinicians and teachers with a scripted deep breathing protocol. It offers some insights to the challenges associated with stress measurement with children and

highlights the importance of continuing to seek ways to measure this with younger children.

Findings from single-subject designs are not generalizable to other samples. However, the results suggest that deep breathing may be beneficial for stress reduction in some children, and further studies with children of various ages, ethnicities, socio-economic levels and behavioral issues warrant investigation. For example, it is possible that deep breathing may have different effects with children experiencing mental health/behavioral challenges, such as ADHD, or those with a history of trauma.

I assessed clinical significance based on information from interviews with the children's teachers at the end of the intervention. Here, I attempted to ascertain meaningful behavioral and/or attitudinal changes in the children after the completion of the intervention. Although the teachers all stated it was difficult to report changes in behavior related to the intervention, they felt that several of the children were able to make the connection between feeling distress and using the technique and several of them were using it on their own in these circumstances. Teachers in both the pre-kindergarten and kindergarten classrooms plan on implementing it into their school day for the entire class; the pre-kindergarten has already begun doing this.

Some barriers to intervention delivery included less than ideal physical space, inconsistency of staffing, child absences, and different pacing of the intervention across teachers, which may have influenced the way the intervention was received.

Assessing the dose of the deep breathing intervention was a challenge. Anecdotally, teachers described witnessing several of the children using deep breathing

during the school day, however use of the at-home practice worksheets proved difficult. The PI received very few of these back and the teachers noted that it was often challenging for students to stop what they were doing to retrieve their folder and color in the bubble (indicating they practiced the deep breathing skill). Some reported barriers to home recording were lost papers, papers eaten by household pets, and spills.

It is possible that the intervention lowered stress levels but the measurement tools did not accurately reflect this. With the exception of a few low readings, the children fell within normative ranges for pulse rate and within normative levels for cortisol which may prevent detection of effects of the intervention. It was also difficult, due to time constraints, to collect enough baseline measurements to achieve stability, thus making interpretation of intervention effects difficult. There were also challenges measuring pulse rate with young children. Although I used a pulse rate monitor designed for use with children (one that minimized the impact of movement), they were required to remain still during the pulse rate monitoring, as well as not speak, both of which were challenging for several of them.

It is important to note that although the children were selected to participate in the intervention based on the highest scores (in total) from the three stress measures, only two scored in the clinical range on the Pediatric Emotional Distress Scale (indicating a higher probability of trauma exposure) and only one scored in the moderate clinical range on the Coddington Life Events Scale (there is no designated clinical cut-off for the Children's Hassles Scale). Interestingly, the child who scored in the clinical range on these two measures (Anna) was also the child who showed significant effects for changes

in pulse rate and perceived stress. The other child who scored in the clinical range on the PEDS was the only other child who showed a significant change, which was in perceived stress (albeit not in the predicted direction). Thus, it is possible that some selected children were identified as "stressed" relative to their peers, but weren't experiencing levels of stress necessary to see intervention effects.

I was unable to easily characterize changes in salivary cortisol from pre- to post-intervention. A dysregulated stress response system can produce cortisol levels that are higher or lower than normal, which may depend on the type of stressors and/or trauma experienced (Carpenter, 2007). Thus, if cortisol levels were higher than normal the expectation would be that the intervention might lower cortisol and if lower than normal cortisol was present, the intervention might increase cortisol. For example, maltreated children in foster care have showed higher rates of low morning cortisol than children who were not maltreated (Bruce, Fisher, Pears, & Levine, 2008). In addition, physical neglect was associated with lower cortisol levels in foster children and emotional maltreatment was associated with higher cortisol levels. Early physical and sexual abuse have been associated with an attenuated diurnal cortisol decrease, specifically those with internalizing symptoms, when compared with non-abused children (Cicchetti, Rogosch, Gunnar, & Toth, 2010).

In this study, all participants had cortisol concentrations within normal ranges for both morning and afternoon, so it is possible that changes in physiological stress may not have been reflected here. There is evidence to suggest that nurturing caregiving can buffer the effects of stressful life events (Ciccchetti, Rogosch, Toth, & Sturge-Apple,

2011; Fisher & Stoolmiller, 2008) so it is possible that although parents were reporting stressful life events and stressors, the effects were buffered by positive parenting. It is also possible that stress effects were buffered by the child/teacher relationship in that students were receiving additional attention from their teacher.

In an effort to measure diurnal slope, where cortisol levels rise before awakening, have a sharp increase approximately 30 to 45 minutes after awakening and continue to decline throughout the day, I collected cortisol on two occasions, either morning or mid-morning, and afternoon. Diurnal slope is an important measure of physiological stress, and atypical diurnal slope may be reflective of a dysregulated stress response system (Gunnar & Quevedo, 2007). A more precise analysis of diurnal slope would have included several collections throughout the day. It is possible that greater differences in diurnal slope would have been observed if this had occurred.

A significant limitation of this study is the lack of a validated self-report measure of perceived stress for this age group. Limitations of the abilities of younger children to report subjective distress that have been highlighted in the literature were prevalent here. As mentioned, we do not know if 5-year-old children can accurately report perceived stress. Although the children were able to understand the concept of rank-ordering, as stated earlier, this does not necessarily translate to being able to identify these feelings in themselves. Previous research found that young children tend to report extremes on a likert-type scale (Goodenough et al., 1997) which was the case for some children in this study. Social desirability bias may be more likely in children than adults (Baxter et al., 2004), although limited studies have examined this with younger children. In addition,

children may be more likely to provide a response, rather than state they don't know (Scott, 2000). It is also possible that the relationship with the individual collecting the data, including the variation in school staff that was present during data collection, influenced the child's ability to accurately report feelings.

In terms of participant selection, parents may not be accurate reporters of child stress symptoms/behaviors (Frick, Silverhorn, & Evans, 1994; De Los Reyes & Kazdin, 2005) thus initial selection stress scores may have been skewed. However, anecdotal comments from teachers (of four out of the five students) indicated that they felt the selection procedures did, in fact, select children they felt would benefit most from a stress reduction intervention.

There was no follow-up for effects in this study. It is possible that longer-term effects were present that were not reflected in the time period in which data was collected. I do not know if any reductions in stress remained present after intervention was completed.

Conclusions/Future Research

This study continues the growing knowledge base of how mind-body interventions may reduce stress symptoms. To my knowledge, this is the first study that attempts to examine the effects of deep breathing as a sole treatment component on stress in five-year-old children. Although the results are inconclusive, it seems that five-year-old children can participate in deep breathing and that it can be implemented relatively easily in a school setting, although flexibility is required.

All three teachers who participated in delivering the intervention stated they could see implementing it on the classroom level and one stated she was already doing so, with the children who participated acting as leaders, after the intervention was completed. All three teachers stated that the children enjoyed participating in the deep breathing, felt the intervention was "kid friendly," and viewed it as a technique that would be easily learned by other children in the class. They also stated they found it relaxing for them, as well.

Future studies should include larger sample sizes and control groups, perhaps focusing on children experiencing higher levels of stress due to issues such as lower SES or maltreatment. In addition, future studies may focus on how children with different characteristics (i.e. hyperactivity, aggression, etc.) respond differently to mind-body interventions. Finding age-appropriate ways to measure perceived stress is paramount in continuing to examine intervention effectiveness for deep breathing and other interventions that may alleviate child stress.

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Appendix B

Table 1-B. Morning and Afternoon Salivary Cortisol Concentrations for Pre- and Post-Intervention and Normative Cortisol Values for Age and Time of Collection

Child	Time of collection	Normative mean Age 4-10 (McCarthy et al.)	Mean Pre	Mean Post	Time of collection	Normative mean Age 4-10 (McCarthy et al.)	Mean Pre	Mean Post	Normative Diurnal Change – Mean (McCarthy et al.)	Diurnal Change-Pre	Diurnal Change-Post
Colin	8:20am	.27 {Range (2 SD + -) 1.16 - .06}	.215	.185	2:20pm	.13 {Range (2 SD + -) .52 - .03}	.105	.095	.27-.13= .14	.22-.11=.11	.19-.10=.09
Christopher	8:25am	.27 {Range (2 SD + -) 1.16 - .06}	.14	.175	2:25pm	.13 {Range (2 SD + -) .52 - .03}	.07	.13	.27-.13=.14	.14-.07=.07	.18-.13=.05
Anna	11:20am	.18 {Range (2 SD + -) .78 - .041}	.25	.25	2:50pm	.12 {Range (2 SD = -) .48 - .03	.18	.105	.18-.12=.06	.25-.18=.07	.25-.11=.14
Jake	10:50am	.22 {Range (2 SD + -) .044-.882}	.055	.07	2:30pm	.13 {Range (2 SD + -) .52 - .03}	.08	.065	.22-.13=.09	.06-.08= -.02	.07-.07=0
Sarah	10:30am	.21 {Range (2 SD + -) .93 - .048}	.085	.06	2:30pm	.13 {Range (2 SD + -) .52 - .03}	.06	.085	.21-.13=.08	.09-.06=.03	.06-.09= -.03

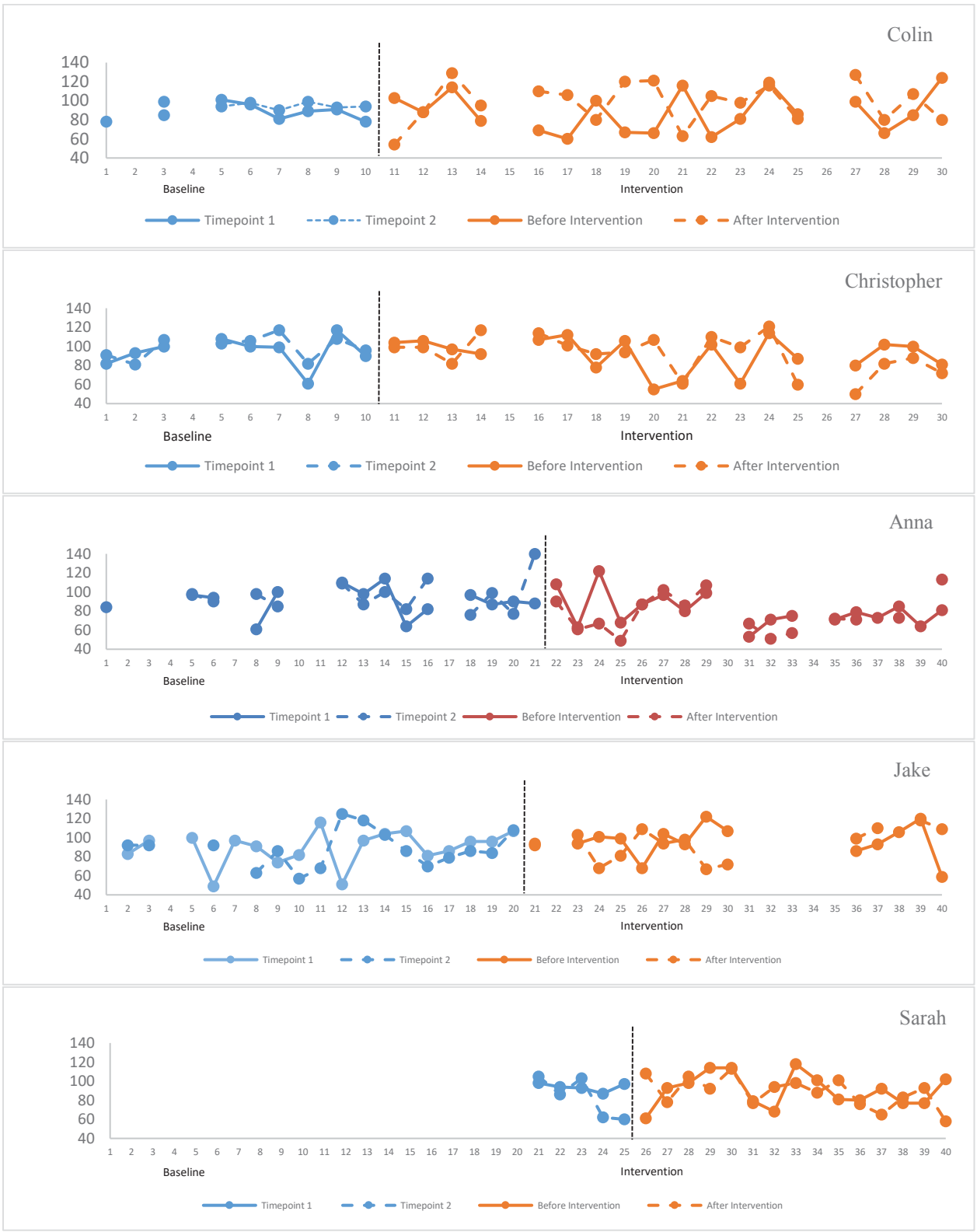

Figure 1-B. Pulse Rate

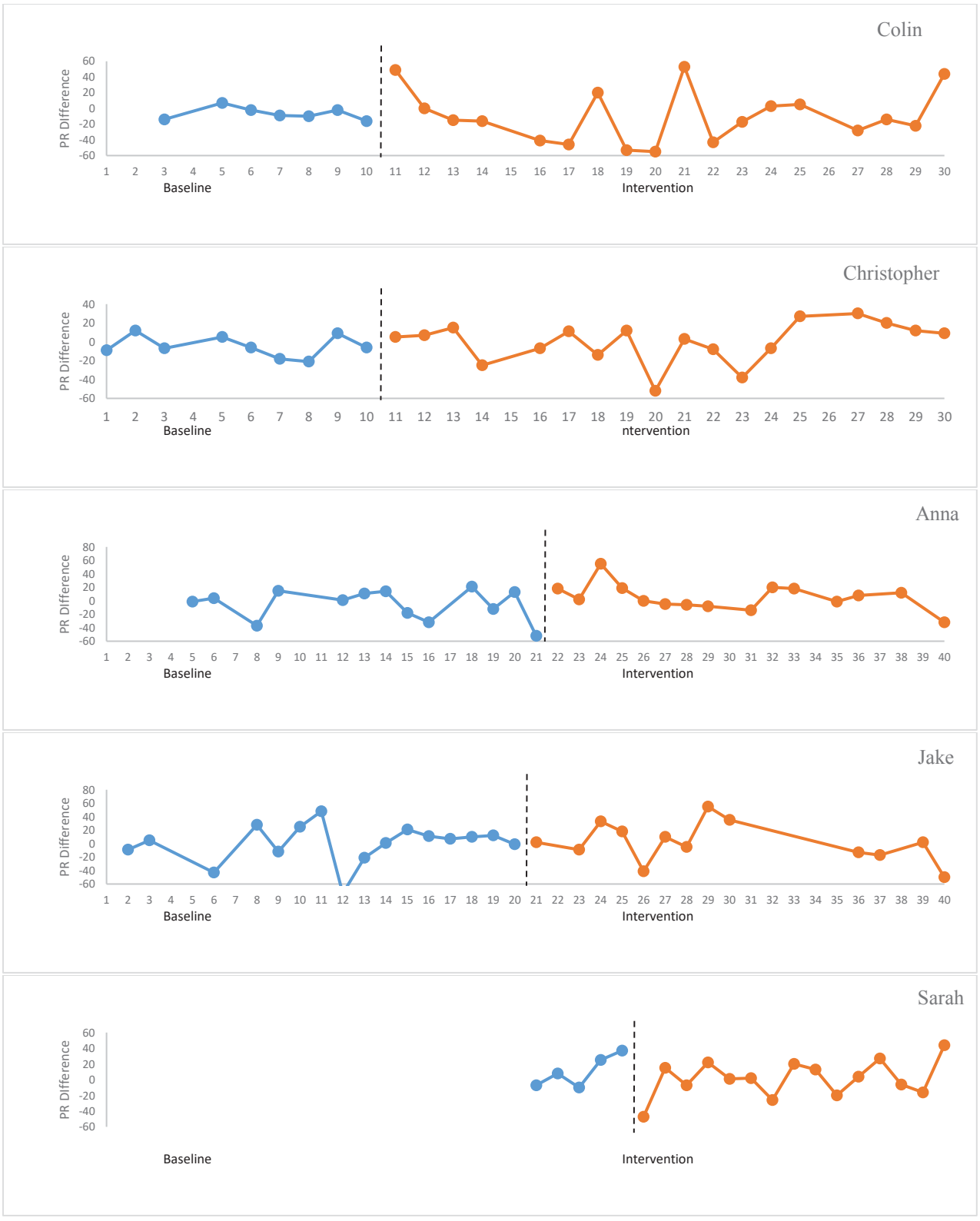

Figure 2-B. Pulse Rate Difference

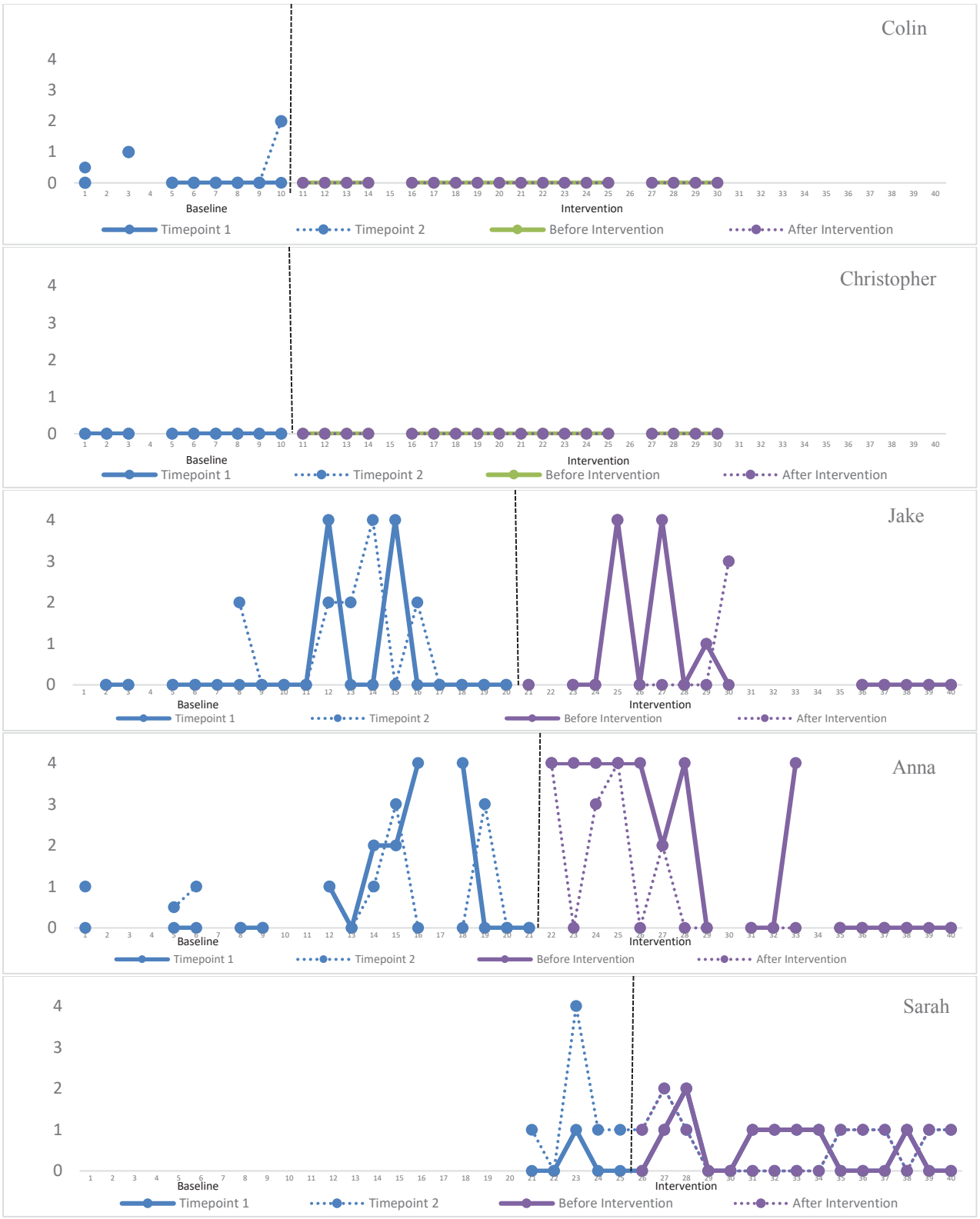

Figure 3-B. Perceived Stress

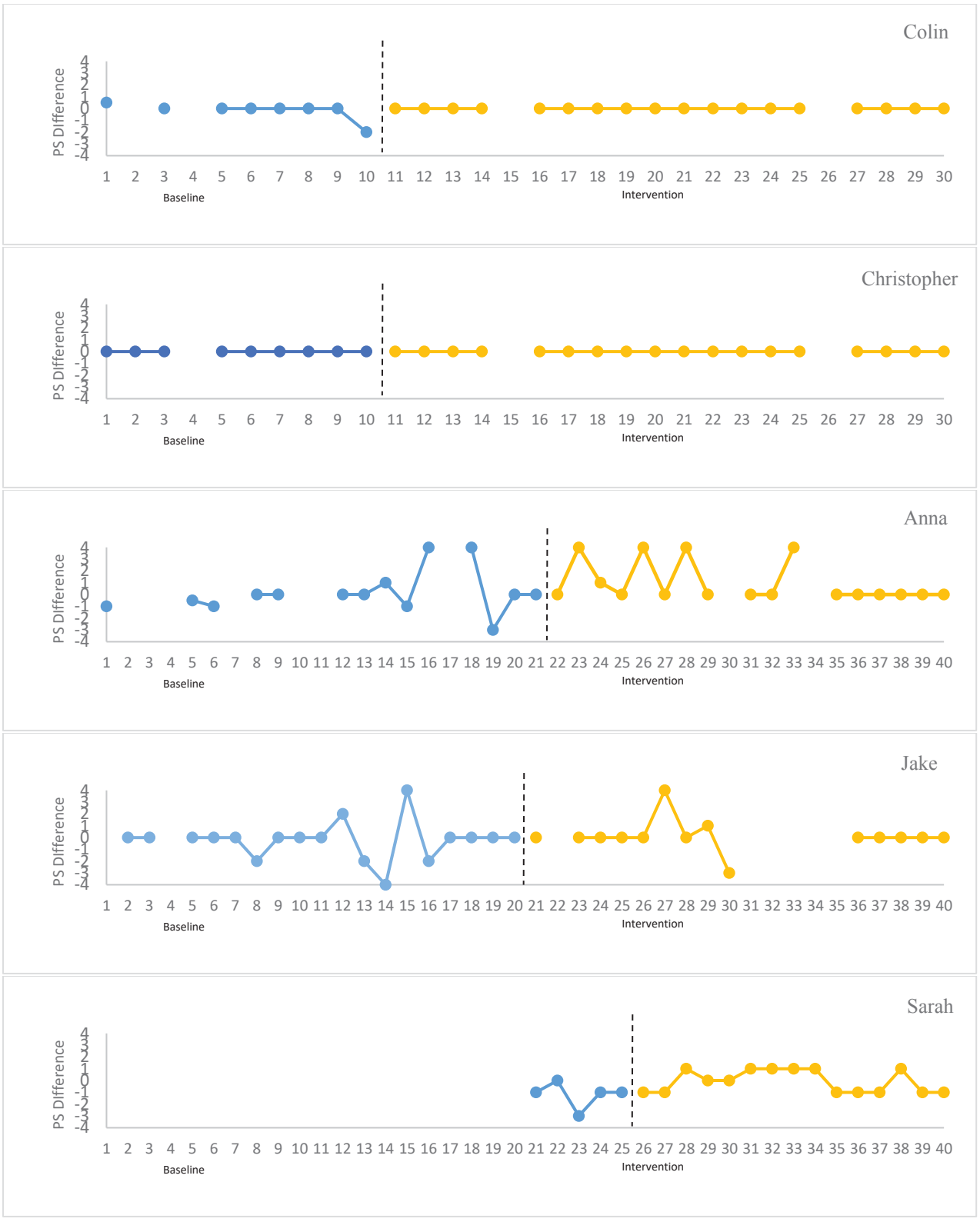

Figure 4-B. Perceived Stress Difference

Conclusion

This dissertation examines key elements of child stress, as viewed through a neuroscience lens, including physiological understanding, measurement, and intervention.

Chapter 1 provides an overview of child stress, focusing on how stress dysregulation impairs brain development, and thus leading to future physical and mental health difficulties. This framework was presented to facilitate an understanding of the neural underpinnings of stress and recognize the importance of physiological aspects of stress, including how they impair the brain both structurally and functionally. This is an important area of attention for social workers, who often focus on emotional and behavioral functioning, with much less emphasis on biology, despite the fact that the bio-psycho-social model is recognized as a core framework in social work practice (Larkin, Feletti, & Anda, 2014*)*.

A true bio-psycho-social focus must aim to incorporate a comparable biological lens into social work practice. Social workers perform a large majority of mental health interventions (NASW, 2017), therefore should play a significant role in intervention development. The knowledge base on how to intervene with children experiencing stress, especially younger children, is limited. One reason for this is because measuring subjective distress in younger children is challenging. There is a lack of validated self-report measures of perceived stress for younger children, especially for those who don't yet read or write. In these cases, parent reports are often used, however parents may not be accurate reporters of child stress symptoms/behaviors (Frick, Silverhorn, & Evans,

1994; De Los Reyes & Kazdin, 2005). Studies have shown that parents underestimate constructs such as worry and anxiety in their children, and their reports often do not correlate with their children's self-report (Lagattuta et al., 2012).

With these measurement challenges in mind, and coupled with our neurobiological knowledge of stress, incorporating physiological measures of stress to assess interventions is critical. To that end, *Chapter 2* examines, via a systematic literature review, use of biological measures in school-based intervention research. Since much of social work intervention research takes place outside of traditional research laboratories, examining use of biological measures in these settings is important. School-based intervention research allows access to children who may not otherwise be identified as at-risk. Many children who experience emotional and behavioral issues do not receive treatment (Bratton, Ray, Rhine, & Jones, 2005) and schools are an effective and efficient venue to reach a large number of potentially underserved children.

I asked the following research question: To what extent are biological measures being used to assess stress and/or anxiety intervention effectiveness in schools, and are they feasible for use in school-based settings? Since use of physiological measures in intervention research is relatively new, I focused on intervention studies published over the last 10 years that incorporated at least one physiological stress measure. I identified 20 studies that used the following physiological stress measures: salivary cortisol (11 studies), serum cortisol (1 study), heart rate (5 studies), ambulatory heart rate (2 studies), heart rate variability (3 studies), resting blood pressure (3 studies), ambulatory heart rate

(3 studies), sodium handling (2 studies), alpha amylase (1 study), and skin conductance (1 study).

I then discussed each biological measure, including its role in the human stress response, logistical measurement considerations, and the feasibility of its use in a school setting. The review reveals that researchers are recognizing the importance of using physiological measures of stress and that many can be implemented relatively easily in school settings, although feasibility issues do exist.

Because of the limited knowledge base regarding how to intervene with younger children experiencing stress, and an increased understanding of physiological processes and stress measurement, a focus on intervention development seemed logical.

To that end, *Chapter 3* presents results of a simple, cost-effective, deep breathing intervention in a school setting. Although there is a dearth of research on deep breathing as a sole intervention with younger children, studies with adults and older children suggest that it may be effective in reducing physiological and perceived stress. Deep breathing is incorporated into several manualized protocols (such as TF-CBT, MBSR, etc.) and virtually all mind-body literature incorporates deep breathing as a component of the intervention. This suggests that young children are capable of performing this technique, which was supported in this study.

Although parent participation is clearly preferable in therapeutic work with young children, the reality is that parents of children, especially children in low-income and/or higher stress environments, are often not available to participate in treatment. Although parental participation would be encouraged, mind-body interventions, such as deep

breathing, with children can be implemented without it.

I used a multiple-baseline-across-individuals design to determine if a 5-minute deep breathing intervention over four weeks reduced stress in five pre-kindergarten or kindergarten children. I examined physiological stress via salivary cortisol and pulse rate, and perceived stress via a faces scale developed for the study. Although self-reports are important to incorporate when examining stress-related variables, relying on them as a sole method of measurement can be misleading and unreliable, especially with younger children.

Although the findings related to physiological and perceived stress are inconclusive (the deep breathing intervention did not significantly alter physiological stress levels in four out of five children), this study contributes important findings related to feasibility of a deep breathing intervention with five-year-olds. Some barriers to intervention delivery included less than ideal physical space, inconsistency of staffing, child absences, and different pacing of the intervention across teachers, which may have influenced the way the intervention was received. It is possible that the intervention lowered stress levels but the measurement tools did not accurately reflect this. With the exception of a few low readings, the children fell within normative ranges for pulse rate and within normative levels for cortisol, which may prevent detection of effects of the intervention. It is possible that higher baseline levels of stress are needed to achieve a benefit from the intervention.

Measurement challenges are noted, in that variability of both pulse rate and cortisol made interpretation challenging. Pulse rate fluctuations made it difficult to

establish the baseline stability necessary to assess intervention changes. And although the small sample was conducive to single-case design, there was not enough power to detect changes in cortisol on the group level, even if changes did exist. It is also possible that the intervention with this sample (higher stress relative to the initial sample, but not clinically high-stressed) wasn't powerful enough to show changes in the physiological stress response over time. The reliability and validity of the perceived stress measure, developed for this study, is unknown and if an established reliable and valid tool was available, it may have detected changes that didn't exist here.

All three teachers who participated in delivering the intervention stated they could see implementing it on the classroom level and one stated she was already doing so, with the children who participated acting as leaders. All three teachers stated that the children enjoyed participating in the deep breathing, felt the intervention was "kid friendly," and viewed it as a technique that would be easily learned by other children in the class. This study provides clinicians and teachers with a scripted deep breathing protocol that can be used in the classroom in an individual or group setting. It offers some insights to the challenges associated with stress measurement with children and highlights the importance of continuing to seek ways to measure this with younger children.

Knowledge Gaps/Future Research

There are a limited number of deep breathing studies with children, making it difficult to conduct an overall assessment of the deep breathing literature with children. However, methodological challenges in this research can be acknowledged. Sample sizes

tend to be small, which may underestimate treatment effects. Use of control groups and random assignment can be challenging in treatment environments, including schools, due to logistical issues and ethical concerns regarding withholding treatment. Larger sample sizes and use of control groups would allow for greater validity in interpreting results.

One of the limitations in synthesizing the mind-body literature in general is that mind-body techniques are often combined. Thus, it is difficult to separate effects of each individual technique. Future research should include examination of techniques in isolation to determine which particular methods are most effective with which populations.

Alteration of neural activity may be predicated on a certain amount of repetition, so a focus on dose and intensity of treatment is paramount (Greenberg & Harris, 2012). According to Baker (2012) a minimum of three to four weeks' practice is recommended for meditation and breathing techniques for the most beneficial physiologic changes. Studies have used a large variation of dose, frequency and duration of breathing interventions, ranging from a single session of deep breathing to several times daily over twelve weeks or more. It is unclear how duration, dose and frequency influence overall outcomes, though several mind-body studies noted that it would have been beneficial for their interventions to be carried out for a longer period of time. Understanding of how dose, duration and frequency of mind-body stress interventions influence psychological and psychosocial stress changes is an important area of exploration, keeping in mind that modified treatment protocols for children need to weigh these issues with developmental limitations, especially with younger children.

Many studies examined offered limited follow-up data, and without this it is difficult to capture the long-term effects of the interventions. It is possible that longer-term effects were present that were not reflected in the time period in which data were collected, as was the case in two of the studies reviewed in *Chapter 3* that examined HRV coherence and salivary cortisol (Bothe et al., 2014; Lozada et al., 2014).

Some studies related to stressful life events and their relationships to adjustment difficulties indicate that some children do not seem to be as negatively affected by life events as others (Dubow & Tisak, 1989). One reason is genetic sensitivity. Genetic predisposition may influence an individual's response to environmental influences, both positive and negative. Certain genetic polymorphisms have been associated with various types of adaptive functioning, as well as mental health issues and developmental problems in children (Obradovic & Boyce, 2009). For example, an individual's genes can lead to resilience or vulnerability as it relates to early adverse experiences and risk of psychopathologies (Kim-Cohen & Turkewitz, 2012) as well as sensitivity to intervention effects. For example, Lupien et al. (2013) found that a stress-coping intervention with middle-school children lowered cortisol only in a sub-group of children who showed high levels of anger. More research examining physiological sensitivities to stress interventions is needed. Also, further studies with children of various ages, ethnicities, socio-economic levels and behavioral issues warrant investigation. For example, it is possible that deep breathing may have different effects with children experiencing mental health/behavioral challenges, such as ADHD.

Events, or situations children find stressful will, of course, vary according to age.

Studies with older children, although limited, have examined this. Adolescents ages 14-17 most commonly cited school, parents, friends and boyfriend or girlfriend problems as stressful (Stark, Spirito, Williams, & Guevremont, 1989). Similarly, children aged 9-14 cited problems with school, parents, siblings, and friends as the most stressful (Spirito, Stark, Grace, & Stamoulis, 1991). A descriptive study on 5th grade students' daily life stressors, using open-ended questions, noted five themes that included: fear of being late, not having sufficient time, physical and mental consequences, experiencing others' stress, and seeing stress as both a positive and negative feeling (Brobeck, Marklund, Haraldsson & Berntsson, 2007). No studies were found examining what stressors are most prevalent for younger children and this is an area in need of further exploration.

Finding age-appropriate ways to measure perceived stress in younger children is critical. In the study presented here (*Chapter 4*) I used a single-item, likert faces scale adapted from the adult Faces Anxiety Scale (McKinley, Coote, & Stein-Parbury, 2003) and the Children's Anxiety/Fear Scale (McMurtry, Noel, Chambers, & McGrath, 2010). To my knowledge, no validated pictorial measurement tools for stress exist for young children. Several studies have found face scales to be the most effective tool for self-report in young children, although virtually all of this research has been related to pain and/or pain-related anxiety/fear or procedural distress (Elfering & Grebner, 2012; Kuttner & LePage, 1989; Woodgate & McClement, 1998). Future work should include development and validation of a pictorial perceived stress measurement tool for younger children who cannot read or write.

Overall, a bio-physiological approach provides a salient avenue to enhance our understanding of human behavior, thus potentially improving social work practice delivery and outcomes (Egan, Combs-Orme, & Barnes, 2011). Comprehensive understanding of the bio-psycho-social framework provides us with a more complete picture of how our client's history and experiences affect functioning, helps us make predictions about future health, thus allowing for early intervention, and enhances our ability to understand differential treatment effects. Assisting clients, especially those who are vulnerable and oppressed, in achieving their highest level of functioning is a core element of social work values. This approach improves our understanding of how physiological processes further inform our understanding of physical and mental health disparities in oppressed groups (Combs-Orme, 2017). We should continue to look to find ways to disseminate this critical material to clinicians and social work educators alike.

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Vita

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